Research Note

Inactivation of Pathogenic Bacteria by Cucumber Volatiles (\(E,Z\))-2,6-Nonadienal and (\(E\))-2-Nonenal†

M. J. CHO, R. W. BUESCHER,* M. JOHNSON, AND M. JANES

Department of Food Science and Institute of Food Science and Engineering, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704, USA

ABSTRACT

The effects of (\(E,Z\))-2,6-nonadienal (NDE) and (\(E\))-2-nonenal (NE) on Bacillus cereus, Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium were investigated. A suspension of each organism of 6 to 9 log CFU/ml was incubated for 1 h at 37°C in brain heart infusion solution that contained 0 to 500 or 1,000 ppm of NDE or NE. Depending on concentration, exposure to either NDE or NE caused a reduction in CFU of each organism. Treatment with 250 and 500 ppm NDE completely eliminated viable B. cereus and Salmonella Typhimurium cells, respectively. L. monocytogenes was the most resistant to NDE, showing only about a 2-log reduction from exposure to 500 ppm for 1 h. Conversely, this concentration of NDE caused a 5.8-log reduction in E. coli O157:H7 cells. NE was also effective in inactivating organisms listed above. A higher concentration of NE, 1,000 ppm, was required to kill E. coli O157:H7, L. monocytogenes, or Salmonella Typhimurium compared with NDE. In conclusion, both NDE and NE demonstrated an apparent bactericidal activity against these pathogens.

MATERIALS AND METHODS

Source of materials. The >96% purity of (\(E,Z\))-2,6-nonadienal and (\(E\))-2-nonenal were obtained from Sigma-Aldrich (St. Louis, Mo.). An emulsion of 0.1% was prepared by mixing NDE or NE (100 μl) with 30 μl of Tween 20, followed by sonicating for 1 h or until they were thoroughly suspended, and bringing the volume to 100 ml with deionized H₂O.

Purification of cultures. Pure cultures of B. cereus (ATCC 11778), E. coli O157:H7 (ATCC 43889), L. monocytogenes (U.S. Food and Drug Administration serotype 1/2a), and Salmonella Typhimurium (ATCC 14028) were obtained from stock cultures maintained by the University of Arkansas Food Microbiology and Safety Laboratory.

Determination of antimicrobial activities. Pure cultures that had been stored at −70°C were subcultured twice in 10 ml of brain heart infusion (BHI; Difco, Becton Dickinson, Sparks, Md.) at 37°C for 24 h before being used. One-milliliter samples of culture solution that contained 10³ to 10⁹ CFU/ml were centrifuged for 10 min at 1,470 × g. Supernatants were decanted, and the cultured cells were resuspended in 1 ml of BHI that contained 0, 100, 250, 500, or 1,000 ppm NDE or NE. These samples were incubated for 1 h at 37°C in Eppendorf tubes. Samples were serially diluted to 10⁻⁷ and plated on BHI agar, then incubated at 37°C. CFU values were determined after incubation for 24 and 48 h. Duplicate samples were prepared for each experiment, which was repeated at least three times to confirm treatment response on separate days.

RESULTS AND DISCUSSION

The effect of NDE (500 ppm) on Salmonella Typhimurium in relation to exposure time (0 to 60 min) is shown in Figure 1. Salmonella Typhimurium was selected for the study because of its relevance to frequent public outbreak

* Author for correspondence. Tel: 479-575-4775; Fax: 479-575-6936; E-mail: buescher@uark.edu.
† Published with the approval of the Director of the Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville, AR 72704, USA.
and its moderate sensitivity to NDE, as was observed in our preliminary study. Viable *Salmonella* Typhimurium declined with increasing time of exposure to NDE. When plated immediately after treatment, about a 2.5-log reduction was observed. After 30 min of exposure to NDE, CFU were reduced by 4.6 log, and no cells were capable of forming colonies after 60 min. On the basis of these results, assessments of antibacterial effects were performed by exposure for 1 h to NDE or NE.

The effects of NDE concentration on survival of *B. cereus*, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium after 1 h of exposure at 37°C are shown in Figure 2. Each type of bacteria responded differently to NDE. Among the bacteria tested, *L. monocytogenes* was the most resistant against NDE, whereas *B. cereus* was one of the most sensitive with 250 ppm causing a 6-log (total) reduction in CFU. The 100 ppm NDE treatment was not sufficient to cause a reduction in the cell population of *E. coli* O157:H7, *L. monocytogenes*, or *Salmonella* Typhimurium. At least 250 ppm of NDE was required to effectively inactivate *Salmonella* Typhimurium (2.2 log) and *E. coli* O157:H7 (1 log), whereas it was ineffective in inactivating *L. monocytogenes*. NDE at 500 ppm caused an almost 5.8-log reduction in *E. coli* O157:H7. Only *L. monocytogenes* was treated with 1,000 ppm NDE, because 500 ppm NDE caused only about a 2-log reduction. At 1,000 ppm, the reduction was enhanced to 6 log.

The antibacterial effects of NE are shown in Figure 3. NE up to 500 ppm had a very slight effect on the bacteria tested, with the exception of *B. cereus*. *B. cereus* was not affected by 100 ppm NE, but 250 ppm caused a 4.3-log reduction in CFU, and at 1,000 ppm there were no CFU remaining. In contrast, the antibacterial activity of NE on *E. coli* O157:H7 and *L. monocytogenes* was similar. NE at 500 ppm caused no reduction in *E. coli* O157:H7 and *L. monocytogenes*, and it caused only a 1-log reduction in *Salmonella* Typhimurium. NE at 1,000 ppm caused a 6-log reduction in *E. coli* O157:H7 and *L. monocytogenes*, whereas as it destroyed all *Salmonella* Typhimurium. *L. monocytogenes* was the most resistant pathogen tested to both NDE and NE, and it was reduced by ~6 log of CFU at 1,000 ppm. *B. cereus* was strongly inactivated by both NDE and NE. However, NDE had much stronger antibacterial activity against all four bacteria. This may be attributed to the higher electronegative arrangement in NDE as opposed to NE, which increases antibacterial activity by interfering with biological processes involving an aldehyde group conjugated to a –C=O– bond in the molecule structure, as was suggested by the results of previous studies (10, 11, 13). Of interest, NDE is also more effective in repelling insect (14).

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coli O157:H7, L. monocytogenes, and Salmonella Typhimurium. NDE and NE compounds are naturally formed in response to the physical disruption of cucumber tissues. Although the effects of the long-term exposure of pathogenic and other microorganisms to low concentration of NDE and NE are unknown, these volatiles may play an important role in controlling microflora ecology associated with cucumbers.

REFERENCES