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Short communication

Efficacy of gaseous ozone for reducing microflora and foodborne pathogens on button mushroom

Ilgaz Akata[a], Emrah Torlak[b,*], Fatih Erci[c]

[a] Ankara University, Faculty of Science, Department of Biology, 06100 Tandogan, Ankara, Turkey
[b] Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, 42090 Meram, Konya, Turkey
[c] Necmettin Erbakan University, Faculty of Science, Department of Biotechnology, 42090 Meram, Konya, Turkey

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ABSTRACT

During growth, mushrooms can be contaminated with both saprophytic and pathogenic microorganisms derived from various points of contamination. This study was performed to evaluate the efficacy of gaseous ozone for reduction of microbial load and elimination of *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 on white button mushroom (*Agaricus bisporus* (J.E. Lange) Imbach). Whole mushroom samples were exposed to gaseous ozone up to 60 min at concentrations of 2.8 and 5.3 mg L^{-1}. The level of yeast and mold population naturally present on mushrooms was reduced more than 1.43 log after ozonation at 5.3 mg L^{-1} for 45 min. Exposure to ozone at 2.8 and 5.3 mg L^{-1} for 60 min yielded 2.44 and 3.07 log reductions in aerobic plate counts, respectively. Initial levels of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 populations on inoculated mushrooms reduced by ranging 2.10 and 2.76 log after 60 min of treatment performed at concentration of 2.8 mg L^{-1}, respectively. Ozonation at 5.3 mg L^{-1} for 60 min reduced the initial counts of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 by 3.61, 2.80 and over 3.41 log, respectively. These results suggest that gaseous ozone treatment can improve the microbial safety and postharvest quality of mushrooms.

1. Introduction

Mushrooms have been consumed throughout the world for many centuries, not only for texture and flavor but also for their chemical and nutritional characteristics (Manzi et al., 1999; Guan et al., 2012). Today, various species of cultivated mushrooms are available all the year round at retail outlets. Also, a variety of edible wild mushrooms are seasonally available in their fresh form. However, world-wide mushroom market has been dominated by the cultivated white button mushroom (*Agaricus bisporus* (J.E. Lange) Imbach) (Venturini et al., 2011).

The rate of postharvest deterioration of mushrooms has been directly related to their initial microbial load (Beelman et al., 1989). Fresh mushrooms are vulnerable to microbial attack and also ideal medium for microbial growth because their high water content and neutral pH (Brennan et al., 2000). Due to the environment in which they grow, mushrooms are likely to be exposed to a wide range of microbial contamination from many sources most notably casing material. In a comprehensive survey conducted by Venturini et al. (2011) in Spain, mesophilic aerobic microorganism counts on fresh mushrooms were reported ranging from 4.9 to 9.4 log CFU g^{-1}. In addition to their high levels, microorganisms on mushrooms tend to increase during postharvest storage (Doores et al., 1987). Fresh mushrooms also have the potential to carry foodborne pathogens derived from different sources. In a study conducted in the USA (Samadpour et al., 2006), the percentages of *Salmonella,* enterohemorrhagic *Escherichia coli* and *Listeria monocytogenes* positive samples for mushrooms were determined as 5%, 4% and 1%, respectively. The highest prevalence was reported for *L. monocytogenes* (11.5%) among foodborne pathogens investigated on mushroom samples in study of Venturini et al. (2011).

Washing of fresh button mushrooms with sanitizers such as sodium hypochlorite and organic acids has become commercially popular, especially due to a consumer preference (Singh et al., 2010). Washing of mushrooms in aqueous sanitizers could result in lower microbial counts and improve initial appearance. However, washing treatments would also result in tissue injury due to mechanical damage and water uptake, which in turn, leads to subsequent bruising and rapid microbial growth (Cliffe-Byrnes and O'Beirne, 2008). Therefore, development of microbial reduction techniques that are less severe and less damaging to mushrooms is an ongoing subject of research. In recent years, some emerging decontamination techniques such as ultraviolet light have been

* Corresponding author. Fax: +90 332 236 21 41.
E-mail address: etorlak@konya.edu.tr (E. Torlak).

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studied to adapt to postharvest mushroom processing (Ha et al., 2011; Guan et al., 2013).

As an alternative to aqueous treatments, potential food applications of ozone in the gaseous state for reducing microbial load have attracted attention in recent years. Ozone, or triatomic oxygen, is formed by addition of singlet oxygen to oxygen molecule (Khadre et al., 2001). Ozone is very effective in destroying microorganisms due to its potent oxidation capacity (Guzel-Seydim et al., 2004b). It has been demonstrated to be convenient for decontamination of foods, because it quickly decomposes into oxygen and hence does not leave undesirable by-products or residues (Graham, 1997). Gaseous ozone was granted generally recognized as safe (GRAS) status by the Food and Drug Administration (FDA) as a direct contact food sanitizing agent. Also, its use in postharvest processing is recognized as allowable by organic certification and regulatory bodies (Selma et al., 2008).

Antimicrobials in the gaseous state are less likely to modify the composition of food matrices compared to their aqueous solutions (Perry and Yousef, 2011). A study was conducted by Escriche et al. (2001) to determine the effect of gaseous ozone on postharvest quality of button mushroom. Ozone treatment prior to packaging at the concentration of 100 mg s^{-1} up to 25 min exhibited no significant differences in terms of texture, maturity index and weight loss. However, there are no published study on the efficacy of the gaseous ozone for the microbial reduction and elimination of foodborne pathogens on mushrooms. Therefore, the objective of this study was to investigate the reduction of microflora and foodborne pathogens (*Salmonella*, *L. monocytogenes* and *E. coli* O157:H7) on whole button mushrooms by gaseous ozone treatment.

2. Materials and methods

2.1. Bacterial strains and preparation of inoculums

Lyophilized cultures of *Salmonella* Typhimurium (ATCC 14028) and *L. monocytogenes* (ATCC 7644) used in this study were supplied from Microbiologics Inc. (Saint Cloud, USA). Culture of *E. coli* O157:H7 (NCIMB 13861) was kindly provided by STA Food Control Laboratory (Mersin, Turkey).

Stock cultures were maintained at −18 °C in brain heart infusion broth (Lab M, Bury, UK) containing 20% glycerol. Working cultures were grown on nutrient agar (Lab M) slants and kept at 4 °C. A loopful of working cultures were transferred into 25 mL tryptic soy broth (Lab M) and incubated overnight at 35 °C. Following incubation, the cultures were centrifuged at 3600 × *g* for 10 min at 5 °C (Hettich, Tuttlingen, Germany), washed twice, and resuspended in 0.1% peptone. The resulted suspensions were diluted in 0.1% peptone to obtain 0.5 McFarland turbidity standard, which represents approximately 1.5×10^8 CFU mL^{-1}. Then, suspensions were further diluted ten-fold in 0.1% peptone and supplemented with Tween 20 (Merck, Darmstadt, Germany) at concentration of 5% (v/v) in order to help to reduce the surface tension (Nascimento et al., 2012). The final suspensions were used as inoculum.

2.2. Inoculation of mushrooms

White button mushroom (*Agaricus bisporus*) samples at the closed cap stage, similar in appearance and size (≈4 cm cap diameter), were supplied by a local producer (Mega Tesnim, Konya, Turkey) and stored at 4 °C until they were used. Before inoculation, representative portions of mushrooms were assayed by the rapid methods (RapidChek, Romer Labs, Newark, DE, USA) for *Salmonella*, *Listeria* and *E. coli* O157:H7 in order to verify absence of contamination.

Mushrooms were firstly exposed to compressed air (Einhell, Landau, Germany) to remove soil particles and organic materials on their skin. Then they divided into four sub-samples and three of them were allocated for inoculation. Effect of ozonation on microorganisms naturally present on mushrooms was evaluated on non-inoculated sub-sample. The stipes were removed and mushrooms were placed cap side up on aluminum trays. Then, inoculums were sprayed as homogeneously as possible onto skin of mushrooms using an atomizer (DeVilbiss Healthcare, Somerset, PA, USA). Each of the sub-samples allocated to inoculation were artificially contaminated with only single pathogen and about 50 μL inoculum was used for each mushroom. The inoculated mushrooms were air-dried for 30 min in the biological safety cabinet (Faster, Ferrara, Italy) before ozonation. Thus, more than 5 log CFU per mushroom inoculation levels of the contamination were achieved on samples.

2.3. Ozone treatment

Inoculated and non-inoculated sub-samples were subjected to ozonation at room temperature (20 ± 2 °C) for four exposure times (15, 30, 45 and 60 min). Each treatment group was comprised of five mushrooms. Ozone treatment was performed under continuous stream of two different constant concentrations in 9.9 L plexiglas desiccator chambers (Belart Products, Wayne, NJ, USA) equipped with two gas ports for the inlet and outlet flow. Ozone was generated directly from atmospheric oxygen by two generators (Opal, Ankara, Turkey) with different ozone generation capacities. Air flow rates in the tubes connected to inlet ports were adjusted to 16.7 mL s^{-1} using a flow meter (Dwyer Instruments, Michigan City, IN, USA).

The ozone concentrations in the air flows were determined as 2.8 and 5.3 mg L^{-1} by the iodometric method (IOA, 1996). Gaseous ozone was bubbled in 200 mL buffered potassium iodide (KI) solution with a diffuser. Then, in order to complete the reaction, the acidity of the KI solution was adjusted with sulfuric acid (4.5 mol L^{-1}) to pH 2. Immediately after, the liberated iodine was titrated to a starch endpoint with freshly standardized sodium thiosulfate solution (0.1 mol L^{-1}). Ozone concentrations were calculated based on ozone/iodine stoichiometry of 1.

The times necessary for the ozone concentrations inside the treatment chambers reached to their maximum levels (2.8 and 5.3 mg L^{-1}) were calculated by a mass balance equation (Silva et al., 1998):

$$C_0 \times \left(1 - e^{(-\nu \times (t/V_t))}\right) = C$$

where V_t is the volume of chamber (mL), ν is the air flow (mL s^{-1}), t is the time (s), C_0 is the concentration of ozone coming from generator (mg L^{-1}) and C is the predicted ozone concentration in the chamber for specified time (mg L^{-1}).

2.4. Microbiological analysis

Microbiological enumerations were performed by plate count technique on plate count agar (PCA, Lab M), dichloran rose bengal chloramphenicol (DRBC) agar (Lab M), xylose lysine deoxycholate (XLD) agar (Liofilchem, Roseto Degli Abruzzi, Italy), agar Listeria according to Ottaviani and Agosti (ALOA, Liofilchem) and chromogenic O157 agar (Chromatic, Liofilchem) for aerobic plate count (APC), yeast and mold (YM), *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 respectively. Mushrooms were aseptically transferred to sterile stomacher bags containing 100 mL of buffered peptone water (Lab M) and pulsified (Pulsifier, Microgen Bioproducts, Camberley, UK). Numbers of surviving microorganisms on mushrooms were determined by plating 1 mL aliquots