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Water saving in fresh-cut salad washing by pulsed light

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ABSTRACT

The possibility to wash salad with recycled water submitted to pulsed light decontamination was studied. Wastewater deriving from lamb's lettuce washing was exposed to pulsed light at increasing fluence up to 17.5 kJ/m^2. Pulsed light dose of 11.0 kJ/m^2 allowed the inactivation of most of the native microflora and the achievement of more than 6-Log reductions in inoculated microorganisms (*Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*). The increase in washing cycles up to 5 did not impair the efficacy of wastewater decontamination promoted by pulsed light (circa 4-Log reduction in native microflora) nor the hygienic level of the washed salad (circa 1-Log reduction in native microflora).

Industrial relevance: The application of pulsed light to decontaminate wastewater deriving from salad washing could decrease the water footprint of fresh-cut vegetables by minimising the overall requirement for water in industrial plants. In addition, it would decrease the risk for residuals of toxic chemicals in fresh-cut vegetables by avoiding the use of sanitizers, such as chlorine.

1. Introduction

Salad entering the fresh-cut industry is generally characterised by a microbial load ranging from 5 to 9 Log units, depending on the type of salad, cultivation system, harvesting and handling procedures among other factors (Barth, Hankinson, Zhuang, & Breidt, 2010; Ölmez & Kretzschmar, 2009). Further cutting operations are well known to cause an increase in microbiological activities with effects on safety and quality (Ragaert, Devlieghere, & Debevere, 2007). In the absence of adequate processing conditions, microorganisms can reach the infective dose, making the product harmful to human health. In this regard, salad consumption has been associated with a number of food poisoning outbreaks (EFSA, 2013; FDA, 2013).

Washing is nowadays the sole technological operation performed to reduce salad microbial load. The turbulent flow of washing water on salad surface mainly promotes the mechanical removal of microorganisms, leading to a reduction in microflora of circa 1 Log unit (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008). As a consequence, washing water becomes highly contaminated, reaching microbial loads analogous to those of the unwashed salad. To accomplish the requirements of water saving, wash water is used in multiple washing cycles and may become not only a vehicle for spoilage microorganisms but also for pathogenic ones. The risk of cross-contamination is conventionally reduced by adding disinfection chemicals, such as chlorine and its related compounds. They are used at levels of 50–200 mg/L of free chlorine to obtain a 6 Log reduction of microbial load and decrease the concentration of water-borne pathogens below the regulatory limits (Codex Alimentarius Commission, 2001; EC, 1998; Gil, Selma, López-Gálvez, & Allende, 2009; Sommer, Lhotsky, Haider, & Cabaj, 2000). However, concern is growing about the possibility of chlorine to react with organic matter and beget carcinogenic and/or mutagenic by-products such as trihalomethanes and haloacetic acids (Krasner et al., 2006; Richardson & Ternes, 2005).

Disinfection of water may also be accomplished by physical treatments with ultraviolet (UV-C) radiation. This technology has been proven to exert strong germicidal effects due to the ability of UV-C light to damage microbial DNA, blocking its transcription and compromising cellular functions (Rame, Chaloupeky, Soikova, & Bencko, 1997; Sastry, Datta, & Worobo, 2000). The sterilising effect of ultraviolet light has been proposed to reduce the microbial flora of wastewater collected from washing of onions, escarole, carrots, spinach and lamb's lettuce (Ignat, Manzocco, Bartolomeoli, Maifreni, & Nicoli, in press; Selma, Allende, López-Gálvez, Conesa, & Gil, 2008). Although highly efficacious, water decontamination by UV-C light usually requires treatment times ranging from many seconds to more than 10 min, depending on processing conditions (e.g. light irradiance, plant geometry, water depth). This disadvantage could be overcome by using pulsed light, which can be considered an improved version of delivering ultraviolet radiation (Gómez-López, Devleghere, Bonduelle, & Debevere, 2005). Pulsed light is actually based on exposure to xenon lamp flashes, which typically last from μs to ms. Light flashes are characterised by an intense broad spectrum of wavelength (200–1000 nm) which includes not only ultraviolet but also visible and infrared light. The latter are known to strengthen the antimicrobial effects of UV-C light by adding a local photothermal effect

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(Dunn, Ott, & Clark, 1995; Fine & Gervais, 2004; Gómez-López et al., 2005; Guerrero-Beltrán & Barbosa-Cánovas, 2004). The high energy flashes can also provide a photophysical disturbance leading to further structural changes to microorganisms (Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2008). When rapid disinfection is required, pulsed light may thus provide a practical advantage over ultraviolet technology, allowing multiple flashes to be delivered within few seconds of treatment. Despite literature data confirming that pulsed light can reduce microbial load and inactivate pathogens in fruit juices and other liquids, to our knowledge, no information is available about its bactericidal efficacy on wastewater deriving from the washing of vegetables (Ogihara et al., 2013; Palgan et al., 2011; Pataro et al., 2011).

This study was designed to investigate the possibility of washing salad by recycling wastewater submitted to pulsed light decontamination. To this purpose, wastewater obtained by washing lamb's lettuce was exposed to increasing doses of pulsed light. The germicidal efficacy of the treatment was evaluated on native microbiota as well as on inoculated pathogens (*Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes*) associated with faecal contamination deriving from irrigation water or postharvest handling. The possibility to reuse pulsed light decontaminated wastewater in multiple washing cycles up to 5 was finally studied.

2. Materials and methods

2.1. Salad washing

Lamb's lettuce was provided by a local farm, stored at 6 °C and processed within 1 day. Lamb's lettuce was washed in tap water (0.3 mg/L free chlorine) at 8 °C for 2 min with 1:10 (w/v) salad/water ratio. Lamb's lettuce leaves were separated from wash water and centrifuged (mod. ACX01, Moulinex, Ecully, France) for 1 min. Water drained from leaves by centrifugation was added to the previously collected wash water. Wash water was treated by pulsed light and reused for up to 5 washing cycles. In each cycle a new batch of lettuce was washed with pulsed light treated water to simulate industrial recycling of wastewater during salad washing. Water and lettuce samples were immediately used for analysis.

2.2. Pulsed light treatment

Pulsed light treatments of wastewater were carried out by using a pulsed light mobile decontamination unit (Claranor, Rouaine, France) equipped with 4 xenon lamps (JA series, Verre et Quartz, Bussy Saint Georges, France) with maximum emission in the range 200–1000 nm (200–400 nm: 41%; 400–700 nm: 51%; 700–1000 nm: 8%). Aliquots of 10 mL of wastewater were placed in 5.5 cm diameter sterile Petri dishes to get a 4 mm thick-sample. Petri dishes were placed on a 5 mm thickness quartz plate at a distance of 10 mm from the lamps positioned above, below and at the two sides of the sample, and exposed at increasing light fluence up to 17.5 kJ/m^2, by modifying capacitor voltage (1000–3000 V). Each light pulse had a duration of 50 μs.

2.3. Temperature

Temperature was measured by a thermocouple probe (Checktemp1, Hanna Instruments Inc., Woonsocket, RI, USA).

2.4. Free chlorine

Free chlorine was determined by a HI38020 kit based on the use of N, N-diethyl-p-phenyldiamine (DPD) (Hanna Instruments Inc., Salaj, Romania).

2.5. Microbiological analysis

For the microbiological analysis water and salad were sampled for microbial plate counts. In particular, before plating, salad samples were added to 9 volumes of Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK) and homogenized for 2 min in a Stomacher Lab-Blender 400 (PBI International, Milano, Italy). Serial dilutions of each suspension were made in MRD (Oxoid) and appropriate aliquots (0.1 mL or 1 mL) were spread on agar plates. Plate Count Agar (PCA, Oxoid) and Pseudomonas Agar base (Oxoid) were used for enumeration of total mesophilic bacteria and *Pseudomonas* spp. respectively; plates were incubated at 30 °C for 48 h. Violet Red Bile Glucose Agar (VRBG, Oxoid) and Coli ID (BioMerieux, Mercy L'Etoile, France) were used for enumeration of Enterobacteriaceae and total coliforms, respectively; plates were incubated at 37 °C for 24 h.

Preliminary trials were carried out on salad leaves and non-inoculated wash water samples to check for *Salmonella* spp. and *L. monocytogenes* presence as previously described (Ignat et al., 2015).

2.6. Bacterial strains and growth conditions

Experiments were performed with pure cultures of *S. enterica* subsp. *enterica* 9898 DSMZ, *L. monocytogenes* 20600 DSMZ and *E. coli* obtained from the Department of Food Science Bacterial Culture Collection (University of Udine, Italy) and stored at −80 °C in Brain Hearth Infusion Broth (BHI, Oxoid) with 30% glycerol until needed. The stock cultures were maintained by regular subcultures in BHI Agar at 4 °C. A loopful of bacteria was transferred to 5 mL of BHI Broth, incubated at 37 °C, collected by centrifugation at 13,000 rpm for 10 min at 4 °C (Beckman, Avanti TM J-25, Palo Alto, CA, USA) and washed with MRD (Oxoid) twice. Each final pellet was suspended in MRD and used for inoculation. Tap water samples were previously filtered with a sterile cellulose acetate filter (ALBET LabScience, Barcelona, Spain) 0.20 μm in pore size and 25 mm in diameter while wash water samples derived from the first washing cycle. The samples were inoculated with each strain separately in order to obtain a final concentration of 10^6–10^7 CFU/mL. The inoculated samples were incubated at 30 °C for 1 h and subsequently treated. Xylose Lysine Desoxycholate Agar (XLD, Oxoid), Palcam Agar (Oxoid) and Coli ID (BioMerieux, Mercy L'Etoile, France) were used for enumeration of *S. enterica*, *L. monocytogenes* and *E. coli* respectively. Plates were incubated at 37 °C for 24 h.

2.7. Statistical analysis

Analyses were performed on at least duplicated samples. Results are reported as mean value ± SD. Linear regression analyses was performed using Microsoft Office Excel 2007. Analysis of variance (ANOVA) was performed with significance level set to $p < 0.05$ (Statistica for Windows, ver. 5.1, Statsoft Inc. Tulsa, USA, 1997). The Tukey procedure was used to test for differences between means.

3. Results and discussion

3.1. Effect of washing on lamb's lettuce and water microflora

The effect of washing on lamb's lettuce and water microflora is reported in Table 1. Total viable count of lettuce resulted in the same magnitude range of literature data relevant to leafy vegetables cultivated on soil (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Selma et al., 2008). In particular, *Pseudomonas* spp. resulted in being the prevalent genus which is in agreement with its diffusion in leafy vegetables and lamb's lettuce (Manzocco et al., 2010; Schwaiger, Helmke, Hölzel, & Bauer, 2011). Enterobacteriaceae and total coliforms were also detected since they are typical inhabitants of salads deriving from soil and manure contamination.