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Inhibition of *Listeria innocua* in Hummus by a Combination of Nisin and Citric Acid

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ABSTRACT

The effect of nisin or citric acid or combinations of these two inhibitors on the inactivation of a cocktail of three *Listeria innocua* strains was investigated in a model brain heart infusion (BHI) broth and hummus (chickpea dip). In BHI broth, citric acid had a limited ability to inhibit *L. innocua* growth. Nisin initially reduced *L. innocua* concentrations by about 3 log cycles; however, *L. innocua* reached concentrations similar to those of the control after 5 days at 22°C. In combination, the effects of 500 IU/ml nisin and 0.2% citric acid were synergistic and resulted in complete elimination of *L. innocua* in the BHI broth. The inhibition of *L. innocua* by nisin (500 or 1,000 IU/g), citric acid (0.1, 0.2, or 0.3%), or their combinations also was evaluated in hummus. Citric acid alone did not affect *L. innocua* growth or the aerobic bacterial plate count. A combination of 1,000 IU/g nisin and 0.3% citric acid was somewhat effective (~1.5-log reduction) in controlling the concentration of *L. innocua* and the aerobic plate count for up to 6 days. This combination also may be useful, in addition to proper hygienic practices, for minimizing the growth of the pathogen *Listeria monocytogenes* in hummus.

Hummus (chickpea dip) is one of the most commonly consumed breakfast foods in the Middle East, and it is increasing in popularity in the United States and Europe *(5)*. Hummus is a low-acid food made from garbanzo beans (*Cicer aretinum* L.), sesame butter, and salt. Other ingredients such as lemon juice or citric acid and garlic can be added as condiments *(39)*. The approximate composition of hummus is 71.0% moisture, 1.7% ash, 6.0% protein, 4.8% fat, 0.7% crude fiber, and 15.7% nitrogen-free extract *(5)*. Because it is a ready-to-eat food, hummus is vulnerable to postprocess contamination. Hummus is a rich source of nutrients with a high water activity and can support the growth of various microorganisms. For example, very high microbial counts have been recovered from hummus produced for local markets in Jordan *(39)*, and high counts of *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* have been recovered from chickpea tempeh *(6, 7)*. Hummus could serve as a potential source of *L. monocytogenes* infection *(30)*. *L. monocytogenes* is widely distributed in the environment *(1, 17)* and has been implicated in several fatal outbreaks of foodborne illness *(8, 15, 25, 30)*; it has been recovered from about 15% of ready-to-eat foods in Portugal *(20)*. *L. monocytogenes* can be easily introduced into hummus from many sources in the processing environment, such as raw materials, food handlers, equipment, and utensils. The presence of *L. monocytogenes* in ready-to-eat foods is considered a risk factor, especially for high-risk individuals, and as a result the U.S. Food and Drug Administration has set a zero tolerance level for *L. monocytogenes* in ready-to-eat food products *(23)*.

Nisin is a heat-stable bacteriocin produced by certain strains of *Lactococcus lactis*. Nisin is primarily active against gram-positive bacteria, including *Clostridium*, *Bacillus*, and *Staphylococcus* species *(28)* and *Listeria* species *(11, 27, 32)*. The Joint Expert Committee on Food Additives, the U.S. Food and Drug Administration, and the European Union recognize nisin as safe for food use *(12)*.

Strains of *L. monocytogenes* vary widely in their sensitivity to nisin *(19)*, with greater sensitivity at low pH *(36)*. Acid-adapted cells (pH 5.5) of *L. monocytogenes* exhibit increased tolerance to nisin *(31)*, and *L. monocytogenes* can develop resistance to nisin *(26)*. However, the antilisterial activity of nisin can be enhanced when nisin is used with other preservation techniques. The combination of suboptimal preservation stresses such as nisin and organic acids may result in a greater effect than that produced by the individual factors alone.

The antimicrobial effect of nisin in combination with low water activity, reduced pH *(18)*, high hydrostatic pressure *(17, 27)*, dielectric heating *(3)*, and chemical antimicrobial treatments *(4)* has been studied. The combination of nisin plus moderate heat or pulsed electric field eliminated *Listeria* spp. in cold-pack lobster meat and skim milk, respectively *(12, 13)*. However, the combination of nisin with other antimicrobial agents such as citric acid has received little attention as a food preservation method.

In this study, nisin and citric acid were evaluated as agents for controlling the ability of *Listeria innocua*, a surrogate for *L. monocytogenes (22)*, to grow in hummus under refrigerated storage for 15 days. Residual concentrations of nisin in the product were recorded during the storage period.

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MATERIALS AND METHODS

Preparation of nisin solution. Nisin powder (MP Biomedicals Inc., Aurora, Ohio) with an activity of 1,000 IU/mg was prepared into a stock solution (10,000 IU/ml) by dissolving an appropriate amount of nisin in 0.02 M HCl. The solution was heated at 80°C for 7 min and kept at −20°C until use.

Bacterial strains and culture media. All cultures used in this study were obtained from the culture collection at the Department of Food Science and Human Nutrition of Washington State University (Pullman). All tested cultures were maintained as frozen stocks at −20°C in 15% glycerol. A stationary-phase culture of a cocktail of three strains of *L. innocua* (ATCC 51742, ATCC 33090, and ATCC 33091) was used to inoculate hummus because cells in this stage are most resistant to preservation stresses *(14, 21, 35). L. innocua* was selected for study because it is more resistant to nisin treatment than are strains of *L. monocytogenes (3). Listeria* strains were cultured individually in brain heart infusion (BHI) broth (Becton Dickinson, Sparks, Md.) at 37°C for 24 h, harvested by centrifugation at 5,000 rpm for 15 min (Accuspin 400, Fisher Scientific Co., Pittsburgh, Pa.), and washed two times with 0.9% saline solution. The final pellet was resuspended in 0.9% saline solution, corresponding to approximately 10^9 CFU/ml.

Product preparation and antimicrobial treatments. The activity of nisin, citric acid, or a combination of both against *L. innocua* was first tested in BHI broth (control). BHI broth containing 500 IU/ml nisin, 0.2% citric acid, or a combination of 500 IU/ml nisin plus 0.2% citric acid was inoculated with the three-strain *L. innocua* cocktail listed at ca. 3.0×10^6 CFU/ml. The samples were kept at 22°C, and *L. innocua* was enumerated every day for 5 days.

Hummus was prepared from ingredients purchased from local grocery stores. Garbanzo beans were soaked overnight in water, drained and cooked in tap water until a soft texture was obtained. The soft beans were blended with 10% tahini (sesame butter), 2% salt and 15 ml of water per 100 g of product. At this point, the hummus was ready for antimicrobial treatments.

Under aseptic conditions, 100-g samples of hummus were treated with 500 IU/g nisin, 1,000 IU/g nisin, 0.1% citric acid, 0.2% citric acid, 0.3% citric acid, or various combinations of nisin and citric acid and then inoculated with the *L. innocua* cocktail at ca. 10^6 CFU/g. Samples of inoculated product (about 100 g) were placed in sterile plastic trays and stored refrigerated (4°C) for 15 days. Inoculated samples that were not treated with nisin or citric acid served as controls. The pH values of the different treatment samples were measured (Accumet AB15 pH meter, Fisher Scientific Co., Singapore) at the beginning and end of the storage period. The water activity of the hummus was measured using an AquaLab water activity meter (Decagon Devices, Pullman, Wash.).

Microbiological enumeration. Five-gram samples of hummus were placed in sterile stomacher bags with 45 ml of sterile 0.1% peptone water, homogenized with a model 400 stomacher (Seward Ltd., London, UK), and then 10-fold serially diluted with 9 ml of sterile 0.1% peptone water. Aerobic bacterial plate counts (APCs) were determined after dilution by spread plating 100 μl of diluted sample onto plates of tryptic soy agar (TSA; Becton Dickinson), which were incubated at 37°C for 36 h.

Surviving and injured *L. innocua* cells were enumerated using the overlay method *(2, 24),* which was designed specifically to improve the recovery of injured cells. TSA spread plates were incubated at 37°C for 2 h to allow the injured *L. innocua* cells to repair and resuscitate, and then about 7 ml of the *Listeria* selective PALCAM medium base (Becton Dickinson) containing the antimicrobic supplement (Becton Dickinson) was overlaid onto the TSA. The plates were then incubated for an additional 34 h at 37°C, and typical black colonies were enumerated. These experiments were conducted in triplicate. Microbiological analyses were conducted at 0, 3, 6, 9, 12, and 15 days, and the results were expressed as log CFU per gram.

Bioassay for nisin activity. Nisin activity was determined using the method of Fowler et al. *(16)* with some modification. Nisin from a 1.0-g hummus sample was extracted with 4 ml of 0.02 M HCl by vortexing for 30 s to ease the dispersion of the hummus into the extracting solution. The pH was adjusted to 2.00 ± 0.02 with 5 M HCl, and the slurry was heated in a boiling water bath for approximately 5 min. The extract was then cooled rapidly to room temperature, and the volume was adjusted to 5.0 ml with 0.02 M HCl. The pH of the extract was then adjusted to 6.5 with 5 M NaOH. Bioassay plates were prepared by autoclaving deMan Rogosa Sharpe (MRS) agar medium (Becton Dickinson) at 121°C for 15 min and then pouring the agar into petri plates to a thickness of 5 mm. The agar was punched with a sterile stainless steel tube to make 8-mm-diameter holes. The holes were filled with 100 μl of the nisin-hummus extract, which was allowed to diffuse into the agar for about 15 min underneath a laminar flow hood. The plates were then overlaid with 8 ml of MRS soft agar (0.7%), which had been seeded with approximately 10^7 CFU of the nisin-sensitive indicator bacteria (*Lactobacillus fermentum* ATCC 9338). These assay plates were then incubated at 37°C for 18 to 24 h. One activity unit was defined as the reciprocal of the highest dilution yielding a definite zone of inhibition. The activity was expressed as IU per gram of hummus.

The standard curve was prepared as described by Bower et al. *(10).* The nisin concentration (log IU per milliliter) was plotted against the square of the corresponding zone width to obtain a regression line; in these experiments, $R^2 > 0.96$. Nisin concentrations were recorded throughout the experiment on days 0, 3, 6, 9, 12, and 15.

Statistical analysis. Each reported value is a mean from three replicate experiments. Data were analyzed with a computer software package (SAS Institute, Cary, N.C.) using and analysis of variance and Fisher's least significant difference test.

RESULTS AND DISCUSSION

Stationary-phase cells of *L. innocua* were used in these experiments because these cells are more resistant to nisin treatment than are cells in the exponential growth phase *(37).* The effect of 500 IU/ml nisin, 0.2% citric acid, or the combination of both agents was studied in a model system of BHI broth (Fig. 1). Neither nisin nor citric acid was effective alone in controlling growth, even for a short period. Nisin treatment resulted in an initial reduction in *L. innocua* counts by approximately 3 log cycles. However, the antilisterial effect started to wane immediately, and a sharp increase in *L. innocua* concentrations was observed during storage at 22°C. Concentrations equivalent to that of the control were reached after only 2 days. The underlying mode of action of nisin is thought to be disruption of membrane function by the formation of pores in the bacterial cell membrane followed by leakage of cellular material and dissipation of the membrane potential *(35, 38).* Addition of nisin to a culture of *L. monocytogenes* can cause a rapid

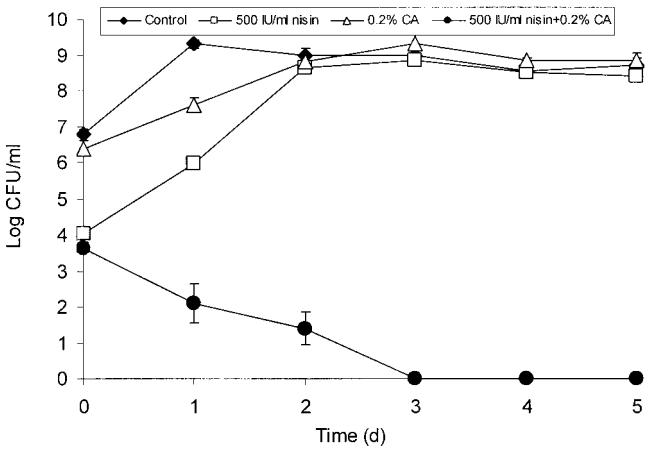

FIGURE 1. *Effect of nisin (500 IU/ml), citric acid (0.2% CA), or their combination (500 IU/ml nisin + 0.2% CA) on a three-strain mixture of* L. innocua *grown in brain heart infusion (BHI) broth at 22°C for 5 days (count before treatment was 6.77 log CFU/g). Control group was BHI broth inoculated with* L. innocua *but not treated. Initial pH values of the inoculated BHI broth were 7.43 (control), 7.23 (500 IU/ml nisin), 5.09 (0.2% CA), and 5.91 (500 IU/ml nisin + 0.2% CA). Each point represents an average of three replicates.*

decrease in the number of viable cells followed by a regrowth to a population similar to that of untreated cells *(33, 36)* because resistant and injured *Listeria* cells recover and resume growth.

Citric acid has very weak antilisterial activity. Treatment with 0.2% citric acid resulted in about a 0.50-log reduction, and the *L. innocua* count then returned to levels similar to that of the control. However, when nisin was used in combination with citric acid, an instantaneous ~3-log reduction in *L. innocua* concentrations was observed, followed by a sharp decline until undetectable levels were reached after 3 days of incubation; no cell recovery was observed through the end of the 5-day storage period.

The growth pattern of *L. innocua* (Table 1) and the APCs (Table 2) indicate that hummus supports microbial growth under refrigerated storage conditions. *Listeria* spp. have been found to grow over a wide range of temperatures (0 to 42°C) *(9)*. *L. innocua* in hummus samples grew slowly but steadily under refrigerated (4°C) conditions. This finding is in agreement with those obtained by Ashenafi *(6)*, who observed growth of *L. monocytogenes* in chickpea tempeh.

The water activity of the hummus as measured in this study was 0.983. This high water activity and the high nutrient content and relatively neutral pH (Table 3) are crucial factors that make hummus a favorable microbial media. Hummus is rich in simple sugars, predominantly sucrose and raffinose, which can be utilized by *Listeria* spp. *(39)*. Treatment of hummus with 500 IU/g nisin resulted in nearly a 0.5-log reduction in *L. innocua* ($P < 0.05$), and treatment with 1,000 IU/g resulted in about a 1-log reduction ($P < 0.05$). However, the listericidal action of nisin did not last for more than 3 days, and concentrations increased to a level similar to that of the control. Organic acids exert antilisterial affects by penetrating bacterial cell membranes.

TABLE 1. *Survival of* Listeria innocua *at 4°C following treatment of hummus with different concentrations of nisin, citric acid, or their combination*

Treatment	*L. innocua* (log CFU/g)[a]					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Control	6.13 ± 0.24 A a	6.66 ± 0.17 B a	7.43 ± 0.44 C a	8.42 ± 0.17 D a	9.20 ± 0.13 E a	9.22 ± 0.06 E b
500 IU/g nisin	5.59 ± 0.19 A cd	6.06 ± 0.52 AB bc	6.21 ± 0.36 C bc	8.52 ± 0.28 D a	9.26 ± 0.06 D a	9.40 ± 0.07 D a
1,000 IU/g nisin	5.18 ± 0.17 A e	5.82 ± 0.11 B c	6.13 ± 0.04 C bc	8.33 ± 0.04 D a	9.09 ± 0.04 E a	9.19 ± 0.13 E b
0.1% CA[b]	6.06 ± 0.11 A a	6.13 ± 0.15 A bc	6.20 ± 0.09 A bc	7.17 ± 0.07 B f	8.65 ± 0.06 C b	9.23 ± 0.09 D b
0.2% CA	5.92 ± 0.03 A a	6.12 ± 0.10 A bc	6.44 ± 0.14 B b	7.41 ± 0.25 C ef	8.54 ± 0.08 D b	8.89 ± 0.05 E c
0.3% CA	5.86 ± 0.02 A ab	5.97 ± 0.36 A bc	6.38 ± 0.28 B b	7.77 ± 0.16 D cd	8.53 ± 0.08 E b	8.84 ± 0.08 E cd
0.1% CA + 500 IU/g nisin	5.51 ± 0.05 A d	6.33 ± 0.09 B ab	6.31 ± 0.12 B b	8.36 ± 0.04 C a	9.06 ± 0.08 D a	8.85 ± 0.05 E cd
0.1% CA + 1,000 IU/g nisin	5.55 ± 0.25 A d	5.31 ± 0.31 A e	6.04 ± 0.18 B bc	7.54 ± 0.20 C de	7.97 ± 0.07 D d	8.74 ± 0.07 E d
0.2% CA + 500 IU/g nisin	5.49 ± 0.06 AB d	5.17 ± 0.27 A e	5.83 ± 0.24 B c	7.96 ± 0.41 C bc	9.22 ± 0.14 E a	9.12 ± 0.05 E b
0.2% CA + 1,000 IU/g nisin	5.64 ± 0.32 A bcd	5.31 ± 0.45 A de	6.36 ± 0.08 B b	7.20 ± 0.16 C f	8.25 ± 0.06 D c	8.94 ± 0.09 E c
0.3% CA + 500 IU/g nisin	5.58 ± 0.01 A d	5.81 ± 0.40 AB cd	6.15 ± 0.46 B bc	8.26 ± 0.16 C ab	9.02 ± 0.42 CD a	8.81 ± 0.08 D cd
0.3% CA + 1,000 IU/g nisin	5.86 ± 0.04 C ab	4.65 ± 0.23 A f	5.34 ± 0.42 B d	6.48 ± 0.07 D g	7.32 ± 0.10 E e	7.88 ± 0.13 F e

[a] Data represent means ± standard deviations of three experiments. Within the same row, means with the same uppercase letter are not significantly different ($P > 0.05$). Within the same column, means with the same lowercase letter are not significantly different ($P > 0.05$).

[b] CA, citric acid.

TABLE 2. *Aerobic plate counts at 4°C following treatment of hummus with different concentrations of nisin, citric acid, or their combination*

Treatment	Concn (log CFU/g)[a]					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Control	6.75 ± 0.62 A a	6.85 ± 0.06 A a	7.47 ± 0.24 B a	8.18 ± 0.12 C c	9.32 ± 0.06 D ab	9.42 ± 0.08 D cd
500 IU/g nisin	5.79 ± 0.04 B bcd	5.46 ± 0.15 A ef	6.40 ± 0.38 C b	8.81 ± 0.11 D a	9.46 ± 0.05 E a	9.71 ± 0.09 E a
1,000 IU/g nisin	5.33 ± 0.29 A de	5.80 ± 0.35 B cd	6.61 ± 0.19 C b	7.78 ± 0.19 D efg	9.24 ± 0.11 E b	9.58 ± 0.06 E ab
0.1% CA[b]	6.13 ± 0.46 AB b	5.95 ± 0.15 A bc	6.33 ± 0.06 B bc	8.03 ± 0.06 C cde	8.66 ± 0.08 D e	9.57 ± 0.06 E ab
0.2% CA	6.04 ± 0.07 A b	6.04 ± 0.21 A bc	6.59 ± 0.06 B b	7.59 ± 0.21 C g	8.47 ± 0.03 D f	8.97 ± 0.07 E e
0.3% CA	5.96 ± 0.04 A bc	5.97 ± 0.04 A bc	6.49 ± 0.18 B b	7.87 ± 0.14 C def	8.36 ± 0.06 D f	8.87 ± 0.07 E e
0.1% CA + 500 IU/g nisin	5.20 ± 0.48 A e	6.20 ± 0.10 B b	6.48 ± 0.13 B b	8.45 ± 0.07 C b	9.03 ± 0.19 D cd	9.53 ± 0.06 E abc
0.1% CA + 1,000 IU/g nisin	5.16 ± 0.09 A e	5.18 ± 0.17 A g	6.07 ± 0.04 B cd	7.54 ± 0.22 C g	8.75 ± 0.05 D e	9.63 ± 0.04 E ab
0.2% CA + 500 IU/g nisin	5.45 ± 0.13 A cde	5.30 ± 0.11 A fg	6.01 ± 0.27 B d	8.06 ± 0.28 C cd	9.08 ± 0.09 D c	9.57 ± 0.10 E ab
0.2% CA + 1,000 IU/g nisin	5.49 ± 0.10 A cde	5.67 ± 0.16 A ed	6.43 ± 0.10 B b	7.75 ± 0.10 C fg	8.38 ± 0.13 D f	9.62 ± 0.07 E ab
0.3% CA + 500 IU/g nisin	5.37 ± 0.27 A de	5.67 ± 0.05 B ed	5.96 ± 0.10 C d	8.00 ± 0.05 D cdef	8.92 ± 0.08 E d	9.29 ± 0.21 F d
0.3% CA + 1,000 IU/g nisin	5.80 ± 0.40 B bcd	5.17 ± 0.06 A g	5.84 ± 0.15 B d	6.63 ± 0.08 C h	7.79 ± 0.07 D g	9.33 ± 0.07 E d

[a] Data represent means ± standard deviations of three experiments. Within the same row, means with the same uppercase letter are not significantly different ($P > 0.05$). Within the same column, means with the same lowercase letter are not significantly different ($P > 0.05$).
[b] CA, citric acid.

TABLE 3. *Initial and final pH values of hummus samples treated with nisin, citric acid, or their combination, inoculated with* Listeria innocua, *and stored at 4°C for 15 days*[a]

Treatment	Initial pH	Final pH
Control	6.28 ± 0.03	6.89 ± 0.17
500 IU/g nisin	6.26 ± 0.01	6.77 ± 0.13
1,000 IU/g nisin	6.25 ± 0.01	6.75 ± 0.12
0.1% CA[b]	5.98 ± 0.02	6.27 ± 0.13
0.2% CA	5.65 ± 0.01	5.98 ± 0.36
0.3% CA	5.41 ± 0.02	5.50 ± 0.05
0.1% CA + 500 IU/g nisin	5.86 ± 0.02	7.10 ± 0.04
0.1% CA + 1,000 IU/g nisin	5.88 ± 0.02	7.00 ± 0.21
0.2% CA + 500 IU/g nisin	5.60 ± 0.01	6.67 ± 0.41
0.2% CA + 1,000 IU/g nisin	5.60 ± 0.01	7.21 ± 0.15
0.3% CA + 500 IU/g nisin	5.33 ± 0.01	6.77 ± 0.08
0.3% CA + 1,000 IU/g nisin	5.32 ± 0.02	5.47 ± 0.05

[a] Data represent means ± standard deviations of measurements from three experiments.
[b] CA, citric acid.

Citric acid (0.1, 0.2, and 0.3%) exhibited limited antilisterial capability despite the fact that there was a noticeable drop in the pH compared with that of the control (Table 3). At a neutral pH, citric acid has limited inhibitory activity against *Listeria* spp. In the hummus, the pH ranged from 5.41 to 5.98, so only a small amount of the acid (<0.41%) would have been present in the active undissociated form that is capable of penetrating the cell membrane of bacteria *(9)*. Even so, *L. innocua* growth was slightly slower in the samples containing citric acid.

Most often, combinations of more than one growth-inhibiting condition result in a greater inhibitory activity against a target microorganism than occurs with any of the suboptimal inhibitory factors alone. Here, different combinations of citric acid and nisin were applied to hummus to inhibit the growth of *L. innocua* and mesophilic microorganisms (Tables 1 and 2). Nisin and citric acid did not exhibit a synergistic effect against *L. innocua* in hummus, as was observed in the BHI broth model system. The nisin–citric acid combination produced a 1- to 2-log reduction in *L. innocua* compared with the control. The combination of 0.3% citric acid + 1,000 IU/g nisin was the most effective treatment for reducing *L. innocua* (ca. 2-log reduction) through about day 9, indicating that the nisin–citric acid combination has a limited ability to inhibit *Listeria* in hummus.

Table 2 shows the APCs of treated hummus. Treatment with nisin alone (500 and 1,000 IU/g) and citric acid alone (0.1, 0.2, and 0.3%) produced significantly different results ($P < 0.05$) for the APC compared with the control, similar to the results for *L. innocua.* With nisin alone, the counts were significantly lower than that of the control for the first 6 days of storage, followed by regrowth to concentrations similar to that of the control. Citric acid treatment resulted in a significant ($P < 0.05$) but not a large initial reduction in the APC, and regrowth was somewhat slower than that for samples treated with nisin alone. The most effective nisin-citric acid combination for retarding the APC was

TABLE 4. *Residual nisin concentrations in hummus stored at 4°C for 15 days*

Treatment	Nisin (IU/g)[a]					
	Day 0	Day 3	Day 6	Day 9	Day 2	Day 15
500 IU/g nisin	447 ± 98	417 ± 84	485 ± 31	438 ± 0	396 ± 36	UD
1,000 IU/g nisin	832 ± 64	811 ± 48	735 ± 13	875 ± 0	500 ± 0	UD
500 IU/g nisin + 0.1% CA[b]	577 ± 32	389 ± 64	425 ± 28	396 ± 36	355 ± 72	UD
1,000 IU/g nisin + 0.1% CA	875 ± 104	839 ± 76	735 ± 79	844 ± 38	625 ± 63	UD
500 IU/g nisin + 0.2% CA	593 ± 36	445 ± 48	344 ± 36	438 ± 63	438 ± 0	UD
1,000 IU/g nisin + 0.2% CA	950 ± 52	895 ± 36	819 ± 31	854 ± 36	583 ± 36	UD
500 IU/g nisin + 0.3% CA	613 ± 21	389 ± 52	469 ± 38	407 ± 36	396 ± 36	UD
1,000 IU/g nisin + 0.3% CA	892 ± 36	867 ± 0	750 ± 0	854 ± 42	664 ± 36	282 ± 45

[a] Data represent means ± standard deviations of three replicates. UD, undetectable.
[b] CA, citric acid.

1,000 IU/g nisin + 0.3% citric acid. This inhibitory effect was more pronounced in this combination possibly because of the lower pH (5.32) (Table 3) compared with that of other treatments. The sensitivity of microbes to nisin can be enhanced by lowering pH *(34)*, and the chemical stability and solubility of nisin increases considerably at low pH *(29)*. The highest residual nisin level was observed when 0.3% citric acid was used with 1,000 IU/ml nisin (Table 4), which indicates that a nisin activity can be retained in an acidic environment.

A noticeable increase in pH was observed for all hummus samples at the end of the storage period. Generally, the increase in pH was inversely related to the concentration of citric acid, i.e., hummus samples containing 0.3% exhibited the smallest increase in pH. This increase in pH took place after a marked increase in *L. innocua* concentration and APCs. When the population of microorganisms is high, there is a switch from sugars to peptides as substrates for growth, resulting in the production free amino acids, ammonia, and amines and consequently a rise in the pH in the surrounding medium. Hummus samples treated with only nisin or nisin plus 0.1% citric acid had an objectionable off-odor and slimy texture with a thin film on the surface after approximately 9 days of storage.

Residual nisin concentrations remained relatively constant with little fluctuations for the first 9 days of storage (Table 4). However, nisin concentration decreased sharply until no detectable nisin was recovered from any treatment except the combination of 1,000 IU/g nisin + 0.3% citric acid. This loss in available nisin was accompanied by an upsurge in *L. innocua* concentration and APC (Tables 1 and 2).

Hummus supports *Listeria* growth. The synergistic antilisterial effects of nisin and citric acid observed in BHI broth were not observed in hummus samples, emphasizing the importance of conducting pathogen control experiments on real foods. A combination of nisin–citric acid (1,000 IU/g nisin + 0.3% citric acid) could be used to control *Listeria* growth, but this treatment should not be used as a substitute for complying with general hygienic practices when handling and preparing ready-to-eat foods such as hummus.

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