



The role of pulsed light spectral distribution in the inactivation of *Escherichia coli* and *Listeria innocua* on fresh-cut mushrooms

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ABSTRACT

Pulsed light (PL) treatments have emerged as a non-thermal method for microbial decontamination on foods surfaces. The aim of this work was to evaluate the bactericidal effect of PL by identifying the spectral range with antimicrobial activity and its effect on the quality of fresh-cut mushrooms (*Agaricus bisporus*). The mechanism responsible for their action on bacterial cells was also studied using Transmission Electron Microscopy (TEM). Results show that the effectiveness of PL-treatment decreases when the UV (ultraviolet) spectral region is blocked (particularly UV-C). PL treatments of full wavelength spectrum (180–1100 nm) and a fluence of 12 J/cm² caused 3 and 2 log reductions in the initial counts of inoculated *Escherichia coli* and *Listeria innocua*, respectively. TEM showed significant damage in cell cytoplasm and cytoplasmic membrane after treatments with full spectrum pulses and a total fluence of 12 J/cm². In contrast, mushroom cells treated with 6 J/cm² did not exhibit apparent changes in their cytoplasmic membrane. Full spectrum treatments had a more pronounced impact on color, texture and headspace gas composition than treatments without UV spectrum profile. This work contributes with new information regarding the effects of the spectral range of PL treatments that the whole UV–Vis range of the spectrum accounts for the lethal effect against microorganisms. On the other hand, it also provides increased knowledge regarding the antimicrobial action of this technology, showing that a photophysical effect exists, leading to changes in the bacterial cytoplasmic membrane and cell content.

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1. Introduction

Changes in the consumers' lifestyle with a preference for more natural, fresh and ready-to-eat products have increased the market of minimally processed foods (Soliva-Fortuny & Martín-Belloso, 2003). Production of sliced mushrooms is growing but there is little information available on their quality, shelf life or safety (Brennan & Gormley, 1998; Brennan, Le Post, & Gormley, 2000). In addition, most research has been carried out with whole mushrooms. Sliced mushrooms are usually marketed in trays over-wrapped with plastic films, which reduce dehydration, and stored under refrigeration temperatures (González-Fandos, 2006). Slicing operations cause spreading of bacteria over cut surfaces, further bruising due to additional handling and disruption of hyphal cells, enabling substrates and enzymes to initiate enzymatic browning

reactions (Brennan & Gormley, 1998). Initial microbial counts on mushrooms are very high, ranging from 6.2 to 7.2 log CFU g⁻¹ at harvest time (Simón, González-Fandos, & Tobar, 2005). Therefore, they are highly perishable and tend to lose quality right after harvest (Kim, Ko, Lee, Park, & Hanna, 2006).

Pulsed light (PL) treatments have been proposed as a feasible alternative to thermal treatments for killing pathogenic and spoilage microorganisms in foods, thus extending their shelf life. Several studies have shown the ability of PL to inactivate *Salmonella* spp., *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes* and *Listeria innocua* in some products (Bialka & Demirci, 2008; Ozer & Demirci, 2006; Palgan et al., 2011; Sauer & Moraru, 2009; Uesugi & Moraru, 2009). The variability of results (2–8 log reductions) is most likely due to the different target microorganisms, the intensity of the treatment, and the properties of the substrates (MacGregor et al., 1998; Paskeviciute, Buchove, & Luksience, 2010; Sharma & Demirci, 2003; Woodling & Moraru, 2005).

In PL treatments, broadband radiation is emitted ranging from ultraviolet (UV) to near-infrared (NIR). UV wavelengths range from 180 to 400 nm, while visible (VIS) from approximately

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400–700 nm and NIR from 700 to 1100 nm (Wekhof, 2001; Woodling & Moraru, 2007). The UV portion of the electromagnetic spectrum includes long-wave UV-A (315–400 nm), medium-wave UV-B (280–315 nm), and short-wave UV-C (180–280 nm) (Wekhof, 2001). While the overall lethal effects of UV light on microorganisms are known, the effect of different wavelength ranges of the spectrum is not fully elucidated. The mechanisms of microbial inactivation proposed to explain the lethal effect of PL are related to the UV part of the spectrum and its photochemical, photothermal and physical effect (Anderson, Rowan, MacGregor, Fouracre, & Farish, 2000; Elmnasser et al., 2007; Takeshita et al., 2003; Wekhof, 2000, 2001), inducing damage to DNA such as the formation of single strand breaks and pyrimidine dimers that prevent DNA unzipping for replication and thus avoiding reproduction of the organism. However, there is little information available about the effects of different spectral ranges when using this technology to decontaminate foods.

The aim of this work was to determine the spectral range of pulsed light (PL) treatments causing microbial inactivation and its effect on quality of fresh-cut mushrooms (*Agaricus bisporus*) inoculated with *E. coli* or *L. innocua*. The effect responsible for their action on bacterial cells using Transmission Electron Microscopy (TEM) was also studied.

2. Materials and methods

2.1. Processing and packaging

Fresh mushrooms (*A. bisporus*) were purchased at a local supermarket in Lleida, Spain and immediately processed. Whole mushrooms as well as the surfaces and tools in contact with them (working area, chopping boards and knives) were washed and disinfected with a solution of 200 µL/L of sodium hypochlorite (pH 7). They were then rinsed with distilled water, finally dried, cut in slices and immediately dipped in a 1% (v/v) aqueous solution of ascorbic acid for 1 min to prevent browning. Six mushroom slices with a weight of approximately 5 g each (ca. 30 g) were placed in polypropylene trays under aseptic conditions to be thermosealed using an ILPRA FoodPack Basic V/6 packaging machine (ILPRA Systems, CP, Vigevano, Italy). A sealing film with permeability to oxygen of $110 \text{ cm}^3 \text{ O}_2 \text{ m}^{-2} \text{ bar}^{-1} \text{ day}^{-1}$ at 23 °C and 0% RH (ILPRA Systems Spain, S.L. Mataró, Spain) was used. Once sealed, the trays were treated by PL. A set of untreated samples was prepared as reference. The packages were stored at 5 °C in darkness until random withdrawal for analysis (each 3 days up to 15 days).

2.2. PL treatments

PL treatments were carried out with a XeMaticA-2L System (SteriBeam Systems GmbH, Germany). The emitted spectrum wavelengths (λ) ranged from 180 to 1100 nm with 15–20% of the light in the UV region. The duration of each pulse was 0.3 ms with a fluence of 0.4 J/cm^2 from one lamp situated 8.5 cm above the sample holder. The distance from the lamp to the sealing film was 6 cm. To evaluate the effect of the composition of the emitted spectrum two types of UV filters were used: a 2-mm thick Pyrex glass filter (PF) that cuts off all light below 305 nm allowing some UV-B (280–320 nm), all UV-A (320–400 nm) and visible light to pass, and a Makrolon polycarbonate plastic filter (MF) that cuts off all light below 400 nm thus allowing only the VIS and NIR to pass through. In this study, the effectiveness of full spectrum (FS) ($\lambda = 180\text{--}1100 \text{ nm}$), FS without UV-C light ($\lambda = 305\text{--}1100 \text{ nm}$) and VIS-NIR light ($\lambda = 400\text{--}1100 \text{ nm}$) treatments on bacterial inactivation and quality parameters of fresh-cut mushroom were compared. Additionally, the effect of fluence (6 or 12 J/cm^2) and

kind of microorganism (Gram positive or Gram-negative) were also investigated.

2.3. Microbiological analysis

2.3.1. Preparation of inoculum

Strains of *E. coli* 1.107 (Laboratoire de répression des Fraudes (LRF); Montpellier, France) and *L. innocua* 1.17 (Laboratoire de répression des Fraudes (LRF); Montpellier, France) as a surrogate of the pathogenic *L. monocytogenes* were provided from the culture collections of the Department of Food Technology, University of Lleida, Spain. The original strains were kept in inclined test-tubes with TSA (Tryptone Soy Agar) (Biokar Diagnostics, Beauvais, France) at a temperature of 5 °C until its use. A stock culture of *L. innocua* was grown in tryptone soy broth (TSB) + 0.6% of extract of yeast (Biokar Diagnostics; Beauvais, France) at 35 °C for 15 h and 180 rpm, to obtain cells near to the stationary phase of growth at the moment of carrying out the inoculation, while a stock culture of *E. coli* was grown in TSB (Biokar Diagnostics; Beauvais, France) at 37 °C for 11 h and 120 rpm (cell in early stationary phase). The maximum growth for *L. innocua* and *E. coli* was 5.4×10^9 and 4.8×10^9 colony forming units/mL (CFU/mL), respectively. Concentrations were then adjusted to 1×10^8 CFU/mL using saline peptone water (Scharlau Chemie, S. A. Barcelona, Spain) in agar and broth dilution assay respectively.

2.3.2. Mushrooms inoculation

Fresh-cut mushrooms (30 g) were inoculated by spreading with 300 µL of *E. coli* or *L. innocua* stock cultures (10^8 CFU/mL) over its entire upper surface with a sterile micropipette. Inoculated mushrooms were packed, treated and stored as explained previously.

2.3.3. *E. coli* and *L. innocua* counts

Mushroom slices (10 g) were removed aseptically from each tray and transferred into sterile plastic bags. Samples were diluted with 90 mL of saline peptone water (0.1% peptone –Biokar Diagnostics, Beauvais, France + 0.85% NaCl – Scharlau Chemie, S.A. Barcelona, Spain) and homogenized for 1 min in a stomacher blender (IUL Instruments, Barcelona, Spain). Serial dilutions were made and spread at reason of 0.1 mL on MacConkey and Palcam agar (Biokar Diagnostics) plates in duplicate for *E. coli* and *L. innocua* counts, respectively. The plates were incubated for 24–48 h at 35–37 °C. Colonies were counted and the results expressed as $\log \text{ CFU g}^{-1}$. Analyses were carried out periodically (each 3 days) during 15 days of storage. The experiments were performed twice. Thus, the reported values are the mean of two determinations made in duplicate $\pm$ standard deviation ($n = 4$).

2.4. Headspace gases analysis

The gaseous composition of the package headspace was determined using a Micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, Netherlands) equipped with a thermal conductivity detector. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 µL were injected for O_2 and CO_2 determination, respectively. The O_2 content was analyzed with a CP-Molsieve 5 A packed column (Chrompack International, Middelburg, Netherlands) ($4 \text{ m} \times 0.32 \text{ mm}$, $\text{df} = 10 \text{ mm}$) at 60 °C and 100° kPa. On the other hand, a Pora-Plot Q column (Chrompack International, Middelburg, Netherlands) ($10 \text{ m} \times 0.32 \text{ mm}$, $\text{df} = 10 \text{ mm}$) was held at 70 °C and 200 kPa for CO_2 quantification. A pair of trays was withdrawn at each time for each different treatment. Two readings were carried out for each one of two treatment replicates ($n = 2 \times 2$).

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