Postharvest Biology and Technology 88 (2014) 46–53

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley

Hakan Karaca [1], Y. Sedat Velioglu *

Ankara University, Faculty of Engineering, Department of Food Engineering, 06110 Diskapi, Ankara, Turkey

ARTICLE INFO

Article history:
Received 26 June 2013
Accepted 15 September 2013

Keywords:
Escherichia coli
Listeria innocua
Leafy green vegetables
Washing treatments
Chlorine

ABSTRACT

The effects of distilled, ozonated (12 mg L^{-1}) and chlorinated (100 mg L^{-1}) water treatments on inactivation of *Escherichia coli* and *Listeria innocua* inoculated on lettuce, spinach, and parsley and on some chemical characteristics (chlorophyll a, chlorophyll b, ascorbic acid, and total phenolic contents and antioxidant activity) of these vegetables were investigated. Chlorine and ozone washes resulted in average log reductions (±standard error) of 2.9 ± 0.1 and 2.0 ± 0.3 for *E. coli* in the vegetables tested, respectively, while the efficiency of ozone (2.2 ± 0.1 log) was very close to that of chlorine (2.3 ± 0.1 log) on *L. innocua*. Aqueous ozone did not cause any detrimental effects on the chemical characteristics of the vegetables. The effect of gaseous ozone treatment (950 μL L^{-1}, 20 min) on microbial inactivation and the chemical characteristics of parsley were also determined. This treatment resulted in 1.0–1.5 log reductions in the numbers of both microorganisms but caused significant losses in important bioactive compounds of parsley. Ascorbic acid and total phenolic contents and antioxidant activity in ozone-treated samples were 40.1, 14.4, and 41.0%, respectively, less than the control samples.

1. Introduction

Treatment of the product with various sanitizing agents is a common practice to prevent potential pathogen contamination and extend the shelf-life of minimally processed fruit and vegetables. Chlorine-based agents are the most commonly used disinfectants for this aim. However, these agents lead to the formation of toxic compounds, such as trihalomethanes, in water and on food contact surfaces. Due to the possible adverse effects of these compounds on health, the use of chlorine-based agents is restricted in many countries (Beltrán et al., 2005). Ozone, an approved sanitizing agent (US-FDA, 2001), is being tested widely for disinfection purposes in food industries. Due to its quick decomposition to oxygen that alleviates concerns about toxic residues, it has attracted much attention for food safety uses. Many studies have shown that high microbial inactivation rates could be obtained with both aqueous and gaseous ozone treatments (Selma et al., 2006; Wei et al., 2007; Hassenberg et al., 2008; Gabler et al., 2010). On the other hand, ozone can also cause some detrimental effects on product physiology and quality such as losses in color, antioxidant constituents, etc., because of its strong oxidizing activity (Allende et al., 2007; Keutgen and Pawelzik, 2008; Vurma et al., 2009; Klockow and Keener, 2009).

Food-borne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. can be involved in worldwide outbreaks. It is usually dangerous to work with the pathogens themselves because of high pathogenicity. For this reason, non-pathogenic strains that behave similarly to the pathogenic organisms under the same experimental conditions (chemical treatment, storage, etc.) could be used to replace the pathogens in challenge studies. For instance, *E. coli* and *L. innocua* were used as models for *E. coli* O157:H7 and *L. monocytogenes*, respectively (Gleeson and O'Beirne, 2005; Vaz-Velho et al., 2006; Fan et al., 2007).

The main objectives of this study were to compare the efficiencies of distilled, ozonated (12.0 ± 0.5 mg L^{-1}), and chlorinated (100 ± 2 mg L^{-1}) water washes in inactivating *E. coli* and *L. innocua* and investigate the effects of these treatments on some chemical characteristics (chlorophylls, ascorbic acid and total phenolic contents, and antioxidant activity) of fresh-cut lettuce, spinach, and parsley. It was also aimed at determining the efficacy of gaseous ozone treatment (950 ± 12 μL L^{-1}) for 20 min in *E. coli* and *L. innocua* inactivation and the effects of this treatment on chemical

* Corresponding author. Tel.: +90 312 596 1166; fax: +90 312 317 8711.
E-mail addresses: hkaraca@pau.edu.tr (H. Karaca), velioglu@ankara.edu.tr (Y.S. Velioglu).
[1] Present address: Pamukkale University, Faculty of Engineering, Department of Food Engineering, 20070 Kinikli, Denizli, Turkey.

0925-5214/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.postharvbio.2013.09.003

characteristics of parsley. In the experiments, we used *E. coli* type 1 and *L. innocua* as non-pathogenic markers of *E. coli* O157:H7 and *L. monocytogenes*, respectively.

2. Materials and methods

2.1. Materials

Lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), and parsley (*Petroselinum crispum* L.) were used to investigate the effects of various washing treatments on microbial inactivation and some chemical characteristics. The material used in the gaseous ozone experiments was parsley. Vegetables used in the study were obtained from a supermarket in Ankara, Turkey. They were washed under running tap water (at ~15 °C) for 2 min to remove soil particles and spun in a salad spinner for 3 min. Lettuce and spinach leaves that were uniform in size and color were selected and midribs were excised with a sharp stainless-steel knife and discarded. The rest of the leaves were cut into 2–2.5 cm wide strips with the knife while parsley samples were prepared for the analysis by picking the small leaves from the main branch. Ten-gram portions of each vegetable were weighed separately as sample units.

Bacterial strains (*E. coli* Type 1 [NRLL B-3008] and *L. innocua* [NRLL B-33314]) were kindly supplied by the Microbiology Laboratory of Food Engineering Department, Ankara University. All culture media and other chemicals (Maximum Recovery Diluent, Tryptic Soy Broth, PALCAM, PALCAM supplement, Fluorocult® Violet Red Bile agar, and organic solvents) were obtained from Merck (Darmstadt, Germany). Potassium indigo trisulfonate was obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Distilled water was supplied from a TKA Pacific UP/UPW water purification system (TKA Water Purification Systems GmbH, Niederelbert, Germany). All materials (culture media, distilled water, glassware, tweezers, coarse filter paper) used in the microbiological experiments were sterilized by autoclaving at 121 °C for 15 min.

2.2. Methods

Two separate experimental components, namely microbiological and chemical analyses, were conducted. Plant materials used for the microbiological analysis were exposed to sterilization and inoculation procedures before treatments. In addition, aseptic precautions were taken for microbiological analysis.

2.2.1. Microbiological analyses

Bacterial strains, maintained at −20 °C in 20% glycerol in tryptic soy broth, were separately grown in tryptic soy broth at 37 °C with two consecutive transfers after 24 h incubation periods. Inoculum cell suspensions were prepared by transferring 10 mL of culture in tryptic soy broth into 1 L of sterile maximum recovery diluents. Populations of *E. coli* and *L. innocua* in the inoculum suspensions, determined by plating triplicate samples, were 6.4×10^7 and 5.6×10^7 colony forming units (cfu) mL^{-1}, respectively.

Vegetable samples were surface-sanitized with a diluted sodium hypochlorite solution (300 mg L^{-1} total chlorine) for 15 min to remove native microbial flora. Residual chlorine level was semi-quantitatively determined by test strips (Quantofix-chlorine, Macherey-Nagel, Duren, Germany). After sanitizing, the leaf pieces were thoroughly rinsed in sterile distilled water and left to drain on sterile filter paper for 30 min. Since *E. coli* and *Listeria* spp. populations were found to be less than 100 cfu g^{-1} vegetable after this sanitization process, the numbers of these bacteria before inoculation were omitted.

Sanitized leaf pieces (10 g) were dipped into the inoculum suspensions (1 L) in a sterile beaker with a magnetic stir bar. After being submerged in the suspension for 10 min, leaves were placed on sterile coarse filter paper and left for 6 h at room temperature (22 ± 1 °C) to allow microorganisms to attach on the plant surface. Inoculated samples were either directly used for microbiological analyses or exposed to various washing treatments or treated with ozone gas. The counts of *E. coli* and *L. innocua* were determined by spread-plating onto Fluorocult® Violet Red Bile and PALCAM agar, respectively. Incubation periods at 37 °C were 18 and 36 h for *E. coli* and *L. innocua*, respectively.

2.2.2. Washing treatments

Chlorine solution was prepared by dissolving 2 g of a commercial product (Ecolab, Ecolab Sanitizing Systems, Turk Henkel, Kocaeli, Turkey) in 1 L of sterile distilled water. Residual chlorine level, determined by chlorine-test strips (Quantofix-chlorine, Macherey-Nagel, Duren, Germany), was 100 ± 2 mg L^{-1} in the solution. Bubbling, the most effective ozonation method in terms of microbial inactivation cited in the literature (Kim et al., 1999; Achen and Yousef, 2001), was used for ozone treatment of vegetables. Ozone was produced by a corona discharge generator (OG 20, Opal, Ankara, Turkey) with a production capacity of 20 g h^{-1} of ozone. Oxygen feed gas produced by an oxygen concentrator incorporated in the generator, was used for ozone generation. To obtain maximum decontamination efficacy with aqueous ozone, bubbling technique was used. Ozone gas, passing through silicone hose, was bubbled into 1 L of water (5 ± 1° C) in a glass beaker by the help of a stainless-steel sparger with 10 μm pore size (Solvent inlet filter, Fisher Scientific, Fair Lawn, NJ, USA). The gas flow was controlled at 827 mL min^{-1} by a Riteflow flow-meter (150 mm, Size 2, Bel-Art Products, Pequannock, NJ, USA). Washing treatments (with distilled, chlorinated or ozonated water) were conducted for 15 min in sterile beakers with magnetic stir bars at refrigerated temperature (5 ± 1 °C). After treating with various sanitizer solutions, samples were rinsed with sterile distilled water (5 ± 1 °C), transferred onto sterile filter paper and left to drain for 30 min under sterile conditions. In order to see the effects of the sanitizers, microbiological enumerations were performed just after washing treatments.

2.2.3. Gaseous ozone treatment

Gaseous ozone treatments were performed in a 4 L glass chamber. The chamber had a glass lid and tight sealing was ensured with clamps outside the chamber. Silicone stoppers (No: 9.5, Cole Parmer Instrument Co., Niles, IL, USA) carrying suspended samples were placed into the holes in the lid at the beginning of the treatment. Ozone gas from the generator was first humidified by passing through a gas-washing bottle containing distilled water. A humidified gas stream was fed through the inlet of the chamber. The exit flow, carrying excess ozone gas from the chamber, was passed to another gas-washing bottle containing 2% potassium iodide for neutralization. Treatment of the samples started after a stable ozone concentration in the chamber (~2 h after introducing ozone gas) was obtained. Ozone treatments were conducted for 20 min with inoculated (for microbiological analyses) and non-inoculated samples (for chemical analyses). Temperature and humidity in the treatment chamber were measured using a commercial sensor (Springfield Precise Temp., Springfield Precision Instruments, Wood Ridge, NJ). The average temperature and humidity values were recorded as 21.0 ± 2.1 °C and 85 ± 5%, respectively.

2.2.4. Chemical analyses

2.2.4.1. Sample preparation. The procedures explained for microbiological analyses (excluding disinfection and inoculation steps) were applied to the samples to be used for chemical analyses but without aseptic precautions. Leaf samples from the treatments were shredded using a laboratory blender (Waring blender) for