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Short communication

Pulsed light decontamination of endive salad and mung bean sprouts in water

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A R T I C L E I N F O

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A B S T R A C T

This study evaluated the Pulsed Light technology (PL) for its efficiency to reduce the microbial loads on mung bean sprouts and endive salad during a simulated wash process for up to 60 s. The microbicidal action of PL proved to be more efficient than the sanitizers electrolyzed water (40 ppm free chlorine) and chlorine dioxide (15 ppm) within the same treatment time. Reductions of the microbial count up to 2.5 log were found in case of PL treated endive salad while both sanitizers only caused maximum reductions by about 1.5 log. A combination of PL and sanitizers did not have pronounced additional effect for both endive salad and mung bean sprouts. Overall, the decontamination treatments were more effective in case of endive salad compared to mung bean sprouts. It was also shown that the microbial loads in the washing water may be kept on a low level during PL treatments as count reductions of approx. 3 log were found in relation to simply washing in tap water. This study reveals that PL may be a suitable approach to reduce microbial hazards of industrially manufactured fresh produce. The application during the washing process not only reduces microbial loads on the product surface but also efficiently inactivates suspended microorganisms in the wash water and may therefore be an alternative to sanitizers for the prevention of cross contaminations.

1. Introduction

The widespread popularity of minimally processed fresh-cut produce goes along with increased microbial hazards arising from frequent contaminations with pathogenic bacteria like *E. coli*, *Listeria* spp. or *Salmonella* spp. (Berger et al. 2010; Harris et al. 2003), which may cause foodborne diseases (Dewaal & Bhuiya, 2007). Recent epidemic outbreaks like e.g. the large EHEC-outbreak in 2011 in Germany, which was most likely due to contaminated sprouts, emphasize the necessity of innovative measures to reduce microbial contaminations on industrially produced vegetable or fruit products. As the application of chemical sanitizers like chlorine is often unwanted in case of fresh produce, because of their possible impact on sensorial properties or the formation of hazardous by-products, other potential surface decontamination technologies are currently in the focus. Pulsed light is an innovative non-thermal technology based on intense broad spectrum light pulses including a high UV content. It has been approved by the U.S. Food and Drug Administration for the treatment of foodstuffs with a maximum fluence of 12 J/cm^2, provided that the emission spectra is between 200 and 1100 nm and the pulse duration is no longer than 2 ms (FDA, 1996). It has already proven to be a highly efficient way for surface decontamination and has been tested in numerous studies for its efficiency to disinfect different food or beverages (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010). For the inactivation of microorganisms on surfaces of vegetables and fruits, PL has mostly been applied in dry state. Only few studies have been conducted where PL is applied during a simulated washing process of fresh produce. The aim of this work was to assess the utility of PL to inactivate microorganisms on mung bean sprouts and endive salad during washing and furthermore to evaluate its effect on the microbial loads in the wash water.

2. Material and methods

2.1. Preparation of inoculum

Listeria innocua DSM 20649, which is often used as a nonpathogenic surrogate for *Listeria monocytogenes* (Lasagabaster

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& De Marañón, 2012), was used as model organism in this study. The test strain was obtained from the German National Resource Center (DSMZ, Germany) and maintained on tryptic soy agar (Oxoid, UK) at 5 °C. Working cultures were made by inoculating 100 ml of tryptic soy broth with cell material from the agar surface and following incubation for 16–18 h at 37 °C in a shaking bath until early stationary phase. 20 ml of the cell culture were centrifuged at 9000 g for 10 min and washed twice with sterile deionized water in order to remove all solutes. The bacterial suspension for inoculation experiments with fresh produce was made by diluting 1 ml of the prepared cell suspension in 1 l of sterilized distilled water which resulted in a cell density of approximately $2*10^6$ CFU/ml.

2.2. Preparation of produce samples

Endive salad heads as well as mung bean sprouts were obtained from local retailer and used immediately. The salad heads were trimmed and cut into pieces of approximately 10 cm^2 with a sharp knife. Salad pieces and mung bean sprouts for experiments with artificial inoculation were dipped in the prepared suspension of *L. innocua* for 5 min at 20 °C under agitation. The produce was then removed and drained until a weight increase of about 10% compared to the initial weight was reached. Samples of 5 g were weighed out and then cold stored for 1 h at 4 °C in order to enable bacterial adhesion. This approach led to a superficial cell density of approximately $5*10^5$ CFU/g produce. Endive and mung bean samples for assessment of the total viable count were prepared the same way without inoculation.

2.3. Pulsed light equipment, sanitizers and treatment conditions

The prepared samples were treated in a lab-scale PL chamber (dimensions: 50 × 50 × 30 cm) (Claranor, France), which was equipped with a reflector composed of three Xenon lamps (emission window: 120 cm^2). The lamps are connected to a capacitor and emit broad spectrum intense light flashes between 200 and 1100 nm. The distance between the reflector and the sample table was manually adjustable (0–20 cm) and the discharge voltage was tunable between 0.5 and 3 kV. The applied fluence was determined with a Solo2 Power and Energy Meter (Gentec, Canada). Chlorine dioxide as well as electrolyzed water (EOW) were used as sanitizers. Electrolyzed water (Anostel) was supplied by the by the company Redokon (Germany) and was used undiluted. The pH was 2.8, the redox potential was 1120 mV and the free chlorine was about 40 ppm. The pH and redox potential were determined with a WTW pH 197 S equipped with a Sentix 91 and a Sentix ORP electrode for pH and redox (WTW, Germany). Chlorine dioxide (Redokon, Germany) was prepared by mixing 9 parts hydrochloric acid (component A) with 1 part sodium chlorite (component B) before incubation at 30 °C for 3 h. The stock solution had a concentration of about 3 g CLO_2/l. Samples of 5 g were individually treated with PL, sanitizers or both in combination. During the treatments, endive salad samples as well as mung bean sprout samples were continuously agitated with a stirrer at 200 rpm.

Pulsed light was applied during a simulated washing process at a distance of 5 cm between the water surface and the flash lamp. A volume of 1.5 l of autoclaved tap water was used as wash water in a round jar resulting in a fluid thickness of 8.5 cm. Discharge voltages of 1.5 kV and 2 kV were used, corresponding to an incident fluence on the water surface of 320 and 580 mJ/cm^2 per light pulse. The treatments were performed at a pulse frequency of 1 Hz for 15–60 s while the sanitizers were only applied for 60 s. EOW was used undiluted and a concentration of 15 ppm of chlorine dioxide was adjusted in sterilized tap water. Concentrations of free chlorine (EOW) as well as chlorine dioxide were measured with colorimetric test kits (Merck, Germany). In cases were PL and sanitizers were combined, the samples were first treated in EOW or chlorine dioxide for 60 s, then removed from the water and immediately exposed to PL for 60 s at 2 kV in sterile tap water as described. The combined treatment was not performed at once because UV light degrades chlorine dioxide. The levels of disinfection were compared to simply washing in water for 60 s under equal conditions. After the treatments, sodium thiosulfate (10 mg/l) was added in order to neutralize the sanitizers. For each treatments at least three replicate samples on two different days were analyzed ($n = 6$). Data is presented as mean values with standard deviation.

2.4. Determination of viable counts

Determination of the viable counts was done by pour-plate-technique based on ISO 4833–1:2013. After the treatments, samples were removed from the water, transferred in bags filled with 45 ml of sterile peptone water and 0.1% Tween 80 and homogenized with a stomacher 400 circulator (Seward, UK) at 260 rpm for 1 min. The resulting sample suspensions were subsequently serially diluted with sterile ringer solution. Appropriate dilutions were plated with tryptic soy agar by use of the pour plate method in triplicates. Water samples were taken and diluted and plated the same way. The total viable count was determined after aerobically incubating the agar plates for 3 days at 30 °C. In case of inoculated samples, Chromocult Listeria selective agar (Merck, Germany) was used and the plates were incubated at 37 °C for 48 h.

3. Results and discussion

3.1. Comparison of PL treatments to sanitizers and combination of both

Pulsed light treatments have been applied in various studies to assess its suitability to disinfect surfaces of vegetables and fruits so far (Oms-Oliu et al. 2010). Different authors have reported about the disinfection of strawberries and raspberries (Bialka & Demirci, 2008), fresh-cut avocado (Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, 2011), cut apple (Gómez, Salvatori, García-Loredo, & Alzamora, 2012), fresh-cut mango (Salinas-Roca, Soliva-Fortuny, Welti-Chanes, & Martín-Belloso, 2016) or mung bean sprouts and endive salad (Kramer, Wunderlich, & Muranyi, 2015), thereby obtaining variable results with count reductions usually ranging between 1 and 4 log. In almost all studies challenge tests were performed in "dry state", with the product surface being directly exposed to PL. There is only few data available for PL treatments which were applied during a washing process, although this approach would allow a simple implementation of the pulsed light technology into running industrial manufacturing processes where washing of the produce is a crucial step due to the possibility of cross contaminations (Gil, Selma, López-Gálvez, & Allende, 2009). We therefore assessed the suitability of PL for microbial decontamination of cut endive salad and mung bean sprouts during a simulated washing process under laboratory conditions, using industrially relevant treatment times. The results showed that PL is more effective in reducing microbial loads on fresh cut salad than equivalent treatments in electrolyzed water (40 ppm free chlorine) or chlorine dioxide (15 ppm). Fig. 1 shows that the total viable count on endive salad decreases by 2 log after a PL treatment for 60 s in tap water. A similar reduction of microbial populations on endive salad was also found by Kramer et al. (2015) when PL was applied in dry state with only up to three single light flashes on both sides. The application of the sanitizers led to maximum reductions of the total count by only 1 log at which the washing in tap water alone caused