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# Surface decontamination of fresh-cut apple by pulsed light: Effects on structure, colour and sensory properties

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ABSTRACT

The effect of pulsed light at increasing fluence (17.5, 52.5, 105.0 and 157.5 kJ/m$^2$) was studied with reference to germicidal efficiency and changes in fresh-like appearance of sliced apple. Independent of fluence, viable counts and inoculated bacteria were reduced by 1 and 3 logs respectively. Fluence significantly affected weight loss, colour and sensory attributes of apple slices during storage at 6 °C. Pulsed light at 17.5 kJ/m$^2$ resulted in apple slices comparable to the untreated samples, with limited quality changes. By contrast, at higher fluence, apple slices underwent dehydration and browning due to loss of cell integrity. Exposure to high fluence treatments was also associated with negative changes in the flavour profile of sliced apple during storage.



## 1. Introduction

Consumer demand for a variety of ready-to-eat food with fresh appearance and health-promoting properties is continuously increasing. In this context, fresh-cut fruit and vegetables are particularly popular. The operations required for fresh-cut vegetable production result in an increase in both microbiological and physiological activities with effects on safety and quality during storage (Ragaert et al., 2007). In particular, microorganisms can easily gain access to the surface of the fresh-cut produce. In the absence of adequate sanitation processes, they can reach infective doses, becoming the cause of food poisoning outbreaks (EFSA, 2011; FDA, 2013). Although the wide application of the HACCP (Hazard Analysis and Critical Control Points) system, documented cases of food-born illness are currently increasing. As a consequence, several non-thermal innovative strategies have been explored to decontaminate the surface of fresh-cut produce. Among these emerging technologies, pulsed light seems to be particularly promising. It involves the use of intense light flashes of a broad spectrum of wavelength (200–1000 nm), including ultraviolet, visible and infrared light (Dunn et al., 1995).

Bactericidal efficacy of pulsed light is mainly attributed to the photochemical effects of its UV light component, able to induce the formation of thymine dimers in microbial DNA inhibiting cell replication (Giese and Darby, 2000; Gómez-López et al., 2007). Literature data has shown that pulsed light can efficiently inactivate *Salmonella* spp., *Escherichia coli* and *Listeria innocua* in raspberries, strawberries, fresh-cut watermelon and mushrooms (Bialka and Demirci, 2008; Ramos-Villaroel et al., 2012a,b), as well as native microflora and inoculated bacteria in sliced apple (Gómez et al., 2012a). Pulsed light required for decontamination (60–1200 kJ/m$^2$) can induce not only photochemical but also photothermal effects, impairing colour, structure and sensory characteristics of fresh-cut products (Oms-Oliu et al., 2010). However, few data are available on pulsed light effects on quality and sensory properties of fresh-cut fruit and vegetables. For this reason, there is a need for optimising treatment intensity to achieve specific decontamination levels without affecting product freshness.

On the basis of these considerations, the aim of the present work was to evaluate the effect of increasing fluence of pulsed light on bacterial inactivation and overall quality of fresh-cut apple. In particular, 'Golden Delicious' apple slices were submitted to pulsed light in a wide range of fluence conditions. The bactericidal effect of pulsed light was initially studied on native microflora and inoculated bacteria (*Lactobacillus brevis* and *Listeria monocytogenes*). Samples were then stored at 6 °C for increasing times up to 7 days, and analysed for native microflora, colour, firmness, weight loss and sensory attributes. Temperature increase and structural modifications in sliced apple were also taken into account to hypothesise possible mechanisms of action of pulsed light.

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## 2. Materials and methods

### 2.1. Sample preparation

Raw apples (*Malus domestica* Borkh., cv. Golden Delicious) of similar size (240 ± 15 g) and stage of ripening (3 on a 1–5 starch index scale) were purchased at the local market and maintained at 6 °C until use. Apples were washed in potable water (0.3 mg $L^{-1}$ residual chlorine), wiped, cored and manually cut into 1 cm thick slices. Hygienic conditions were strictly preserved during handling operations. Apple slices were submitted to pulsed light treatments 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%). Samples were placed on a 0.5 cm 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 from 0 to 157.5 kJ/$m^2$. Increasing fluence was obtained by delivering to the sample increasing numbers of pulses. Pulse duration was 50 μs and the repetition rate was 0.5 Hz.

Differently processed apple slices were introduced in sterile Petri dishes and stored in darkness at 6 °C for up to 7 days. At increasing times during storage, samples were removed from the thermostated cell and submitted to analyses.

### 2.2. Microbiological analysis

Microbiological determinations were carried out as follows: 25 g of sample were diluted 1:5 (w/v) with Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK) and homogenised for 1 min at normal speed in a Stomacher (PBI International, Milano, Italy). Serial dilutions of each suspension were made in MRD (Oxoid) and analysed for microbial counts. Appropriate aliquots (0.1 mL or 1 mL) were spread on agar plates. Plate Count Agar (PCA, Oxoid) was used for enumeration of total mesophilic bacteria; plates were incubated for 48 h at 30 °C. Oxytracycline-Glucose-Yeast Extract (OGY) agar (Oxoid) for enumeration of yeasts and moulds; plates were incubated for 72 h at 28 °C. Violet Red Bile Glucose (VRBG, Oxoid) for enumeration of *Enterobacteriaceae*; plates were incubated for 24 h at 37 °C.

### 2.3. Bacterial strains and inoculation of apple slices

*Lactobacillus brevis* 20054 DSMZ and *Listeria monocytogenes* 20600 DSMZ were respectively used as spoilage and pathogenic strains for inoculation. The strains were maintained at −80 °C in Tryptone Soya Broth (TSB, Oxoid) with 30% glycerol added as a cryogenic agent. The original strains were kept in TSB at 37 °C for 24 h and subsequently were spread on Tryptone Soya Agar (TSA, Oxoid) and incubated under the same conditions. Fresh cultures were prepared by inoculating one colony from the pure culture within 10 mL of TSB and incubated at 37 °C for 24 h to give an initial inoculum of $10^8$–$10^9$ CFU/mL. Fresh-cut apple slices were inoculated with 100 μL of *L. brevis* or *L. monocytogenes* fresh cultures over the entire upper surface by spreading with a sterile micropipette to obtain an inoculum of about $10^5$–$10^6$ CFU/$cm^2$. Man Rogosa Sharpe (MRS) Agar (Oxoid) with 0.025% Delvocid (DSM, Heerlen, the Netherlands) was used for enumeration of *L. brevis*. Plates were incubated for 48 h at 30 °C. Palcam Agar (Oxoid) was used for enumeration of *L. monocytogenes* and plates were incubated for 24 h at 37 °C. Results were expressed as log CFU/$cm^2$.

### 2.4. Temperature

The temperature of apple slices during pulsed light treatment was monitored using a T-type thermocouple placed 2 mm under the surface of the fruit and connected to a portable data logger (mod. 502A1, Tersid, Milano, Italy).

### 2.5. Colour

Colour of the apple slices was analysed using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardised against a white tile before measurements. Colour was expressed in $L^*$ and $a^*$ Hunter scale parameters. Apple colour changes were measured by following the changes in $L^*$ and $a^*$ parameters.

### 2.6. Firmness

Firmness was measured by a puncture test using an Instron 4301 (Instron LTD, High Wycombe, UK). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, UK). On the test day, apple slices were punctured with a 1.5 mm cylindrical probe. Crosshead speed was set at 5 cm/min. Force–distance curves were obtained from the puncture tests and firmness was taken as the force (*N*) required to puncture the slicesby 0.5 cm.

### 2.7. Weight loss

Weight loss was determined by weighing the apple slices before and after the storage period. Weight loss was expressed as the percentage of weight loss respect to initial weight.

### 2.8. Histological preparation

For light microscopy, apple samples were fixed in 4% (v/v) formaldehyde buffered solution for 7 days. After fixation, samples were processed by an automatic histoprocessor (TISBE tissue processor, Diapath, Martinengo, Italy) to embed the tissue in paraffin (ParaplastPlus, Diapath, Martinengo, Italy). Serial 5 μm sections were cut to obtain specimens transverse to the fibre direction by a programmable microtome (Reichert-Jung 2050, Nussloch, Germany). For histological evaluation the paraffin sections were stained with toluidine blue (Sigma–Aldrich, Milano, Italy). The specimens were finally examined at 20× by light microscope (Leica DMRB, Leica Microsystems GmbH, Solms, Germany) and images acquired by a digital camera (Leica, ICC50, Solms, Germany) using a LAS-EZ (Leica, Solms, Germany) software.

### 2.9. Sensory analysis

A panel of 12 Italian assessors, equally distributed between males and females, of age between 25 and 48, was selected and trained. They all had a minimum of 2 years experience in discrimination and descriptive sensory analysis. A quantitative descriptive analysis method was used and the sensory lexicon was developed using the consensus method (Lawless and Heymann, 2010). A preliminary test was performed to identify the sensory changes most likely to appear as a result of pulsed light treatment. In particular, a treated sample (157.5 kJ/$m^2$) and a fresh one (untreated apple) were presented to the judges, who were asked to write down the descriptors which differentiated the samples. The samples were presented to the judges just after preparation. The assessors agreed