Disinfection of Iceberg Lettuce by Titanium Dioxide–UV Photocatalytic Reaction

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Abstract

Securing the physical quality and microbial safety of fresh foods has been a major focus in the food industry. To improve quality and increase the shelf life of fresh produce, disinfection methods have been developed. Titanium dioxide (TiO₂) photocatalytic reactions under UV radiation produce hydroxyl radicals that can be used for disinfection of foodborne pathogenic bacteria. We investigated the effects of TiO₂-UV photocatalytic disinfection on the shelf life of iceberg lettuce. Counts of natural microflora (total aerobic bacteria, coliforms, psychrotrophic bacteria, and yeasts and molds) and inoculated pathogenic bacteria (Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella Typhimurium) on iceberg lettuce were determined after 20-min treatments with TiO₂-UV, UV radiation, a sodium hypochlorite (NaOCl) solution, and tap water. TiO₂-UV treatment reduced the number of microorganisms by 1.8 to 2.8 log CFU/g compared with reductions of 0.9 to 1.4 and 0.7 to 1.1 log CFU/g obtained with UV radiation and NaOCl treatments, respectively. Treatment with tap water was used as a control and resulted in no reductions. Counts of microflora for iceberg lettuce at 4 and 25°C were determined during a 9-day period. TiO₂-UV treatment resulted in 1.2- and 4.3-log increases in the counts of total aerobic bacteria at 4 and 25°C, respectively, compared with 1.3- to 1.6-log and 4.4- to 4.8-log increases due to UV radiation and NaOCl treatments.

High consumer demand for fresh-cut produce has encouraged research into methods to keep fresh produce safe from microbiological hazards for longer periods (10, 19). Maintenance of quality and control of microbial populations are problems in the fresh-cut produce industry (25). Disinfection methods used to solve these problems include treatments with ionizing radiation (21, 22), UV radiation (6), electrolyzed oxidizing water (11, 17), organic acids (1), gaseous or aqueous ozone (1, 3, 4, 10, 28, 29), high-intensity pulsed light (13), sodium hypochlorite (14, 21), and chlorine dioxide (24). However, factors to be considered in any processes for food disinfection include limited efficacy, high equipment cost, instability of agents, physiological injury to produce, and consumer safety (1, 5, 8, 11, 16, 21–23, 26, 28, 29). The traditional use of chlorinated water to remove bacterial contaminants from vegetables may not completely remove or kill all bacterial pathogens (1, 9). Chlorinated water has attracted public health concerns because of the generation of trihalomethanes during treatment (1, 5, 16, 26, 28).

The TiO₂ photocatalyst reaction generates hydroxyl radicals via reductive and/or oxidative pathways. Electron-hole pairs, an electron in a conduction band (e⁻cb) and a hole in a valence band (h⁺vb), are generated on the TiO₂ photocatalyst surface by UV radiation (12, 15). The e⁻cb changes oxygen to a hydroxyl radical via the reductive pathway, and h⁺vb changes hydroxyl ions or water to hydroxyl radicals via the oxidative pathway (7, 31). Hydroxyl radicals cause damage to microorganisms, including viruses, bacteria, fungi, and algae (2, 7, 12, 15, 20). Some authors (7, 12) have proposed that cell death by TiO₂ photocatalyst reaction is caused by a significant disorder in cell permeability and structural damage to the cell wall. Partial decomposition of the cell wall allows penetration of photogenerated reactive oxygen species into the cytoplasmic membrane, which leads to the peroxidation of membrane lipids, the direct oxidation of coenzyme A, and damage to intracellular macromolecules (12, 18, 31).

The effect of the TiO₂-UV photocatalytic reaction on the disinfection of wash water used for fresh-cut vegetables was evaluated (27). Vegetable wash water was circulated through a heterogeneous photocatalytic system with a TiO₂ photocatalyst fiber illuminated with a 40-W UV-C lamp. Total bacterial reduction in the TiO₂-UV–treated wash water was 4.1 to 4.8 log CFU/ml after a 10-min treatment. Untreated wash water had no reduction. Practical applications of TiO₂ photocatalytic disinfection have attracted interest in the food industry (30, 32). The bactericidal effects of TiO₂-UV treatment against bacteria grown in a liquid medium and on carrots have been reported (6). TiO₂-UV disinfection resulted in significantly greater reductions in...
the counts of total aerobic bacteria on fresh carrots and in the counts of foodborne pathogenic bacteria on inoculated carrots than did UV disinfection alone. However, development of a more effective TiO$_2$-UV photocatalytic disinfection method to obtain a shorter treatment time was recommended. To increase the disinfection efficiency, modification of the TiO$_2$ coating, increasing the UV lamp wattage, supplying oxygen gas to the reactor, and adjusting the pH of the wash water were recommended.

The UV lamp wattage (light intensity) was increased compared to that used in our previous study and air was supplied to mix the sample in the TiO$_2$-UV photocatalytic reactor in the present study. The objectives of this study were to assess the effect of the TiO$_2$-UV photocatalytic reaction on counts of natural microflora (total aerobic bacteria, coliforms, psychrotrophic bacteria, and yeasts and molds) and inoculated pathogenic bacteria (Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella Typhimurium) on iceberg lettuce after treatment and during storage at both 4 and 25°C.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** *E. coli* (ATCC 25922), *L. monocytogenes* (KCCM 40307), *Salmonella Typhimurium* (ATCC 14028), and *S. aureus* (KCCM 11335) were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and the Korean Culture Center of Microorganisms (KCCM; Seoul, Korea). Stock cultures were stored at −85°C in nutrient broth (Difco, Becton Dickinson, Sparks, MD) containing 20% (vol/vol) glycerol and grown overnight in 50 ml of sterile nutrient broth with shaking at 200 rpm. Strain cultures were transferred to 200 ml of fresh medium and incubated with shaking for 24 h. 

**FIGURE 1.** Schematic diagram of the TiO$_2$-UV photocatalytic reactor.

**FIGURE 2.** Bactericidal effects of tap water (♦), NaOCl solution (○), UV alone (□), and the TiO$_2$-UV photocatalytic reaction (▲) on bacteria naturally occurring on iceberg lettuce. (A) Total aerobic bacteria; (B) coliform bacteria; (C) psychrotrophic bacteria; (D) yeasts and molds. Error bars represent the standard deviation of four replications. Different lowercase letters indicate significant differences between treatments (P < 0.05).
at 200 rpm for 24 h. Bacterial cells were harvested by centrifugation (4,000 × g for 10 min at 20 °C) with a 0.85% (wt/vol) NaCl solution. The colony counts for bacterial in the inocula were obtained using the spread plate method on nutrient agar after serial dilution in a 0.85% NaCl solution.

Preparation of iceberg lettuce. Fresh iceberg lettuce (Lactuca sativa L.) was purchased at a local market. The core and outer leaves were removed, and the other parts were cut into pieces (3 by 3 cm) with an alcohol-sterilized knife. The lettuce pieces were then rinsed with running tap water for 1 min to reduce surface contamination and stored at 4 °C until used for treatments. These cut pieces were randomly mixed to provide a homogeneous sample and used in experiments for analysis of bacterial counts on both fresh and inoculated samples.

Inoculation of iceberg lettuce. Prepared cut iceberg lettuce was treated with 254-nm UV radiation in a clean bench (VS-1400LS, Vision Scientific, Bucheon, Korea) for 30 min to reduce the number of natural microorganisms. Each sample (4 °C) was then inoculated with E. coli, L. monocytogenes, S. aureus, and Salmonella Typhimurium by immersion in a bacterial inoculum solution of 7 log CFU/ml at a sample:inoculum solution ratio of 1:3 (wt/vol). Samples were agitated with a gloved hand for 3 min, drained on sterile paper to remove excess inoculum, and air dried in a clean bench for 30 min to allow the attachment of cells.

TiO₂-UV photocatalytic reactor. The TiO₂-UV photocatalytic reactor was a stainless steel vessel operated at an 80-liter working volume (Fig. 1). The reactor consisted of an air pump and UV lamps with a wavelength of 254 nm (35 W, 25 mW/cm²; Sankyo Denki, Tokyo, Japan) surrounded by TiO₂-coated quartz tubes (coating thickness of 0.7 to 0.9 μm; Taekyeng UV Co., Namyangju, Korea) to protect the UV lamps from direct contact with the aqueous solution. Five UV lamp and quartz tube (24.5 mm outside diameter, 900 mm long) units were placed at the top of the reactor. Another five UV lamp and quartz tube (24.5 mm outside diameter, 1,100 mm long) units were placed at the bottom of the reactor. The TiO₂-coated quartz tube units were used to determine TiO₂-UV disinfection, and uncoated quartz tube units were used to determine UV disinfection. All UV lamp and quartz tube units located at both the top and bottom of the reactor were waterproof and submerged in water during the reaction. Four air blower units located at the bottom of the reactor were used to mix the samples.

Determination of antibacterial activity. The viable cell count method was used to determine the antibacterial activity in the TiO₂-UV photocatalytic reactor. Two kilograms of iceberg lettuce was dipped into the sterile water of the reactor at a sample:water ratio of 1:40 (wt/vol) and mixed with the air blower. The iceberg lettuce was treated with TiO₂-UV, UV alone, a sodium hypochlorite solution (NaOCl, 150 ppm), or tap water and sampled (approximately 25 g) at 0, 5, 10, 15, and 20 min. The lettuce was then rinsed with tap water for 1 min.

For analysis of bacteria on the lettuce, samples (25 g) were diluted 10 times in a sterile 0.85% NaCl solution in sterile plastic stomacher bags (Nasco, Fort Atkinson, WI) and homogenized with

FIGURE 3. Bactericidal effects of tap water ( ), NaOCl solution ( ), UV alone ( ), and the TiO₂-UV photocatalytic reaction ( ) on (A) Escherichia coli, (B) Listeria monocytogenes, (C) Staphylococcus aureus, and (D) Salmonella Typhimurium on inoculated iceberg lettuce. Error bars represent the standard deviation of four replications. Different lowercase letters indicate significant differences between treatments (P < 0.05).
a stomacher (AES, Combourg, France) for 2 min. Homogenized samples of both fresh iceberg lettuce containing microflora (total aerobic bacteria, psychrotrophic bacteria, coliforms, and yeasts and molds) and iceberg lettuce inoculated with foodborne bacteria (E. coli, L. monocytogenes, S. aureus, or Salmonella Typhimurium) were serially diluted in the 0.85% NaCl solution, resulting in 10-fold dilutions.

One milliliter of diluted sample was poured onto MacConkey agar (Difco, Becton Dickinson) for analysis of E. coli and Salmonella Typhimurium, onto Oxford Listeria selective agar (Oxoid, Basingstoke, UK) for analysis of L. monocytogenes, and onto Petrifilm Staph Express count plates (3 M, Maplewood, MN) for analysis of S. aureus. Populations of total aerobic bacteria and psychrotrophic bacteria in each diluted sample were determined by pouring samples onto nutrient agar followed by incubation at 36°C for 24 h (total aerobic bacteria) or at 7°C for 10 days (psychrotrophic bacteria). The number of coliforms was determined using the pour plate method on a Petrifilm coliform count plate (3 M) incubated at 30°C for 48 h. Colonies of yeasts and molds were visible on potato dextrose agar (Difco, Becton Dickinson) after 3 days of incubation at 25°C. The colonies on the plates for E. coli, Salmonella Typhimurium, and S. aureus were counted after incubation at 36°C for 24 h, and those for L. monocytogenes were counted after incubation at 36°C for 36 h. Duplicate plates were used for each dilution. Suspect colonies of E. coli and Salmonella Typhimurium were identified via biochemical characterization with an API 20E kit (bioMérieux, Marcy l’Etoile, France).

All experiments were performed four times, and CFU per gram were converted to log counts before statistical analysis. Average values with error bars indicating standard deviations were used for data analysis.

Changes in product quality during storage. Samples treated with TiO$_2$-UV, UV alone, the NaOCl solution, or tap water for 20 min were aseptically divided into 20-g portions for microfloral analysis (total aerobic bacteria, psychrotrophic bacteria, coliforms, and yeasts and molds) and iceberg lettuce inoculated with foodborne bacteria (E. coli, L. monocytogenes, S. aureus, or Salmonella Typhimurium) were serially diluted in the 0.85% NaCl solution, resulting in 10-fold dilutions.

One milliliter of diluted sample was poured onto MacConkey agar (Difco, Becton Dickinson) for analysis of E. coli and Salmonella Typhimurium, onto Oxford Listeria selective agar (Oxoid, Basingstoke, UK) for analysis of L. monocytogenes, and onto Petrifilm Staph Express count plates (3 M, Maplewood, MN) for analysis of S. aureus. Populations of total aerobic bacteria and psychrotrophic bacteria in each diluted sample were determined by pouring samples onto nutrient agar followed by incubation at 36°C for 24 h (total aerobic bacteria) or at 7°C for 10 days (psychrotrophic bacteria). The number of coliforms was determined using the pour plate method on a Petrifilm coliform count plate (3 M) incubated at 30°C for 48 h. Colonies of yeasts and molds were visible on potato dextrose agar (Difco, Becton Dickinson) after 3 days of incubation at 25°C. The colonies on the plates for E. coli, Salmonella Typhimurium, and S. aureus were counted after incubation at 36°C for 24 h, and those for L. monocytogenes were counted after incubation at 36°C for 36 h. Duplicate plates were used for each dilution. Suspect colonies of E. coli and Salmonella Typhimurium were identified via biochemical characterization with an API 20E kit (bioMérieux, Marcy l’Etoile, France).

All experiments were performed four times, and CFU per gram were converted to log counts before statistical analysis. Average values with error bars indicating standard deviations were used for data analysis.

Statistical analysis. Statistical analyses were conducted using version 9.2 of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Experimental results were analyzed by analysis of variance and mean separation with the Student-Newman-Keuls test.

RESULTS

Disinfection of foodborne bacteria on iceberg lettuce. The disinfection effects of the TiO$_2$-UV photocatalytic reaction on the natural microorganisms on fresh iceberg lettuce and foodborne pathogenic bacteria on inoculated
iceberg lettuce are shown in Figures 2 and 3. The antibacterial effects of the NaOCl solution treatment, which is current industry standard practice for washing fruits and vegetables, and the UV radiation treatment were determined for comparison.

At 0 min, samples contained approximately 6.3, 3.8, 5.4, and 5.8 log CFU/g total aerobic bacteria, coliforms, psychrotrophic bacteria, and yeasts and molds, respectively. Reductions of 1.0, 1.0, 0.8, and 0.7 log CFU/g for total aerobic bacteria, coliforms, psychrotrophic bacteria, and yeasts and molds, respectively, were achieved by treatment with the NaOCl solution for 20 min. Sample treatment in the TiO$_2$-UV photocatalytic reactor for 20 min resulted in 2.5-, 1.8-, 1.8-, and 1.9-log reductions, respectively, compared with 1.1-, 1.4-, 0.9-, and 1.1-log reductions due to UV treatment alone for 20 min (Fig. 2).

The counts of E. coli, L. monocytogenes, S. aureus, and Salmonella Typhimurium at 0 min on inoculated iceberg lettuce were approximately 6.2, 6.5, 6.3, and 6.8 log CFU/g, respectively. Treatments with the NaOCl solution, UV only, and TiO$_2$-UV for 20 min reduced these counts to 0.9, 1.4, and 2.6 log CFU/g for E. coli, 0.8, 1.0, and 2.5 log CFU/g for L. monocytogenes, 0.9, 1.4, and 2.3 log CFU/g for S. aureus, and 1.1, 1.4, and 2.8 log CFU/g for Salmonella Typhimurium, respectively (Fig. 3). These results indicate that Salmonella Typhimurium was the most sensitive bacterium to the TiO$_2$-UV photocatalytic reaction. Salmonella Typhimurium in liquid medium (initial concentration of 7 log CFU/ml) was completely inactivated within 30 s by the TiO$_2$-UV photocatalytic reaction compared with 40 and 60 s for the liquid cultures of E. coli and Bacillus cereus in our previous study (6).

**Microbial quality during storage.** Total counts of aerobic bacteria after treatment with tap water, NaOCl, UV only, and TiO$_2$-UV increased by 1.2, 1.6, 1.3, and 1.2 log CFU/g, respectively, after storage at 4°C for 9 days (Fig. 4). Counts of coliforms, psychrotrophic bacteria, and yeasts and molds increased by 2.1, 2.0, and 2.1 log CFU/g with NaOCl, 2.9, 2.3, and 3.9 with UV only, and 2.0, 1.3, and 3.3 log CFU/g with TiO$_2$-UV, respectively, after storage at 4°C for 9 days (Fig. 4).

The total aerobic bacteria count after treatment with tap water, NaOCl, UV only, and TiO$_2$-UV increased by 3.5, 4.8, 4.4, and 4.3 log CFU/g, respectively, after storage at 25°C for 9 days (Fig. 5). Counts of coliforms, psychrotrophic bacteria, and yeasts and molds increased by 4.7, 2.4, and 3.5 log CFU/g with tap water, 4.6, 2.6, and 4.0 log CFU/g with NaOCl, 2.9, 2.3, and 3.9 with UV only, and 2.0, 1.3, and 3.3 log CFU/g with TiO$_2$-UV, respectively, after storage at 25°C for 9 days (Fig. 5).
TABLE 1. Effects of different nonthermal disinfection methods on counts of total aerobic bacteria on fresh produce

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh produce</th>
<th>Condition</th>
<th>Maximum log reduction</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂-UV</td>
<td>Carrot</td>
<td>254-nm UV, 16 mW/cm²</td>
<td>1.8</td>
<td>6</td>
</tr>
<tr>
<td>UV</td>
<td>Carrot</td>
<td>254-nm UV, 16 mW/cm²</td>
<td>1.1</td>
<td>6</td>
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<tr>
<td>NaOCl</td>
<td>Romaine lettuce</td>
<td>600 ppm</td>
<td>0.5</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Spinach</td>
<td>600 ppm</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>151 ppm</td>
<td>1.2</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Strawberry</td>
<td>151 ppm</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iceberg lettuce</td>
<td>100 ppm</td>
<td>1.6</td>
<td>1, 10, 23</td>
</tr>
<tr>
<td>Electrolyzed water</td>
<td>Cucumber</td>
<td>32 ppm</td>
<td>1.4</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Strawberry</td>
<td>32 ppm</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Ozonated water</td>
<td>Cucumber</td>
<td>5 ppm</td>
<td>0.7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Strawberry</td>
<td>5 ppm</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Citric acid + ascorbic acid</td>
<td>Iceberg lettuce</td>
<td>4 ppm</td>
<td>1.6</td>
<td>1, 10, 23</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Iceberg lettuce</td>
<td>0.25% citric acid + 0.5% ascorbic acid</td>
<td>1.0</td>
<td>23</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Iceberg lettuce</td>
<td>5,000 ppm</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,000 ppm</td>
<td>1.6</td>
<td>1</td>
</tr>
</tbody>
</table>

DISCUSSION

We used a TiO₂-UV photocatalytic reaction for disinfection of foodborne pathogenic bacterial on iceberg lettuce. The TiO₂-UV photocatalytic reaction resulted in large reductions in the numbers of both natural microorganisms and inoculated pathogenic bacteria. In contrast, the NaOCl solution and UV radiation alone had only limited disinfection effects. The natural microflora on iceberg lettuce had a slower growth rate during storage for 9 days at 4°C after TiO₂-UV treatment than after UV radiation, NaOCl, or tap water treatments.

Bacteria in iceberg lettuce can be protected against disinfection treatments by attachment in pores or exposed vascular tissues on the cut lettuce surface. However, TiO₂-UV treatment for 20 min resulted in the highest reduction (approximately 2.5 to 2.8 log CFU/g) in total aerobic bacteria and inoculated pathogenic bacteria compared with UV radiation and NaOCl treatments. The bactericidal effect of the TiO₂-UV treatment in our study was superior to that of other nonthermal disinfection methods (UV radiation, ozonated water, electrolyzed water, and sanitizer solutions including sodium hypochlorite, citric acid, and lactic acid) on the total aerobic bacterial counts on various kinds of fresh produce (Table 1).

After 9 days of storage at both 4 and 25°C, the NaOCl solution treatment resulted in a larger increase in the counts of total aerobic bacteria, coliforms, psychrotrophic bacteria, and yeasts and molds than did the TiO₂-UV treatment. These results indicate that damage inflicted by the NaOCl solution treatment increased the risk of microbial contamination of lettuce pieces during storage. Water and nutrients from tissue fluids that remained on the NaOCl-treated lettuce probably enhanced microbial growth during subsequent storage, resulting in reduced shelf life.

The results of this study indicate that the TiO₂-UV photocatalytic system is an effective disinfection method. One practical application of this method would be small-scale disinfection of shredded vegetables in a restaurant. For a larger scale application in the food industry, a continuous conveying system using the same principles could be designed.

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REFERENCES


