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Inactivation of hepatitis A virus and norovirus on berries by broad-spectrum pulsed light

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ABSTRACT

Foodborne diseases are still a major global health and economic burden, and are mainly caused by viral pathogens, such as human norovirus and hepatitis A virus, which may remain infective for long times on food contact surfaces and on produce. The strategies of viral inactivation applied in the industry are not generally suitable for delicate foods such as berries. Brief exposure to high-intensity white light (UV to IR) has been shown to inactivate many bacteria. The effectiveness of this treatment against foodborne viruses on fresh produce is largely unknown. We show that pulsed light treatment causes a moderate drop in the luminosity (L*, which ranges from bright (high) to dark (low)) of blueberries (to 36.31 ± 0.99 from 42.47 ± 1.17) and affects the luminosity of lettuce slightly but does not affect the appearance of strawberries, blackberries or raspberries. Hepatitis A virus and murine norovirus 1 are thus reduced by 2 log cycles. Viral inactivation on blackberries was less effective. These results will help food industries evaluate the suitability of pulsed light disinfecting technology for specific fruits and vegetables.

1. Introduction

Foodborne illness is currently estimated to affect 600 million persons per year, causing 420,000 deaths as well as costing 110 billion USD in lost productivity and medical expenses (World Health Organization, 2020). Although food hygiene standards are getting more and more rigorous, outbreaks of viral foodborne illness are increasing in frequency (David et al., 2007; Government of Canada, 2016; McIntyre et al., 2012; Smith et al., 2019; Swinkels et al., 2014). The two most frequent viral causal agents are human norovirus and hepatitis A virus (HAV) (Thomas et al., 2015; World Health Organization, 2015), both are known to persist for long times on food contact surfaces and to resist most common disinfectants (Bae et al., 2014; Cook et al., 2016, 2018; Sattar et al., 2000). Vegetables and fruits and especially berries have been involved in several incidents of norovirus or hepatitis A transmission (Enkirch et al., 2018; European Food Safety Authority, 2013, 2014; Scavia et al., 2017). Frequent exportation of these foods has created an urgent need for effective means of inactivating these viruses directly on the product during processing.

To limit the risk of viral contamination, food industries generally rely on good hygienic practices and conventional inactivation methods such as chemical disinfectants, heat treatment or UV radiation. However, these methods are often not suitable for delicate fruits and vegetables. One technology that appears to be effective without degrading product quality is pulsed light treatment, an inactivation method based on exposing the food product to bursts of high-intensity white light (wavelengths ranging from 200 to 1100 nm and hence including UV) lasting microseconds (Elmnasser et al., 2007). This method has been accepted by the US Food and Drug Administration (FDA) since 1996 (U. S. Food and Drug Administration, 1996) and strict regulations are in place limiting pulse duration (2000 μs max) and fluence or total energy absorbed per unit of product surface (12 J/cm^2).

Pulsed light has been tested on juice, syrup, milk (Rowan, 2019), water (Vimont et al., 2015), strawberries, blueberries, and raspberries (Huang and Chen, 2015; Huang et al., 2017) and other products. Studies of its effect on sensory properties are limited. Browning has been shown to occur on tomatoes (Valdivia-Nájar et al., 2018), in apple juice (Muñoz et al., 2012) and on mushrooms (Oms-Oliu et al., 2010), eggs (Manzocco et al., 2013) and ham (Wambura and Verghese, 2011). On the other hand, pulsed light has also been shown to slow down the ripening of tomatoes (Dhakal and Baek, 2014; Scotta et al., 2017). Its impact on the sensory properties of berries is largely unknown.

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The lethality of pulsed light for bacteria such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* and others has been shown (Elmnasser et al., 2007; Ghasemi et al., 2003; Rowan et al., 1999). Its effects on viruses have been studied much less (Huang and Chen, 2015; Huang et al., 2017; Huffman et al., 2000; Jean et al., 2011; Lamont et al., 2007; Pexara and Govaris, 2020; Roberts and Hope, 2003), especially in foods. We and others have shown previously that it can inactivate human norovirus surrogates, such as the Tulane virus (Huang and Chen, 2015; Huang et al., 2017) and murine norovirus 1 (MNV-1) in drinking water or sewage (Vimont et al., 2015). We have found barely any data on the use of pulsed light to inactivate viral pathogens directly on foods. The aim of this study was to investigate the impact of pulsed light on food sensory properties such as texture, color, and weight and to measure the extent to which it can inactivate a norovirus and HAV on produce, namely strawberry, raspberry, blueberry, blackberry, and lettuce.

2. Materials and methods

2.1. Pulsed light equipment and parameters

A benchtop pulsed light system (Xenon, Corp. Wilmington, MA, model X-1100 LH-810/910) was used to apply all treatments. This model emits a spectrum between 200 nm and 1000 nm. The parameters were set as follows: 1 pulse lasting 546 μs was emitted at an intensity of 830 J and 2700 V to produce a fluence of 0.72 J/cm^2. Up to 16 pulses were emitted, all at intervals of 1186 ms. The most intense treatment was thus within FDA-approved limits (2000 μs pulse duration and total fluence of 12 J/cm^2). The distance between the lamp and the fruit or vegetable surface was 7.5 cm. The effect of heat generation by the lamp was minimized by placing an icepack inside the device for approximately 1 min in between treatment.

2.2. Berries and vegetables

All berries (strawberries, blueberries, raspberries, blackberries) and lettuce were purchased as fresh as possible on the same day at a local supermarket and stored at 4 °C until the experiment. All samples were withdrawn randomly from their original container. Outer (dark green) and inner (light green) leaves of lettuce were treated separately.

2.3. Effect of pulsed light on sensory properties of the tested products

The foods were treated at fluences of 2.15 J/cm^2, 6.44 J/cm^2, and 11.45 J/cm^2. One set of experiment consisted of 10 blueberries, 5 strawberry halves (symmetrical), 5 lettuce leaf squares of 2.5 cm^2, 5 raspberries or 5 blackberries were placed in the sample holder. Two sets of experiments were performed. Food characteristics were tested at room temperature. Color and/or mass were always measured before texture. Representative pictures were also taken from both sets of experiments.

2.3.1. Color analysis

Product color was measured on a CR-300 colorimeter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA). The color parameters were luminosity or L*, which ranged from bright (high) to dark (low), *a* (redness versus greenness) and *b* (yellowness versus blueness). Three measurements were taken per product, each at a different location on the sample. Blueberry color was measured in a closed chamber usually used for sugar and flour.

2.3.2. Weight loss analysis

All samples were weighed before and after treatment to measure the impact of a possible thermal component of pulsed light on product water content.

2.3.3. Texture evaluation

A TA-XT2 texture analysis instrument (Stable Micro Systems, Godalming, Surrey, UK) was used with a Kramer shear cell. The basic cell was used for lettuce, raspberries, and blackberries, whereas the heavy-duty plate was used for blueberries and strawberries. Prior to any measurements, the loading cell and the force of the head part were calibrated, and the height of the cell was adjusted accordingly.

2.3.3.1. Strawberries. The loading cell was calibrated with a 5 kg weight. Strawberries were cut in half symmetrically. Five halves were tested on the heavy-duty plate. A 1/4-inch aluminum sphere was positioned above the shoulder side of the strawberry. A compression test was then performed at a speed of 5 mm/s for up to 6 mm of penetration into the fruit. This protocol, adapted from a previous study (Ordidge et al., 2012), gives the maximum force that the strawberry can withstand without tearing of the tissue.

2.3.3.2. Blueberries. The loading cell was calibrated with a 5 kg weight. Ten blueberries were tested. The fruit was placed in an Eppendorf cap (used as a holder) on the heavy-duty plate. The maximum compression force test was performed with a 2 mm cylinder head at a speed of 0.8 mm/s for penetration of up to 7 mm into the berry as described previously (Stückrath et al., 2008).

2.3.3.3. Lettuce. The loading cell was calibrated with a 50 kg weight. Lettuce leaves were dried, cut into five 2.5 cm^2 squares and laid out flat in the holder. The Kramer shear cell was positioned over the lettuce at a height of 25 mm and lowered 20 mm at a speed of 1.5 mm/s. This protocol gave the maximal shear force withstood by the leaf, based on a previous study (Rico et al., 2006).

2.3.3.4. Raspberries and blackberries. The loading cell was calibrated with a 50 kg weight. Five raspberries or blackberries were used. The Kramer shear cell was positioned over the berries at a height 25 mm and passed through them (30 mm stroke) at a speed of 5 mm/s, giving the maximum shear force withstood by the berries, based on previous studies (Giongo et al., 2019; Sousa et al., 2007).

2.4. Inactivation of HAV and MNV-1 using pulsed light

2.4.1. Sample preparations

HAV inactivation tests were performed with strawberries, raspberries, and blackberries. All products were tested in triplicate. Testing of 9 strawberries (18 halves), 18 raspberries and 18 blackberries constituted one experiment. Three experiments were performed on separate days. Prior to any experiment, all berries were washed 3 times with distilled water and once with deionized water. With a sterilized scalpel, strawberries were cut in half, the bottom part (sepal side) of blackberries was removed to increase Petri adherence, and raspberries were left intact. Samples were then placed in sterile Petri dishes (cut side on the dish surface) 3 per dish (considered to be one sample once pooled together after viral recovery), 6 dishes per fruit, and were treated with UV light for 15 min under sterilized laminar flow hood to inactivate potential contaminating microorganisms.

2.4.2. Sample treatments

The treated samples were then exposed to 16 pulses for a total fluence of 11.45 J/cm^2. The same protocol was used for experiments with MNV-1 on the same food matrices at the same viral load as for HAV (30 μL of suspension containing 10^5 pfu/mL). Cytopathogenic HAV strain HM-175, was obtained from the Bureau of Microbial Hazards, Health Canada, Ottawa, ON, and propagated as previously described (Mbithi et al., 1992). MNV-1 (ATCC VR-1937; https://www.atcc.org/products/vr-1937) was purchased from the ATCC (Cedarlane, Burlington, ON, Canada) and propagated as previously described (Gonzalez-Hernandez

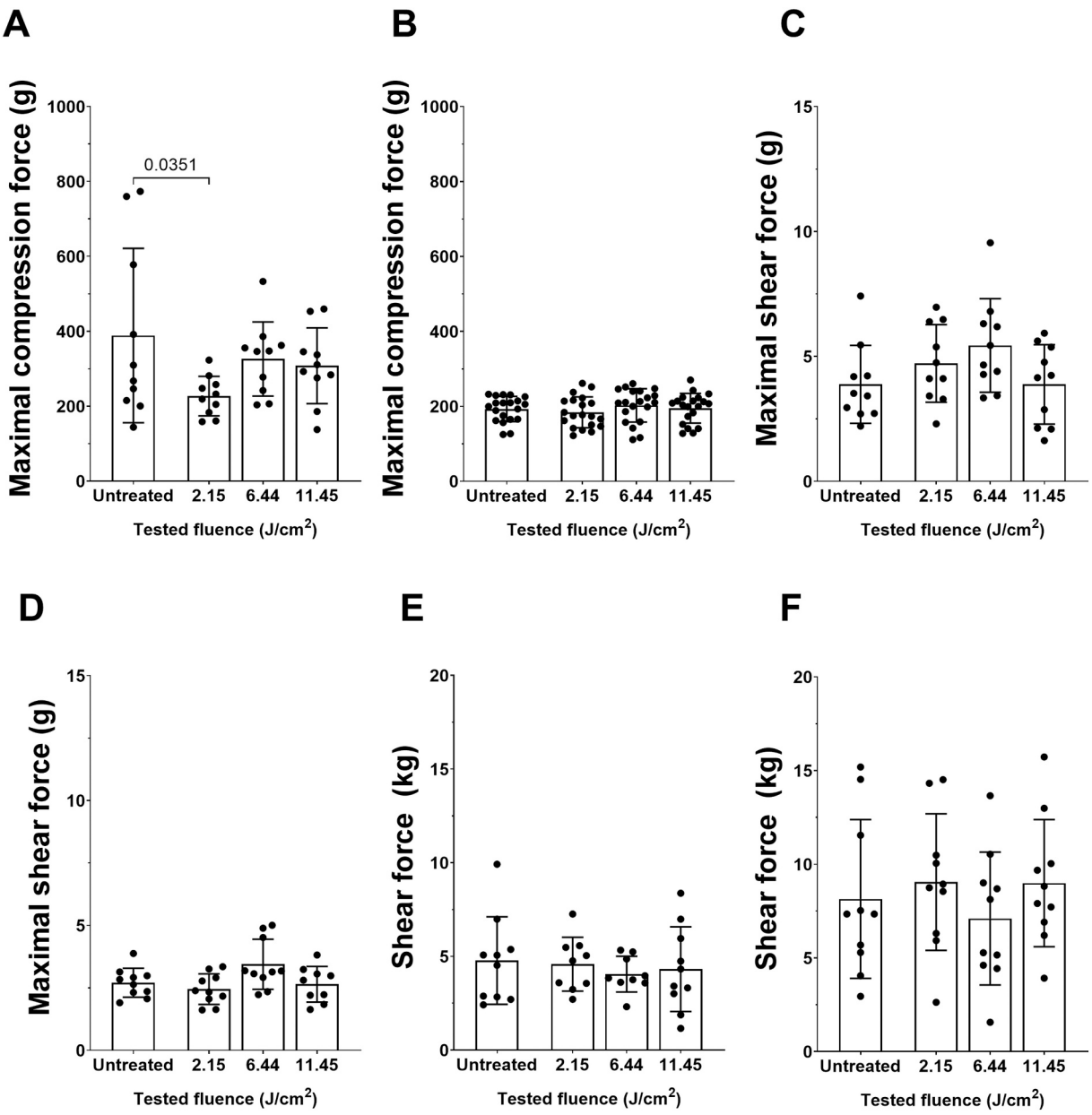

Fig. 1. Impact of pulsed-light disinfecting treatment on the texture of berries and lettuce. Maximal compression force under different tested fluences withstood by (A) strawberry halves ($n = 10$) and (B) blueberries ($n = 20$) were measured using a Kramer shear cell with the heavy-duty plate. Maximal shear force under different tested fluences withstood by (C) lettuce outer leaf ($n = 10$), (D) lettuce inner leaf (n = 10), (E) raspberries (n = 10) and (F) blackberries (n = 10) were measured using a Kramer shear cell. Values are mean ± sd.

et al., 2012). The contamination process is as follows: 30 μL of suspension containing 10^5 pfu/mL was used to artificially contaminate each piece of fruits (3 per dish, total of 90 μL per dish, which equal one sample). Briefly, viruses were pipetted on the top shoulder of strawberries, on the tip of blackberries and on the tip of raspberries. All samples were allowed to dry for about 90 min under the flow hood before pulsed light treatment.

2.4.3. Controls

A contaminated Petri dish of fruit (1 per produce) with 30 μL of HAV suspension (10^5 pfu/mL) but not treated with pulsed light served as a positive processing control. The positive processing control was used to establish the maximal recovery of viruses per sample. An uncontaminated (30 μL of EBSS buffer) Petri dish of fruit (1 per produce) but treated with pulsed light served as a negative processing control. The negative processing control was used to 1) assess the potential impact of juice flow on our cell lines during plaque assays and to 2) evaluate if we contaminated our sample during pulsed light treatments. An extra uncontaminated and untreated fruit dish (1 per produce) was left under the flow hood and served as a negative contamination control. All samples were also allowed to dry for about 90 min under the flow hood before pulsed light treatment.

2.4.4. Viral recovery after pulsed light treatment

HAV and MNV-1 were recovered from the berries using Earle's Balanced Salts Solution 1× pH 7–7.4 (Fisher Scientific, Ottawa, ON, Canada) as an elution buffer (Ansari et al., 1988), added to each Petri dish in 5 portions of 60 μL for a total volume of 300 μL. Since we had 3 fruits per dish, this totaled 900 μL of elution buffer per type of berry and constituted one sample. The recovered buffer was diluted serially to 10^{-4} with PBS 1× (Corning, Tewksbury, MA, USA) for plaque assay. Positive and negative processing controls and the extra negative control

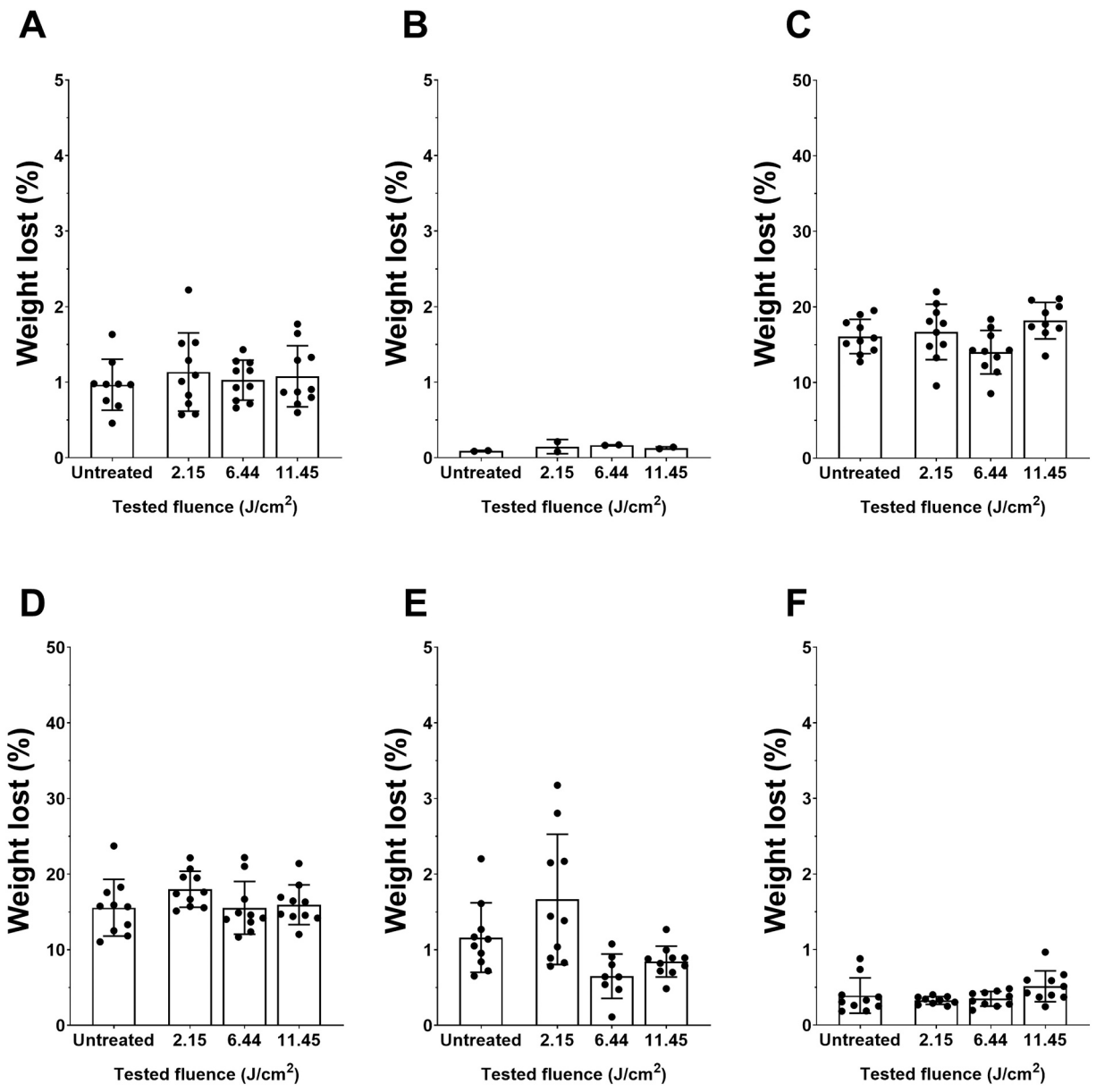

Fig. 2. Impact of pulsed-light disinfecting treatment on the weight of berries and lettuce. Percentage weight loss under different tested fluences of (A) strawberry halves ($n = 10$), (B) blueberries ($n = 20$), (C) lettuce outer leaf (n = 10), (D) lettuce inner leaf (n = 10), (E) raspberries (n = 10), and (F) blackberries (n = 10). Values are mean ± sd.

were also recovered with EBSS buffer using the same method described for HAV and MNV-1 samples.

2.4.5. HAV propagation and plaque assay

FRhK-4 cells (HAV strain HM host cells, Bureau of Microbial Hazards, Health Canada, Ottawa, ON) were cultured in Eagle's minimal essential medium (EMEM, Wisent, St-Bruno, QC, Canada) with 10% fetal bovine serum (FBS, Wisent, Canada) and 1% penicillin/streptomycin according to a protocol published previously (Mbithi et al., 1992) with slight modification. Cells were brought to confluence in a T75 flask, counted, diluted, and plated in 12-well plates (Corning CellBind, Tewksbury, MA, USA) at 125,000 cells/well then grown for 24 h at 37 °C with 5% CO_2 to confluence. They were infected with 300 µL of diluted viral elution and incubated for 90 min at 37 °C with 5% CO_2 in accordance with previously published protocol (Trudel-Ferland et al., 2021; Mbithi et al., 1992). Briefly, each well was then covered with an overlay mix and 1% agarose and the plate was incubated for 8 days at 37 °C with 5% CO^2. A negative control (PBS only) and a positive control (HAV stock) were run. All samples were tested in duplicate.

2.4.6. MNV-1 propagation and plaque assay

RAW 264.7 cells (ATCC TIB-71) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Wisent, Canada) with 10% fetal bovine serum (FBS, Wisent, Canada) and 1% penicillin/streptomycin. Cells were brought to confluence in a T75 flask, then counted, diluted, plated in 12-well plates (Corning CellBind, USA) at 850,000 per well, grown for 24 h at 37 °C with 5% CO_2 to confluence as described previously (Vimont et al., 2015; Gonzalez-Hernandez et al., 2012). They were infected with 300 µL of sample suspension (dilution), incubated for 90 min at 37 °C with 5% CO_2, overlaid with 0.8% low-melting-temperature agarose (Sea Plaque, VWR, Mississauga, ON, Canada) and incubated for about 72 h as described previously (Vimont et al., 2015; Gonzalez-Hernandez et al., 2012). Negative (PBS only) and positive (MNV-1 stock) controls were run. All samples were tested in duplicate.

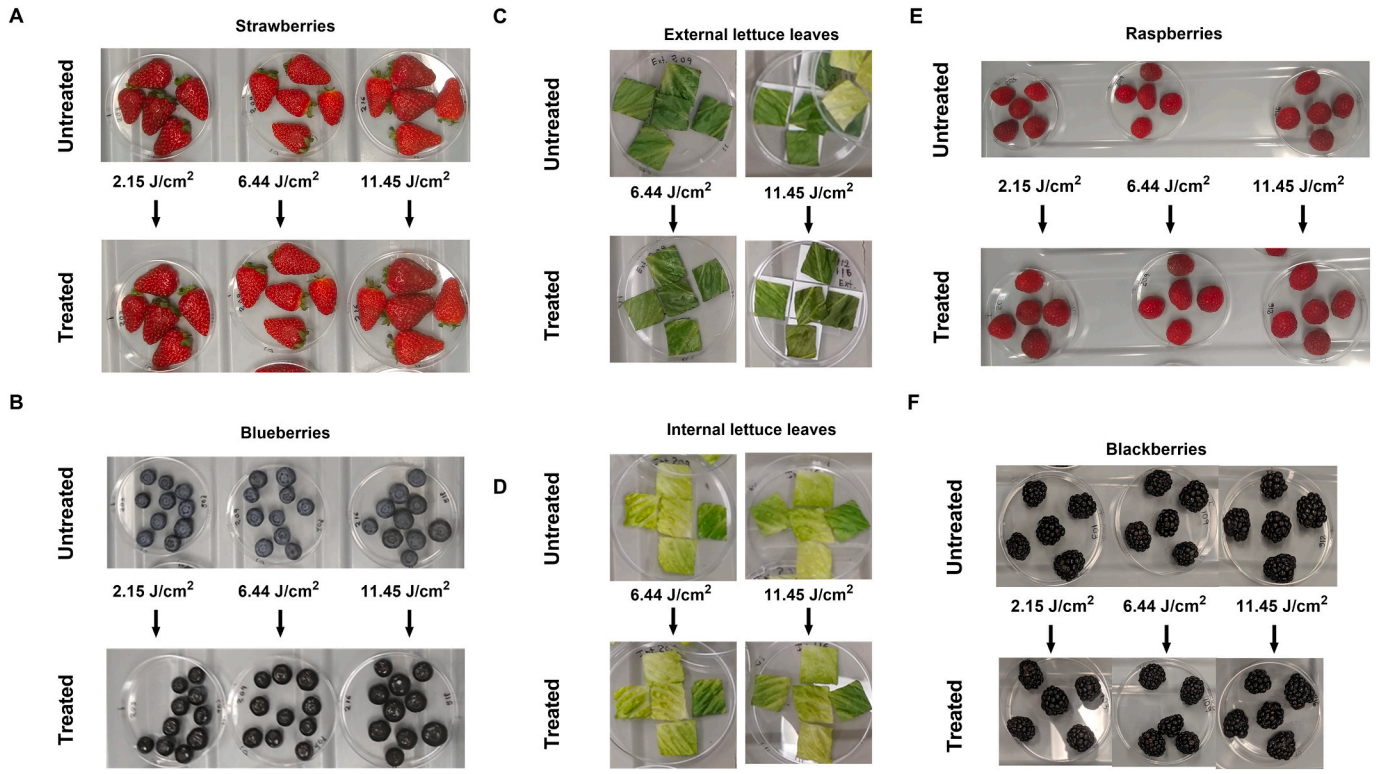

Fig. 3. Impact of pulsed-light disinfecting treatment on the overall appearance of berries and lettuce. Visual aspect under different tested fluences of (A) strawberry halves ($n = 5$), (B) blueberries ($n = 10$), (C) lettuce outer leaf (n = 5), (D) lettuce inner leaf (n = 5), (E) raspberries (n = 5), and (F) blackberries (n = 5).

2.4.7. Fixation and staining of cells and calculation of viral titer

Infected cells were fixed with 3.7% formaldehyde in 0.85% saline for at least 5 h then stained with 0.1% crystal violet (Sigma, Oakville, ON, Canada) for 30 min to assay for HAV (Vimont et al., 2015) or with 1% crystal violet for 10 min to assay for MNV-1 (Gonzalez-Hernandez et al., 2012). Plaques were counted, and wells with 3 to 30 were used to calculate viral titer in pfu per mL as 100 × the number / the plated volume (0.3 mL). The reduction in titer due to the treatment was calculated as $\log_{10}$ (C/S) where C is the titer of the positive control and S is the titer in the diluted sample suspension.

2.5. Statistical analysis

All statistical analysis was performed using GraphPad Prism 9 (V9.1.0, GraphPad Software, San Diego, CA, USA). Multiple comparisons were performed with ANOVA followed by a Dunnett post-test (compared to untreated samples) for texture, weight loss and luminosity.

3. Results

3.1. Impact of pulsed light on the texture of berries and lettuce

Pulsed light had very little effect on the maximal compression force that strawberries and blueberries could withstand (Fig. 1A and B) or the maximal shear force in the case of lettuce (Fig. 1C and D) and raspberries and blackberries, regardless of fluence. There was a slight but significant difference ($p = 0.0351$) in texture between untreated and treated strawberries, namely a softening at 2.15 J/cm^2. Why this was not also the case at higher fluence is unclear.

3.2. Impact of pulsed light on berry and lettuce leaf weight

Weight loss percentage of berries and lettuce was analyzed pre- and post-exposure to different fluences. Our results show that there are no significant weight loss differences between PL-treated and PL-untreated berry samples (Fig. 2). However, a general weight loss of lettuce has been noted independently of treatments.

3.3. Visual and color impact of pulsed light on berries and lettuce

Pulsed light caused no apparent change in the appearance of strawberries, raspberries, or blackberries (Fig. 3A, E, and F). However, the treatment did darken the apical surface of blueberries (Fig. 3B). Some browning of lettuce was also noted, on both inner and outer leaves (Fig. 3C and D).

As shown in Fig. 4, pulsed light treatment did not affect the luminosity of strawberries, raspberries, or blackberries (Fig. 4A, E, F) but did in the blueberry and lettuce cases (Fig. 4B, C and D). Blueberry luminosity was reduced significantly ($p \leq 0.05$) at all three fluences, to 36.97 ± 0.67, 36.53 ± 0.77, 35.42 ± 1.52 from 42.47 ± 1.17. Lettuce suffered a smaller but significant loss when exposed to 6.44 J/cm^2 or 11.45 J/cm^2. Blackberries were found significantly brighter after treatment at 11.45 J/cm^2, but this is not likely perceptible by the consumer. Based on these observations, blueberry and lettuce were not included in the viral inactivation experiments.

3.4. Inactivation of foodborne viruses on berries by pulsed light

Using the lamp at a fluence of 11.78 J/cm^2 and 7.5 cm from strawberries and raspberries, pulsed light treatment reduced HAV titers by respectively 2.10 ± 0.08 and 1.97 ± 0.16 log cycles and MNV-1 titers by 1.61 ± 0.38 and 1.89 ± 0.30 (Fig. 5A & B). Reductions obtained on blackberries were smaller (1.25 ± 0.57 and 1.37 ± 0.62).

4. Discussion

Pulsed light has been tested as a disinfecting agent on several food

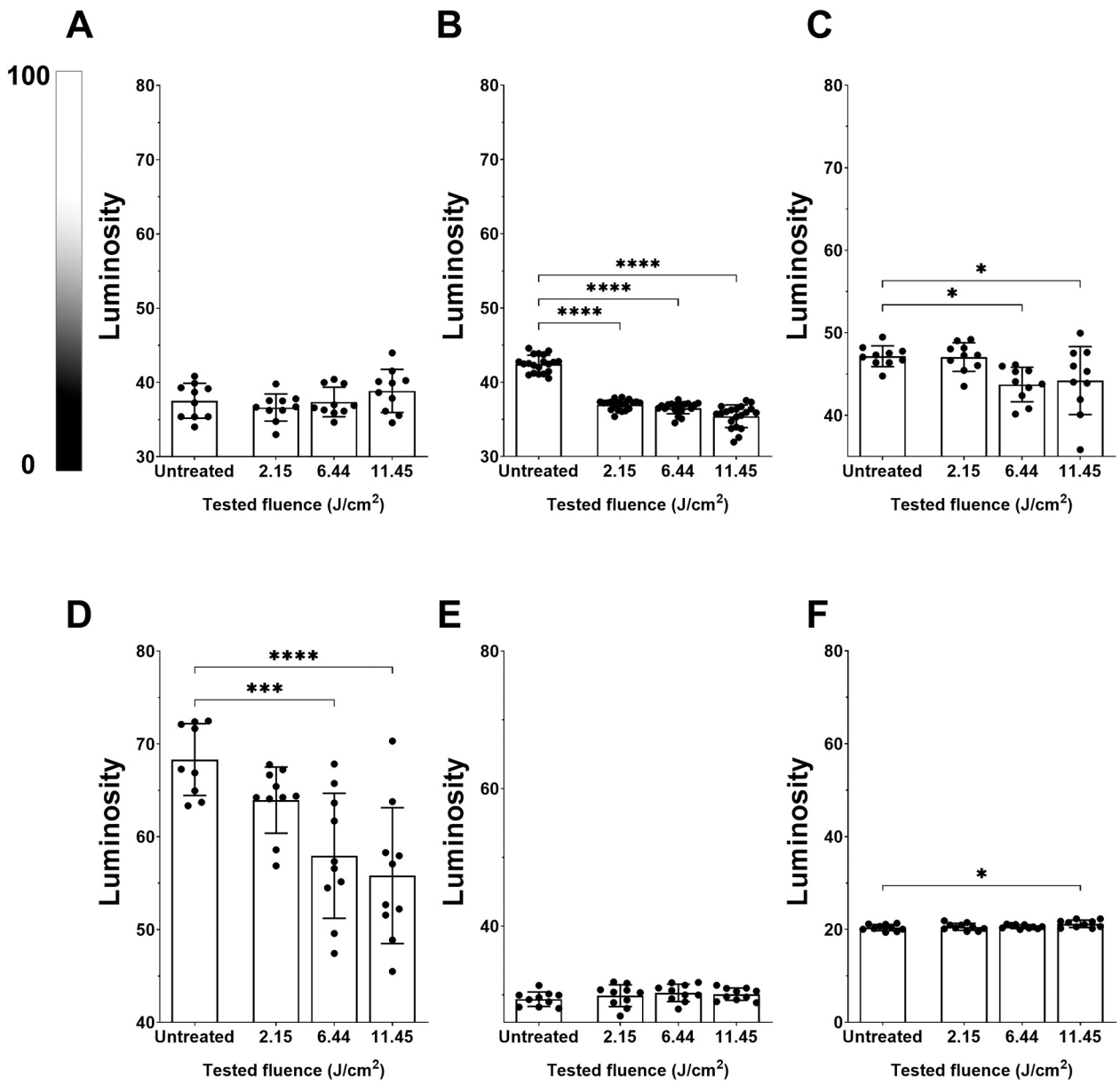

Fig. 4. Impact of pulsed-light disinfecting treatment on the luminosity of berries and lettuce. Luminosity values under different tested fluences of (A) strawberry halves ($n = 10$), (B) blueberries ($n = 20$), (C) lettuce outer leaf (n = 10), (D) lettuce inner leaf (n = 10), (E) raspberries (n = 10), and (F) blackberries (n = 10). Luminosity (measured using a Konica model CR-300) ranges from 100 (white) to 0 (black). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$. Values are mean ± sd.

matrices (Huang and Chen, 2015; Rowan, 2019). Its impact on sensory properties such as texture, color, taste, weight loss has been found negative or positive from one study to another (Dhakal and Baek, 2014; Muñoz et al., 2012; Scotta et al., 2017; Valdivia-Nájar et al., 2018). It can alter the texture of fresh produce such as mushrooms (Ramos-Villarroel et al., 2012) and avocado (Ramos-Villarroel et al., 2013). At the fluences tested in our study, it appears not to affect the texture of berries and lettuce very much. This suggests that consumers would not likely distinguish between treated and untreated berries. In some cases, a texture stabilizer could be used to mask such changes (Bhavya and Umesh Hebbar, 2017). However, for fresh strawberries, differences in the distance from the lamp due to fruit size variability could cause product visual quality inconsistencies. This might be less of a problem with berries that are to be frozen, since they could be sorted by size, and freezing changes their appearance considerably in any event. The virucidal effectiveness of pulsed light on frozen produce remains to be investigated.

The physics of pulsed light includes a small thermal component (Bialka and Demirci, 2008; Huang and Chen, 2015; Luksiene et al., 2013), which could decrease the water content of the food produce surface and hence the weight of berries and lettuce. In a study of fresh sliced tomatoes, the weight loss over time was greater in the pulsed-light-treated product (Valdivia-Nájar et al., 2018). The thermal component of the treatment certainly increases with proximity to the lamp and with exposure time and fluence (Bialka and Demirci, 2008). However, most studies that show significant heat release from pulsed light lamps involved fluences that exceeded FDA recommendations. Based on previous findings by our group (Vimont et al., 2015), we waited 1 min between exposures to limit the possible photothermal effect of the treatment. Under these conditions, weight losses of 0.5–1% reported previously for pulsed-light-treated blueberries (Cao et al., 2017) did not occur. Storage conditions, berry seasonal variations, lamp

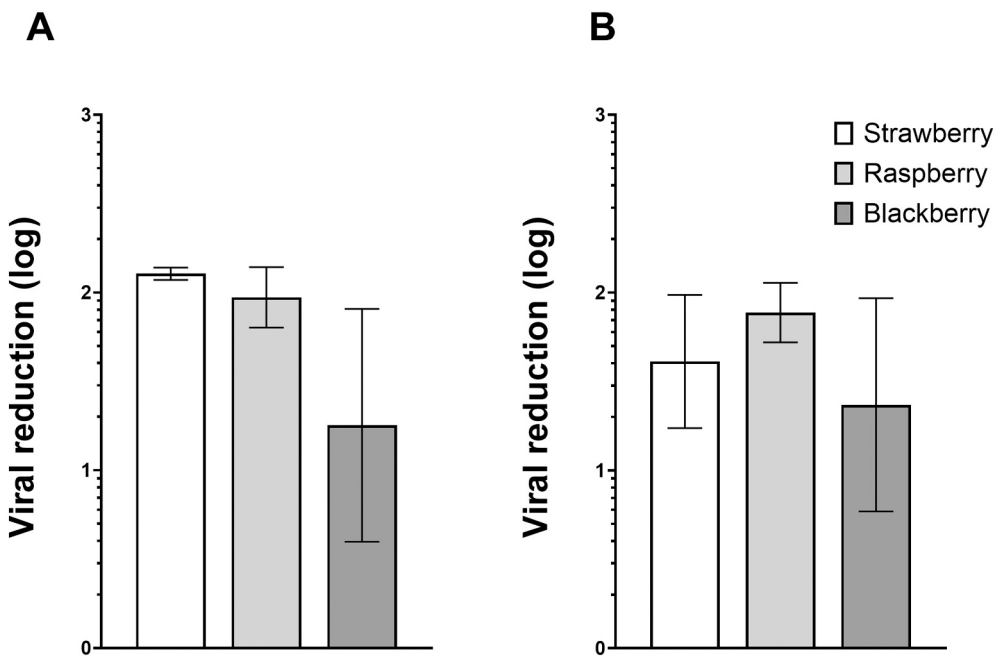

Fig. 5. Impact of pulsed-light disinfecting treatment at a fluence of 11.45 J/cm^2 on the infectivity of HAV and MNV-1 adherent to strawberries, raspberries and blackberries. Logarithmic viral reduction of (A) HAV (n = 3), (B) MNV-1 (n = 3) on strawberry halves (white), on raspberries (light grey), and blackberries (dark grey). Values are mean ± sd. Experiments were performed in triplicate.

distance and equipment configuration could explain these contrasting results. We nevertheless conclude that our photo-disinfection treatment did not have any impact on the weight of the berries and lettuce tested in our study.

Pulsed light has been shown previously to affect the color of food matrices by changing the luminosity and/or the browning index (Charles et al., 2013; Fine and Gervais, 2004; Gómez et al., 2011; Ignat et al., 2014; Muñoz et al., 2012). However, it has also been reported not to affect the browning index and pH of juices (Muñoz et al., 2012). Our results on strawberries and raspberries are consistent with the very few studies of the impact of pulsed light on the color of these berries (Bialka and Demirci, 2008; Huang and Chen, 2015; Luksiene et al., 2013). A decrease of ~1 unit in the L value of blueberries has been noted at least once (Cao et al., 2017), which is again consistent with our results, although our decreases are greater, due likely to differences in experimental conditions.

The few studies of the use of pulsed light to inactivate viruses have focused mainly on suspensions in buffer (Lamont et al., 2007; Roberts and Hope, 2003) or food contact surfaces (Jean et al., 2011) and usually with fluences exceeding 12 J/cm^2. Our group confirmed years ago an earlier study showing that one factor needing to be taken into consideration is the potential presence of organic matter associated with berries, which reduces treatment efficacy in suspensions and on food contact surfaces (Jean et al., 2011; Roberts and Hope, 2003). More than routine washing of berries therefore may be necessary to obtain effective inactivation of viruses by pulsed light.

In the present study, inactivation of HAV and MNV-1 was inconsistent on blackberries in comparison with strawberries and raspberries. This may be due to surface characteristics. Blackberry drupelets are loose and large compared to those of raspberries (Caballero et al., 2016) whereas strawberries have a continuous non-drupelet structure. This made blackberries not only more difficult to cover with the viral suspension, but also more apt to provide adherent virus with spaces not exposed directly to the lamp. Food matrices having structures that provide such shading therefore might be poor candidates for disinfection by pulsed light. Differences in berry surface hydrophobicity could add variability to the results obtained using diluted aqueous suspensions of test virus. Finally, additional elution buffer could be evaluated as it may have impacted viral recovery (e.g. TGBE pH 9; Dubois et al., 2002).

Our results suggest that pulsed light could be used to inactivate foodborne viruses on some (strawberry and raspberry) but not all berries. We have suggested previously that the inactivating mechanism of pulsed light on viruses acts by disrupting the capsid structure and degrading viral proteins and RNA (Vimont et al., 2015). In its current state, pulsed light technology does not appear to be difficult to implement on conventional berry conveyors. Although the inactivation obtained in the present study (~2 log) might be considered modest, it could be combined with routine inactivation methods to reduce substantially the overall microbiological risk associated with delicate berries. In the case of fresh strawberries and raspberries, this would be achieved with no negative impact on texture, color, overall appearance, or product weight. At least these foods could be taken off the list of frequent contributors to foodborne disease and the associated worldwide economic burden and healthcare costs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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