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Microbiological and physical properties of pennywort (*Centella asiatica*) leaves using pulsed light technology

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Abstract

Pennywort (*Centella asiatica*) is a herbaceous vegetable commonly consumed raw as 'ulam' or salad. Consumption of raw leafy green vegetables is one of the pathogenic mechanisms that could cause foodborne outbreaks. The aim of the present work was therefore to investigate the effect of pulsed light (PL) treatment at fluences of 1.5, 4.2, 6.9, 9.6, and 12.3 J/cm² on the microbiological and physical quality of pennywort stored at 4 ± 1°C. *Escherichia coli* (*E. coli*) were inoculated onto the pennywort leaves before being exposed to PL and viewed using scanning electron microscopy (SEM). PL fluences of 6.9, 9.6, and 12.3 J/cm² significantly reduced the microbial count; however, the highest inactivation was obtained by using fluences of 9.6 and 12.3 J/cm². The color of pennywort was not significantly affected by PL treatment applied at lower fluences of 1.5, 4.2, and 6.9 J/cm²; however, at higher fluence, 9.6 and 12.3 J/cm², the color was affected. PL at 1.5, 4.2, 6.9, and 9.6 J/cm² was able to retain the texture appearance of the leaves. To conclude, PL at 6.9 J/cm² showed the best fluence to reduce total aerobic mesophilic count while retaining the physical properties of pennywort leaves and extend the shelf life to about four days. The inactivation of *E. coli* population was significantly higher at PL fluence of 6.9 J/cm². It was observed that PL caused the destruction to the surface of *E. coli's* cell membrane. The reductions of samples inoculated with *E. coli* were better than those achieved in native microbiota. Furthermore, the present work also demonstrated that PL treatment was able to reduce the microbial count on pennywort leaves.

Introduction

The consumption rate of raw fresh produce such as salad, vegetable, and fruit among the public is increasing. The consumers' interests in high-quality fresh fruits and vegetables expand rapidly thus causing the market to offer highly nutritious, convenient, and fresh-like quality foods (Romero *et al.,* 2017). Pennywort (*Centella asiatica*), which belongs to family Apiaceae or Umbelliferae, is one of Asian herbs that possess various therapeutic effects such as promoting corneal epithelium wound healing (Ruszymah *et al.,* 2012). It has also been reported to possess other pharmacological effects in treating inflammation, asthma, tuberculosis, leprosy, psoriasis, keloid, and gastric ulcer (Zheng and Qin, 2007). Pharmacological evaluations of pennywort in different experimental models have demonstrated the antioxidant effects (Veerendra Kumar and Gupta, 2003) as well as ameliorating effects on learning and memory deficits (*Rao et al.,* 2005).

Minimally processed salads are subjected to several preliminary operations including washing, sorting, cutting, and peeling or slicing (Karagözlü *et al.,* 2011). Lettuce and salad (all varieties), leafy green vegetables (LGVs) such as spinach, cabbage, raw watercress, and fresh herbs are accorded the highest priority regarding fresh produce safety from a global perspective (FAO/WHO, 2008). In Malaysia, pennywort is usually available and sold in the wet markets and is seldom available in the supermarket. It is sold in bunches as plants with roots or lose leaves, or cut and packed in bags. It is usually consumed raw as '*ulam*'. Several findings have indicated the prevalence of *E. coli* O157:H7 (Chang *et al.,* 2013), Listeria monocytogenes (Ponniah *et al.,* 2010), *Campylobacter* sp. (Chai *et al.,* 2007), and *Salmonella* serovars (Awang Salleh *et al.,* 2003) that have potential hazards associated with the consumption of '*ulam*' or raw vegetables. There are also

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reports on contamination cases associated with celery (Vandamm *et al.,* 2013), lettuce (Delaquis *et al.,* 2007) and cabbage (Wachtel *et al.,* 2002).

A non-thermal food preservation technique, namely pulsed light (PL), may be suitable to reduce the microbial population in fresh-cut vegetables. PL is used for rapid inactivation of microorganisms on food surface, and involves the use of intense and short-duration pulses of broad-spectrum light (Elmnasser *et al.,* 2007; Gómez-López *et al.,* 2007; Oms-Oliu *et al.,* 2010a). The mode of action of PL is attributed to the unique effects of high peak power and a broad spectrum of light. The inactivation may occur through several chemical mechanisms including the cleavage of DNA, protein denaturation, and cellular material alterations. The presence of natural openings such as stomata and cracks or crevices provides the potential attachment areas for pathogens and microorganisms (Takeuchi *et al.,* 2001). The germicidal effects of PL appear to be due to both photochemical and photothermal effects (Elmnasser *et al.,* 2007). The intense PL treatments were effective in reducing the naturally-occurring microbial loads on spinach around log 0.4 – 2.2 CFU/g, depending on the fluence applied (Agüero *et al.,* 2016). Moreover, PL treatment was reported to protect and preserve the condition of a product without causing detrimental effects on the overall quality and sensory characteristics (Koutchma *et al.,* 2009). Pennywort is usually eaten raw and is highly perishable. It has short storage time and can be the transmitting agent for several pathogens. In some cases, it is intensively linked to foodborne outbreaks. Therefore, the present work was aimed to investigate the effect of PL treatment at different fluences on the microbiological and physical properties of pennywort. The inactivation of artificially inoculated *E. coli* and the morphological changes in the plant cell following PL treatment were also observed.

Materials and methods

Plant material and preparation of pennywort samples

Fresh *pennywort* at commercial maturity (three to four months) were purchased from a commercial farm in Paya Rumput, Melaka. Bruised and yellow leaves were cut using a sterilized stainless-steel knife and discarded. The remaining intact green leaves were washed for approximately 2 min under running tap water to remove soil and dirt (Faour-Klingbeil *et al.,* 2016). The pennywort leaves were plucked off the stems. Those stems were removed but the petioles (5 cm from the top) were used. The plucked leaves and petioles were air-dried at room temperature (25 ± 2°C) for 20 min, packed into polypropylene bags, and sealed prior to PL treatment.

Apparatus

PL treatment was carried out using a XeMatic-2 L-A system (SteriBeam Systems GmbH, Baden-Württemberg, Germany), It consisted of two lamps, one above and another below a quartz table that was placed at the center to hold samples with a maximum energy emission of 350 J. The distance between the light and sample shelf was 10 cm. The emitted spectrum wavelengths ranged from 180 to 1,100 nm. The total fluence per pulse emitted was 0.3 J/cm².

Pulsed light (PL) treatment
Effect of PL fluences

The samples were exposed to PL for 1, 3, 5, 7, and 9 min at varying number of pulses (5, 14, 23, 32, and 41), respectively. The fluence was calculated using Eq. 1:

$$\text{Fluence } (\text{J/cm}^2) = \text{number of pulses} \times \text{total fluence per pulse} \quad \text{(Eq. 1)}$$

where, total fluence per pulse = 0.3 J/cm².

Storage study

The effect of storage stability using different PL fluences (1.5, 4.2, 6.9, 9.6, and 12.3 J/cm²) on microbiological and physical properties of pennywort was investigated. Samples were randomly withdrawn for analysis every four days up to 16 days of storage at 4 ± 1°C. Microbiological, color, texture, and water activity analyses were conducted. Results were compared with a set of untreated samples (control) stored under the same conditions as those of treated samples.

Methods of analysis
Study on typical microbiota

Microbiological analysis of pennywort leaves was performed by determining the total aerobic mesophilic plate count and yeast and mold count based on the standard methods prepared by Fan and Song (2008). To enumerate the microorganism, a ratio of 1:10 (sample:peptone water, w/v) was used and mixed with 0.1% sterile buffered peptone water. The sample was homogenized using a Stomacher lab blender (Interscience, Saint-Germain-en-Laye, France) under sterile conditions for 2 min in a stomacher bag (BagMixer 400). The aliquot was used for

various serial dilutions, spread-plated onto plate count agar (PCA, Merck®, Germany), and incubated (24 - 48 h) at 35 ± 2°C for total aerobic mesophilic bacteria count. Potato dextrose agar (Merck, Darmstadt, Germany) was used to determine the yeast and mold count with incubation at 25 ± 1°C for 5 d. The results were expressed as log colony-forming units per gram (log CFU/g).

Study on the inoculation of E. coli on pennywort leaves

Bacterial strains, culture conditions, and preparation of inoculum

The bacterial strain used in this experiment was *E. coli* which was obtained from Laboratory of Food Safety and Quality 2, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM). The *E. coli* was isolated from ready-to-eat food and identified using IMViC (indole-methyl red-Voges-Proskauer-citrate) test and Most Probable Number coupled Polymerase Chain Reaction (MPN-PCR) method according to Loo *et al.* (2013). The preparation of inoculum was based on the method described by Shynkaryk *et al.* (2016) with some modifications. Briefly, a loop of slant culture of *E. coli* (at 4°C) was inoculated into LB broth (Difco, Becton-Dickinson, Sparks, MD) and incubated overnight at 37°C. The bacterial cells were harvested by centrifugation at 4,200 *g* (15 min) for three consecutive times, and the cell pellets were suspended in 0.1% (w/v) sterile peptone water (Difco, Becton-Dickinson, Sparks, MD). The bacterial cell concentration was adjusted by spectrophotometric analysis (Thermo Spectronic Genesys 5 Spectrophotometer, Thermo Fisher Scientific, Waltham, MA) to log 8 CFU/mL (absorbance reading of 0.5 at 600 nm), which was then confirmed by plating.

Inoculation of pennywort leaves and enumeration of microorganisms

All pennywort samples were UV-treated (254 nm) on both sides in a biosafety cabinet for 10 min to reduce the impact of microbial populations before each analysis (Huang and Chen, 2014). The UV treatment did not influence the structure of the leaves significantly in which the firmness of the leaves after UV treatment was similar to the one without UV treatment, which ranged 301 - 368 N. Also, the UV treatment reduced native microorganisms from log 6.41 CFU/g to log 5.35 - 6.38 CFU/g, respectively for PL treated at 12.3 - 1.5 J/cm². 5 g of samples were inoculated with 500 µL of *E. coli* culture over the entire surface (Agüero *et al.,* 2016) of pennywort leaves using a sterile micropipette in order to obtain an initial count of about ~log 8 CFU/g on the pennywort samples. The 500 µL of culture containing *E. coli* cells of ~log 8 CFU/g was defined based on 5 g of pennywort samples. The culture was spread over the surfaces of around 10 - 12 pennywort leaves which made up 5 g samples. Following inoculation, the pennywort samples were air-dried in a biosafety cabinet at 22 ± 2°C for 2 h to facilitate bacterial attachment. Next, the plastic packaging was sealed and treated with PL.

Enumeration of E. coli

Triplicate samples were taken from each treatment. Samples of 5 g were aseptically mixed with 45 mL of peptone water in a stomacher bag, and homogenized (BagMixer 400, Interscience, Saint-Germain-en-Laye, France). Serial dilutions of the homogenates were plated onto eosin methylene blue agar (Merck®, Germany) or MacConkey agar (Merck®, Germany) and incubated for 24 h at 37 ± 1°C. Distinctive metallic green sheen colonies or red colony were counted as *E. coli* and the results were expressed in log CFU/g.

Water activity analysis

Water activity on the surface of pennywort was determined using the AquaLab Series 3 TE water activity analyzer (Decagon Devices, Inc., Washington, USA).

Physical analysis

Firmness

The firmness of pennywort was measured using a TX-XT2i texture analyzer (Stable Micro Systems Ltd., Surrey, UK). Settings used were set according to São Josė and Vanetti (2015) with some modifications. The pennywort textures were determined using a Kramer cell shearer, with blades attached to the testing instrument with the setting of pre-test speed at 1.00 mm/s, test speed at 0.50 mm/s, the post-test speed at 10.00 mm/s, distance at 100% strain and trigger force at 5 g. The results of firmness analysis were expressed as the maximum load in Newtons (N).

Color

A Minolta colorimeter (Minolta Spectrophotometer CR-300, Minolta Camera, Japan) was used to determine the values of L*(lightness), a*(green-red chromaticity), and b*(blue-yellow chromaticity) parameters. The instrument was calibrated with a standard white tile. The calculation for the chromaticity (*C**) and hue angle (h°) was performed using Eq. 2 and Eq. 3:

$$C^* = \left| (a^*)^2 + (b^*)^2 \right|^{½} \quad \text{(Eq. 2)}$$

$$h° = \tan^{-1} (b^*/a^*) \quad \text{(Eq. 3)}$$

Statistical analysis

All treatments were performed in triplicate. For each treatment, sample analysis was repeated three times. The data were analyzed using software Minitab (v 16.0) based on analysis of variance, and expressed as mean value ± standard deviation. The confidence level for statistical significance was set at a p-value of 0.05. Tukey's test was used to determine the significant difference of the data. The microbial counts were transformed into logarithms before computing means and standard deviations. The population densities were reported as log CFU/g.

Results and discussion

Effect of PL fluences on the microbial stability of fresh-cut pennywort

Figure 1 shows the effect of PL fluence on total aerobic mesophilic count (AMC) and yeast and mold count (YMC) of pennywort as well as the changes throughout storage. Our finding indicates that PL fluences of 6.9, 9.6, and 12.3 J/cm² significantly reduced the AMC and YMC. The highest inactivation ranged log 0.79 - 1.06 CFU/g for AMC and log 0.16 - 0.61 CFU/g for YMC, and were obtained by using fluences of 9.6 and 12.3 J/cm², respectively. Pennywort presented high initial microbial populations for AMC and YMC (log 6.41 and 5.72 CFU/g, respectively). Most AMC in organic and conventional vegetables ranged from log 6 to 7 CFU/g, and most YMC ranged from log 5 to 6 CFU/g (Maffei *et al.,* 2013). Similar findings were reported for spinach, fresh-cut vegetables, ready-to-eat salads, and sprout samples with microbial counts greater than log 6 CFU/g (Agüero *et al.,* 2016). There are many factors that may influence the higher initial microbial count including type and variety of plants, specific conditions during pre-harvest including soil, climate, and crop management condition, and several post-harvest factors such as temperature and humidity during harvesting and distribution of raw materials (Agüero *et al.,* 2016).

Our finding indicates that both AMC and YMC similarly increased with longer storage time for all PL treatment with counts of around log 6.52 - 8.08 CFU/g and log 6.83 - 8.06 CFU/g on day 16, respectively. PL decreased microbial count with increasing fluence during storage of pennywort. Based on the Spanish legislation for minimally

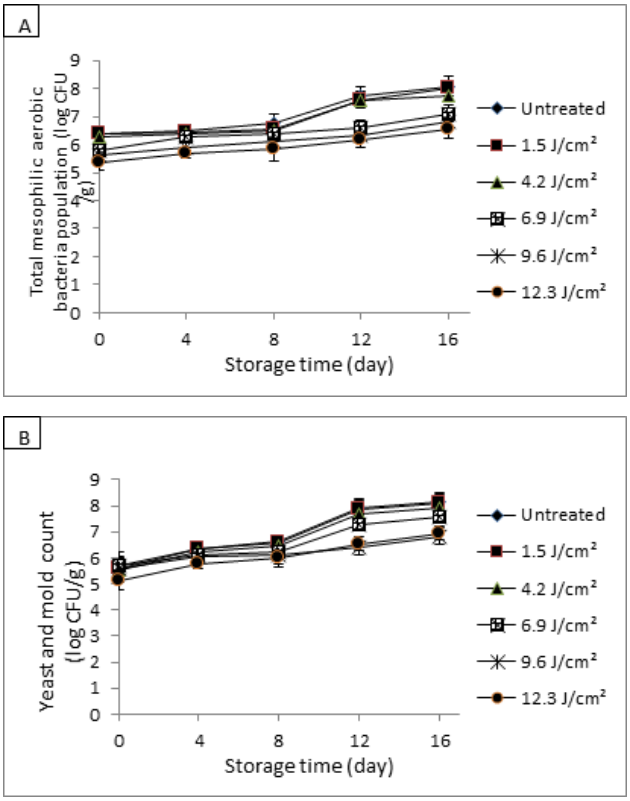


Figure 1. Total Mesophilic Aerobic Bacterial Count (A) and Yeast and Mold Count (B) of pennywort untreated (control) and treated with pulsed light stored at 4 ± 1°C. Markers are means of three replicates (n = 3) with vertical bars representing the standard deviation (± SD).

processed vegetables (RD 3484/2000), the maximum allowable microbial counts are log 7 CFU/g for aerobic bacteria during storage (Oms-Oliu *et al.,* 2010a). On the other hand, untreated and PL-treated (1.5 and 4.2 J/cm²) samples did not exceed the limit of acceptability for aerobic microorganism after eight days of storage. While for the sample treated at 6.9 J/cm² at day 12. Thus, PL extended the pennywort shelf life by about four to eight days depends on the PL fluences. Pennywort treated with PL at 9.6, and 12.3 J/cm² remained lower than the limit at about log 6.81 and 6.52 CFU/g, respectively, even at day 16; however, the color become darker.

Water activity (a_w) can be a significant factor in affecting microbial growth. Most foods with a_w above 0.95 will provide sufficient moisture to support the growth of bacteria, yeasts, and molds. The a_w in untreated pennywort (control) was found to be insignificantly different ($p > 0.05$) as compared to all treated samples of about 0.95 - 0.98 (data not shown). This concludes that the primary factor in inactivating the microbial population was the effect of PL treatment, and not a_w.

In the present work, PL treatment had significant lethal effects against AMC and YMC.

The primary mechanism of action for PL is mostly related to the UV part of the spectrum and its photochemical and photothermal effects (Anderson *et al.,* 2000; Takeshita *et al.,* 2003; Elmnasser *et al.,* 2007). In addition, PL causes sublethal damage to the microorganisms by making the cells to be more sensitive towards subsequent stress such as low-temperature storage (Koh *et al.,* 2016). Moreover, findings indicated that PL treatment has higher effectiveness than ultraviolet light or irradiation since PL exhibits broader spectral range with a high peak power (McDonald *et al.,* 2000). Our findings indicated no significant difference ($p > 0.05$) in AMC and YMC for samples treated with 1.5 and 4.2 J/cm² as compared to control (untreated) sample throughout the storage. The AMC and YMC for these three samples were in the range of log 6.27 - 8.08 CFU/g and log 5.61 - 8.17 CFU/g, respectively. Further increased in the PL fluence up to 12.3 J/cm² showed no further significant microbial reduction ($p > 0.05$) even though the lowest microbial counts (log 6.52 CFU/g) were noticeable on day 16 as compared to other fluences and the control sample. Similarly, no further microbial inactivation was observed in fresh-cut cantaloupe when treated with higher PL fluence of 15.6 J/cm² (Koh *et al.,* 2016). Ignat *et al.* (2014) also observed no further reduction in microbial population when fresh-cut apple was treated by PL at higher fluence up to 15.75 J/cm². This finding is in agreement with Gómez *et al.* (2012b) who also reported that PL treatment using high fluence did not offer additional microbial inactivation in fresh-cut apple treated at 71.6 J/cm². The efficacy of PL technology was observed in the initial microbial population reductions and along the storage. At the end of storage, PL-treated pennywort exhibited lower counts than the control samples. These results were also reported by other authors who worked with PL treatments in different food substrates (Oms-Oliu *et al.,* 2010b; Agüero *et al.,* 2016).

From our observations, treatment with PL fluences of 6.9, 9.6 and 12.3 J/cm² significantly reduced the microbial count. However, pennywort treated with 9.6 J/cm² showed the highest inactivation of microbial populations and lesser than the maximum fluence limit approved by the FDA which allows a complete cumulative treatment of 12 J/cm² (FDA, 1996) during storage. Our findings indicated that PL treatment was effective to significantly reduce the microbial counts. Until at the end of the storage, the microbial count for PL-treated pennywort was lower as compared to untreated pennywort leaves.

Effect of PL fluences on the physical properties of fresh-cut pennywort

Firmness

Texture is an important quality attribute in the consumer acceptability of fresh vegetable, and it is a combination of parameters including appearance, texture, flavor, and nutritional value (Kader, 2013). The effect of PL treatment on the firmness of fresh-cut pennywort is presented in Figure 2 with the range of 208.74 - 368.66 N. A continuous decrease in the firmness was found in all packages when stored at 4 ± 1°C. The firmness of PL-treated pennywort decreased to a greater extent than untreated pennywort at the beginning of storage. However, at the end of storage, there was no significant effect ($p > 0.05$) on the firmness between the samples. This indicated that senescence or cell degradation of pennywort sample treated with PL was slower as compared to the untreated sample. In general, the insignificant alteration ($p > 0.05$) in the texture of pennywort stored at 0 d denoted that little or no senescence or cell degradation had occurred in untreated, 1.5, 4.2, 6.9, and 9.6 J/cm² PL-treated pennyworts. On the other hand, significant effect ($p \leq 0.05$) was observed for the sample treated with 12.3 J/cm² on the firmness of pennywort at day 0 between treated and untreated samples.

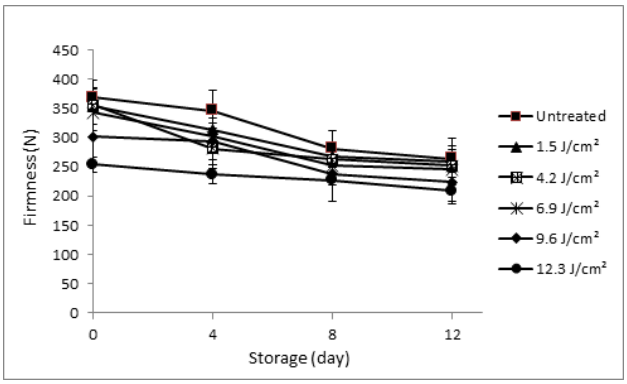


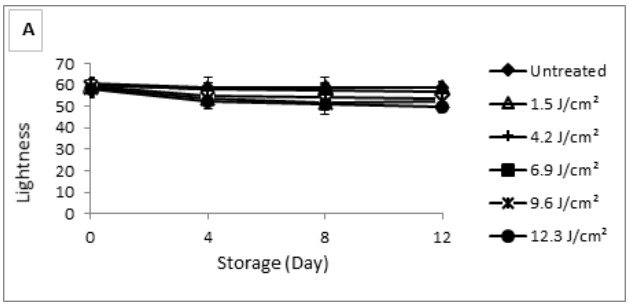
Figure 2. Effect of pulsed light fluences on firmness (N) of fresh-cut pennywort during storage at 4 ± 1°C. Markers are means of three replicates (n = 3) with vertical bars representing the standard deviation (± SD).

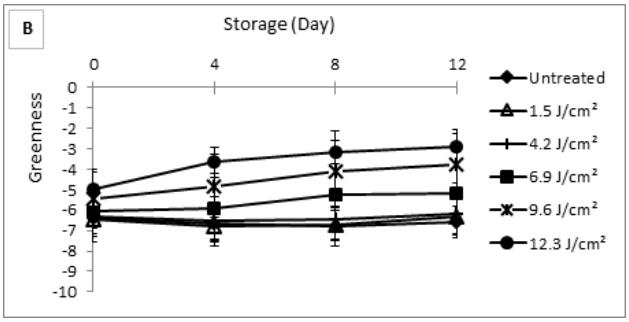
Similarly, PL can dramatically affect the textural properties of fresh-cut mushrooms due to the thermal damage caused by the use of high fluences (Oms-Oliu *et al.,* 2010a). In many studies, it was reported that an intense PL treatment of plant-based foods might negatively affect several quality parameters like, e.g., the texture of mushrooms (Oms-Oliu *et al.,* 2010a) or sensory attributes of cut apple (Ignat *et al.,* 2014). Most of the significant deterioration was

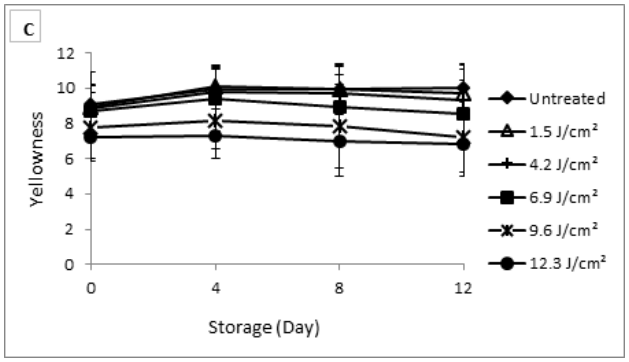
found during storage and not immediately after PL treatment. Therefore, storage trials of PL exposed products are not only crucial for the maintenance of microbial reductions but also to investigate the impact on the shelf life. As the effect on quality parameters varies significantly among products and is mostly dose dependent, it is hence of great importance to find process parameters which minimizes the adverse effect on the physical quality of samples while providing substantial microbial inactivation. Throughout storage, findings indicated that the cell degradation of pennywort sample treated with PL was slower as compared to the untreated sample. Our study indicates that PL fluences of 1.5, 4.2, 6.9, and 9.6 J/cm² did not seem to have an effect on this firmness attribute as compared to the untreated sample.

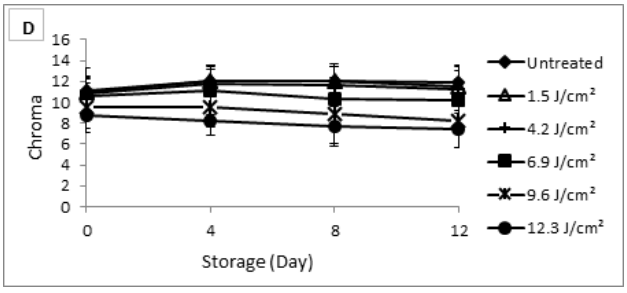
Color

The primary sensory characteristics losses that occur along storage are changes in color, texture, and flavor. In the present work, the L*, a*, b* values, chroma, and hue angle were monitored as indicated in Figure 3. In general, the color parameters decreased for samples except for greenness (a*) which increased during storage. Overall, parameters for PL-treated samples decreased slower as compared to untreated pennywort which proved that PL was useful to slow down the color changes of stored pennywort leaves. Findings indicate that the color parameters changed significantly when treated with 9.6 and 12.3 J/cm² during storage. Previous study using an intense PL treatment negatively affected the color of salad (Kramer *et al.,* 2015). The color parameters for lightness remained not significant in the range of 49.65 - 60.34 but showed slight decreases over storage. Similarly, Artés-Hernández *et al.* (2009) also reported minor losses of lightness over storage for spinach after being exposed to UV-C doses. The color parameters for hue angle remained in the range of 113.74 - 125.9, which showed a slight decrease in storage similar to broccoli treated with UV-C light (Costa *et al.,* 2006). Bermúdez-Aguirre and Barbosa-Cánovas (2013) also reported that UV-C negatively affected the color of lettuce and generated tissue browning. UV irradiation causes increased of tissue browning of salad, which was reported by Allende *et al.* (2006) for Red Oak leaf lettuce. Browning is mainly triggered by a decompartmentation process, allowing phenolic compounds to come in contact with oxidation enzymes (Charles *et al.,* 2013). Although PL is designated to be a non-thermal decontamination process, products with low thermal capacity like salad leave still undergo considerable heating. Gómez e*t al.* (2012a) explained that PL treatment caused damage to cellular membranes and resulted in decompartmentation which allows for enzyme-substrate contact and is subsequently causing browning of tissue. Furthermore, photothermal effects caused by PL treatment could lead to non-enzymatic browning. Thermal damage in products exposed to high doses of PL can cause substantial structural modifications. To sum up, the color of pennywort was not significantly affected by PL treatment which was applied at the lower fluences of 1.5, 4.2, and 6.9 J/cm²; however, at higher fluences of 9.6 and 12.3 J/cm², the color was adversely affected. Therefore, the optimum fluence to maintain the microbial and physical quality for pennywort was at PL fluence of 6.9 J/cm².









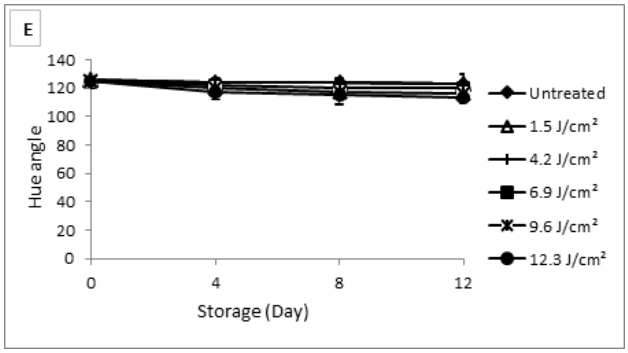


Figure 3. Effect of pulsed light fluences on color properties of fresh-cut pennywort during storage at 4 ± 1°C for: (A) = lightness (L*); (B) = greenness (a*); (C) = yellowness (b*); (D) = chroma (C*); and (E) = hue angle (H°). Markers are means of three replicates (n = 3) with vertical bars representing the standard deviation (± SD).

Effect of PL fluences on E. coli inactivation of fresh-cut pennywort

Figure 4 shows the *E. coli* counts obtained on pennywort leaves after PL treatments at increasing fluences. Untreated sample was the pennywort inoculated with *E. coli* without any PL treatment. The *E. coli* count for PL-treated sample was significantly ($p \leq 0.05$) lower as compared to untreated pennywort. The reduction of *E. coli* population (log 1.53 - 2.37 CFU/g) was observed as the PL fluence increased. PL treatments at 1.5, 4.2, 6.9, 9.6, and 12.3 J/cm² significantly ($p \leq 0.05$) inactivated the *E. coli* counts which were significantly lower as compared to untreated (control) pennywort. This implies that PL effect kills the cells of *E. coli* and the effect was not sublethal even when the lowest fluence was applied. Samples treated with fluences of 1.5 and 4.2 J/cm² showed significant difference in *E. coli* inactivation counts; however, the highest inactivation was achieved at PL fluences of 6.9, 9.6, and 12.3 J/cm². The inactivation of *E. coli* count was increased as the PL fluences increased. Ramos-Villarroel *et al.* (2012) reported on the reduction of these microorganisms which were inoculated on fresh-cut mushrooms in the range of log 2.66 - 3.03 CFU/g. However, higher resistance of microorganism's cell wall structure affected the effectiveness of PL. Lower reduction of native microbiota growing on pennywort was observed than those achieved in the *E. coli* counts from the inoculated samples. The best PL fluence in reducing native microbiota was at 9.6 J/cm² while for *E. coli* inactivation was at 6.9 J/cm². This result is related to the potential internalization of indigenous microorganisms. The type of microorganisms is a major factor to be considered when applying the PL treatments. Gómez-López (2005) did not observe any sensitivity patterns among different groups of microorganisms after studying 27 bacterial, yeast, and mold species. Previous research in various food substrates demonstrated the efficiency of PL to inactivate bacteria (Bialka and Demirci, 2008). However, the comparison between microorganisms is often difficult due to the influence of various factors on the effectiveness of PL. Several critical data such as the type of microorganism, inoculum size, food compositions, and natural competitive microbiota differ among studies. The present work shows that the effectiveness of PL depends on the entire spectral distribution and fluence although the factors mentioned above are important. Finally, the result indicated that PL is effective in significantly reducing ($p \leq 0.05$) the *E. coli* count.

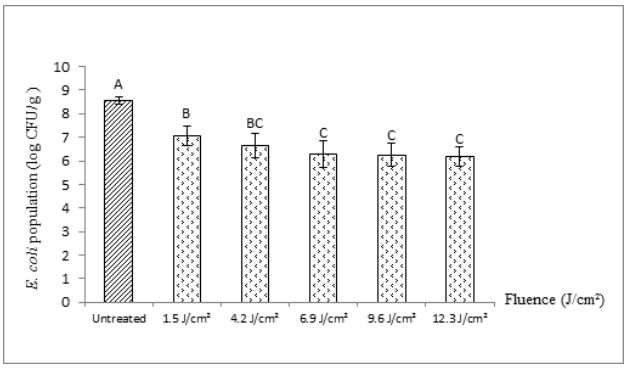


Figure 4. Escherichia coli population of pennywort at pulsed light fluences of 1.5, 4.2, 6.9, 9.6, and 12.3 J/cm². Columns are means of three replicates (n = 3) with vertical bars representing the standard deviation (± SD).

Our findings indicated that PL treatment was effective to significantly reduce the natural microbial counts in pennywort leaves throughout storage. Previous studies on the effectiveness of PL demonstrated that this technology resulted in significant reductions on the levels of microbial pathogens, molds, and yeasts (Hillegas and Demirci, 2003; Takeshita *et al.,* 2003; Gómez-López *et al.,* 2007). More efficient reductions were also shown in the inoculated sample. Similarly, PL has been demonstrated to reduce the microorganisms that are naturally present (Gómez-López *et al.,* 2005) as well as inoculated microorganisms such as Saccharomyces cerevisiae cells which were inoculated onto wheat flour and black peppers, *Aspergillus niger* spores inoculated in cornmeal, and *E. coli* O157:H7 inoculated on alfalfa seeds (Sharma and Demirci, 2003).

Scanning Electron Microscopy of E. coli

The effects of PL fluences of 1.5, 6.9, and

12.3 J/cm² treatments on the *E. coli* inoculated on pennywort leaves were investigated using SEM. Figure 5 (A), no visible morphological damage was observed on *E. coli* cells without PL treatment (control). For PL treatment at 1.5 J/cm² as shown in Figure 5 (B), there was a combination of damages on the *E. coli* cells supported by the significant reduction of *E. coli* count as compared to untreated pennywort leaves (control). Some of the *E. coli* cells appeared similar before and after treatment as compared to Figure 5 (A) and (B), suggesting that PL did not induce severe deformation or membrane disruption of *E. coli* in our testing conditions at low fluence of 1.5 J/cm², but there was minimal damage occurred. Figure 5 (C) of 6.9 J/cm² and Figure 5 (D) of 12.3 J/cm² show that there was a combination of some shape destructions observed on the *E. coli's* cell membranes. Therefore, findings indicated that PL at 6.9 J/cm² and 12.3 J/cm² might cause alterations on the cellular membrane including disruption and leakage of cell content which led to growth inhibitions of *E. coli* in pennywort. Other researchers proposed that the impact of PL may not depend wholly on DNA destruction as the lethal effect of PL can also be due to photothermal effect (Wekhof, 2000). The photothermal effect is noticeable along the photochemical effect. The relative contribution of the two inactivation mechanisms depends on the energy dosage (fluence) and the target microorganism (Gómez-López *et al.,* 2007). Takeshita *et al.* (2003) particularly found that PL caused damage to the membranes, proteins, and other macromolecules within microorganisms; thereby increasing the efficiency of microbial inactivation.

Figure 5 (E and F) shows that *E. coli* can hide in the stomata and PL sterilization only occurs on the surface of the microbe. This proves that some *E. coli* might survive when exposed to PL by hiding inside the stomata which will affect the efficiency of PL. In addition, different bacteria exhibited preferential attachments on various surfaces (cut edge and stomata *versus* intact tissue) or plants (Barak *et al.,* 2008). The presence of natural openings (e.g., stomata, hydathodes, nectathods, lenticels, stem scars, and calyx) and cracks or crevices (Takeuchi *et al.,* 2001) provides the potential attachment areas for pathogens and microorganisms. Similar to the present work, Ramos-Villarroel *et al.* (2012) reported significant damage to the cell membrane of microorganisms resulted from pulsed ultraviolet treatment. Alterations in the bacterial cell membrane resulting from PL treatment were also reported in another study (Kramer and Muranyi, 2014). Previous findings supported that initial electron micrograph may indicate possible alterations or damage on the bacterial cell membrane structure as a result of PL treatment (Ramos-Villarroel *et al.,* 2012; Kramer and Muranyi, 2014). However, these morphological changes which caused alteration or damage to the bacterial cell wall and the structures of cell membranes should be interpreted with caution and may not solely be attributable by PL. There may be other contributory factors along with the DNA structural damage (thymine dimer formation) that contributes to cell injury and cell death (Koutchma *et al.,* 2009).

Conclusion

To sum up, PL at 6.9 J/cm² is the best fluence to reduce the microbial count while retaining the physical properties of pennywort leaves and extend the shelf life to about four days. Lower reduction of the total aerobic mesophilic count was observed than those achieved in the inoculated *E. coli* count on pennywort leaves. The inactivation of *E. coli* population was significantly higher at PL fluence of 6.9 J/cm² for the inoculated sample, and it was observed that PL induced morphological changes of the surface of *E. coli's* cell membrane using SEM. Our findings indicated that PL-treated sample had reduced microbial count and slower degradation as compared to untreated pennywort leaves throughout storage. Hence, PL is a potential technology that could be used to maintain the quality of fresh-cut pennywort and further investigation to improve this method needs to be carried out.

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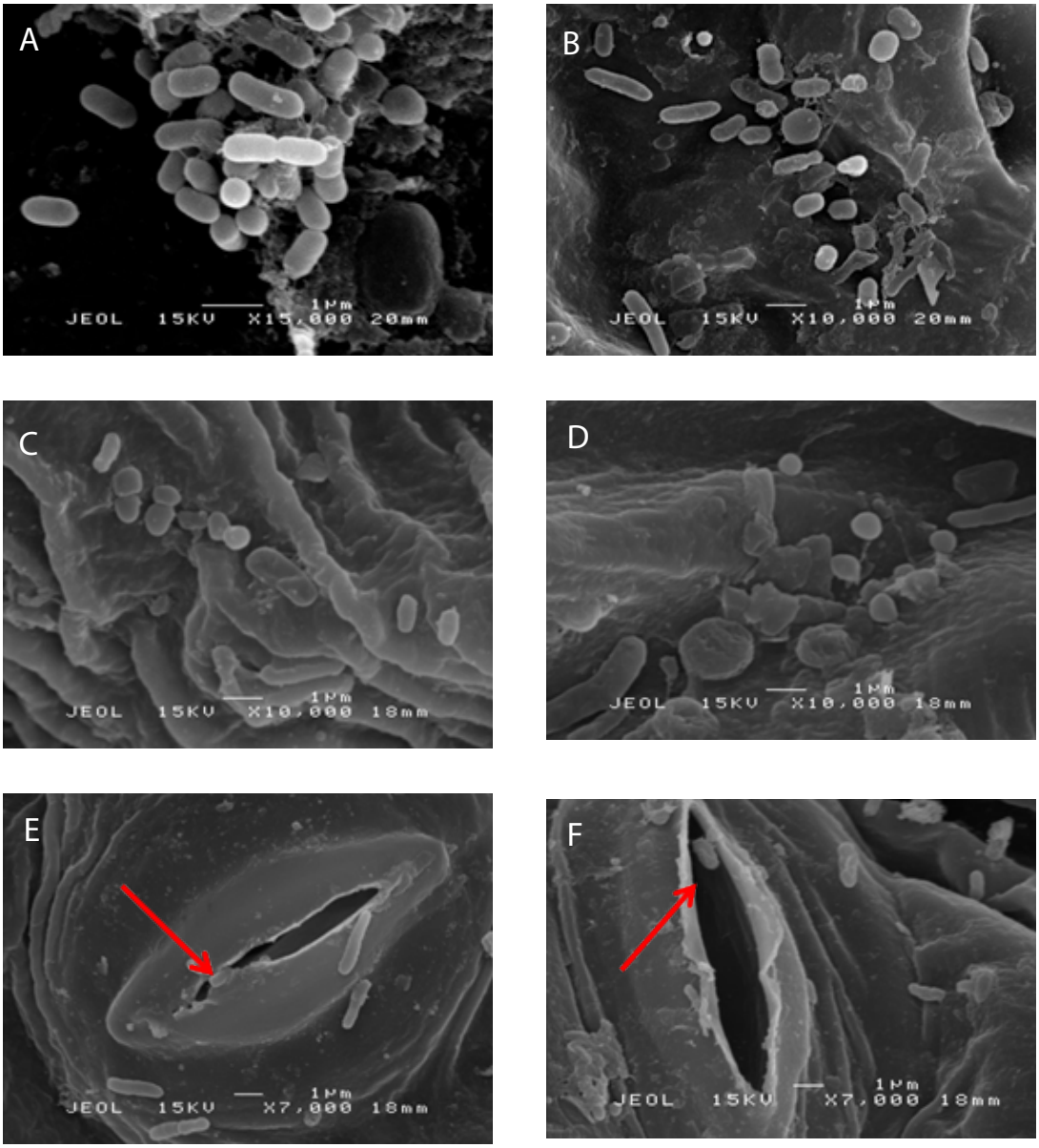

Figure 5. Scanning electron microscopy (SEM) of Escherichia coli: (A) = untreated samples; (B) = treated by pulsed light (PL) at 1.5 J/cm², (C) = treated by PL at 6.9 J/cm², (D) = treated by PL at 12.3 J/cm² and E. coli hiding in stomata, (E) = untreated samples; and (F) = treated by PL at 12.3 J/cm².

References

Agüero, M. V., Jagus, R. J., Martin-Belloso, O. and Soliva-Fortuny, R. 2016. Surface decontamination of spinach by intense pulsed light treatments: impact on quality attributes. Postharvest Biology and Technology 121: 118–125.

Allende, A., McEvoy, J. L., Luo, Y., Artes, F. and Wang, C. Y. 2006. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed "Red Oak Leaf" lettuce. Food Microbiology 23(3): 241–249.

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A. and Parish, O. 2000. Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. IEEE Transactions on Plasma Science 28(1): 83–88.

Artés-Hernández, F., Escalona, V. H., Robles, P. A., Martínez-Hernández, G. B. and Artés, F. 2009. Effect of UV-C radiation on quality of minimally processed spinach leaves. Journal of the Science of Food and Agriculture 89(3): 414–421.

Awang Salleh, N., Rasul, G., Hassan, Z., Reezal, A., Isa, S. H., Nishibuchi, M. and Radu, S. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. Food Control 14(7): 475–479.

Barak, J. D., Liang, A. and Narm, K.-E. 2008. Differential attachment to and subsequent contamination of agricultural crops by *Salmonella enterica.* Applied and Environmental Microbiology 74(17): 5568–5570.

Bermúdez-Aguirre, D. and Barbosa-Cánovas, G. V. 2013. Disinfection of selected vegetables under nonthermal treatments: chlorine, acid citric, ultraviolet light and ozone. Food Control 29(1): 82–90.

Bialka, K. L. and Demirci, A. 2008. Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on raspberries and strawberries. Journal of Food Science 73(5): M201–M207.

Chai, L. C., Robin, T., Ragavan, U. M., Gunsalam, J. W., Bakar, F. A., Ghazali, F. M., ... and Kumar, M. P. 2007. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. International Journal of Food Microbiology 117(1): 106–111.

Chang, W. S., Afsah-Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y. L., Loo, Y. Y., … and Son, R., 2013. Quantification of *Escherichia coli* O157:H7 in organic vegetables and chickens. International Food Research Journal 20(2): 1023–1029.

Charles, F., Vidal, V., Olive, F., Filgueiras, H. and Sallanon, H. 2013. Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. Innovative Food Science and Emerging Technologies 18: 190–195.

Costa, L., Vicente, A. R., Civello, P. M., Chaves, A. R. and Martínez, G. A. 2006. UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biology and Technology 39(2): 204–210.

Delaquis, P., Bach, S. and Dinu, L. D. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. Journal of Food Protection 70(8): 1966–1974.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A. and Federighi, M. 2007. Pulsed-light system as a novel food decontamination technology: a review. Canadian Journal of Microbiology 53(7): 813–821.

Fan, L. and Song, J. 2008. Microbial quality assessment methods for fresh-cut fruits and vegetables. Stewart Postharvest Review 4(3): 1–9.

Faour-Klingbeil, D., Kuri, V. and Todd, E. C. D. 2016. The influence of pre-wash chopping and storage conditions of parsley on the efficacy of disinfection against *S. Typhimurium.* Food Control 65: 121–131.

Food and Agriculture Organization (FAO) and World Health Organization (WHO). 2008. Microbiological hazards in fresh fruits and vegetables. Rome: FAO/WHO.

Food and Drug Administration (FDA). 1996. CFR - code of federal regulations: 21CFR179.41. Retrieved on February 22, 2017 from website: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.41

Gómez, P. L., García-Loredo, A., Nieto, A., Salvatori, D. M., Guerrero, S. and Alzamora, S. M. 2012a. Effect of pulsed light combined with an antibrowning pretreatment on quality of fresh cut apple. Innovative Food Science and Emerging Technologies 16: 102–112.

Gómez, P. L., Salvatori, D. M., García-loredo, A. and Alzamora, S. M. 2012b. Pulsed light treatment of cut apple: dose effect on color, structure, and microbiological stability. Food and Bioprocess Technology 5: 2311–2322.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V. and Debevere, J. 2005. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. International Journal of Food Microbiology 103(1): 79–89.

Gómez-López, V. M., Ragaert, P., Debevere, J. and Devlieghere, F. 2007. Pulsed light for food

decontamination: a review. Trends in Food Science and Technology 18(9): 464–473.

Hillegas, S. L. and Demirci, A. 2003. Inactivation of *Clostridium sporogenes* in clover honey by pulsed UV-light treatment. Retrieved from website: https://cigrjournal.org/index.php/E-jounral/article/view/427/420

Huang, Y. and Chen, H. 2014. A novel water-assisted pulsed light processing for decontamination of blueberries. Food Microbiology 40: 1–8.

Ignat, A., Manzocco, L., Maifreni, M., Bartolomeoli, I. and Nicoli, M. C. 2014. Surface decontamination of fresh-cut apple by pulsed light: effects on structure, colour and sensory properties. Postharvest Biology and Technology 91: 122–127.

Kader, A. A. 2013. Postharvest technology of horticultural crops - an overview from farm to fork. Ethiopian Journal of Science and Technology (Special Issue 1): 1–8.

Karagözlü, N., Ergönül, B. and Özcan, D. 2011. Determination of antimicrobial effect of mint and basil essential oils on survival of *E. coli* O157:H7 and *S. typhimurium* in fresh-cut lettuce and purslane. Food Control 22(12): 1851–1855.

Koh, P. C., Noranizan, M. A., Karim, R. and Nur Hanani, Z. A. 2016. Microbiological stability and quality of pulsed light treated cantaloupe (*Cucumis melo* L. *reticulatus* cv. Glamour) based on cut type and light fluence. Journal of Food Science and Technology 53: 1798–1181.

Koutchma, T. N., Forney, L. J. and Moraru, C. I. 2009. Ultraviolet light in food technology - principles and applications. United States: CRC Press.

Kramer, B. and Muranyi, P. 2014. Effect of pulsed light on structural and physiological properties of *Listeria innocua* and *Escherichia coli.* Journal of Applied Microbiology 116(3): 596–611.

Kramer, B., Wunderlich, J. and Muranyi, P. 2015. Pulsed light decontamination of endive salad and mung bean sprouts and impact on color and respiration activity. Journal of Food Protection 78(2): 340–348.

Loo, Y. Y., Puspanadan, S., Goh, S. G., Kuan, C. H., Chang, W. S., Lye, Y. L., ... and Son, R. 2013. Quantitative detection and characterization of Shiga toxin-producing *Escherichia coli* O157 and non-O157 in raw vegetables by MPN-PCR in Malaysia. International Food Research Journal 20(6): 3313–3317.

Maffei, D. F., Silveira, N. F. A. and Catanozi, M. D. P. L. M. 2013. Microbiological quality of organic and conventional vegetables sold in Brazil. Food Control 29(1): 226–230.

McDonald, K. F., Curry, R. D., Clevenger, T. E., Unklesbay, K., Eisenstark, A., Golden, J., … and Morgan, R. D. 2000. A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. IEEE Transactions on Plasma Science 28(5): 1581–1587.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O. and Soliva-Fortuny, R. 2010a. Postharvest biology and technology effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). Postharvest Biology and Technology 56(3): 216–222.

Oms-Oliu, G., Martín-Belloso, O. and Soliva-Fortuny, R. 2010b. Pulsed light treatments for food preservation. A review. Food and Bioprocess Technology 3: article ID 13.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., … and Malakar, P. K. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. Food Control 21(5): 774–778.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O. and Soliva-Fortuny, R. 2012. Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. Postharvest Biology and Technology 69: 32–39.

Rao, S. B., Chetana, M. and Uma Devi, P. 2005. *Centella asiatica* treatment during postnatal period enhances learning and memory in mice. Physiology and Behavior 86(4): 449–457.

Romero, L., Colivet, J., Aron, N. M. and Ramosvillarroel, A. 2017. Impact of ultraviolet light on quality attributes of stored fresh-cut mango. The Annals of the University of Dunarea De Jos of Galati - Food Technology 41(1): 62–80.

Ruszymah, B. H., Chowdhury, S. R., Manan, N. A., Fong, O. S., Adenan, M. I. and Saim, A. B. 2012. Aqueous extract of *Centella asiatica* promotes corneal epithelium wound healing *in vitro*. Journal of Ethnopharmacology 140(2): 333–338.

São Josė, J. F. B. and Vanetti, M. C. D. 2015. Application of ultrasound and chemical sanitizers to watercress, parsley and strawberry: microbiological and physicochemical quality. LWT - Food Science and Technology 63(2): 946–952.

Sharma, R. R. and Demirci, A. 2003. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. Journal of Food Science 68(4): 1448–1453.

Shynkaryk, M. V., Pyatkovskyy, T. I., Yousef, A. E. and Sastry, S. K. 2016. Gaseous ozone treatment of baby spinach within the existing production

chain for inactivation of *Escherichia coli* O157:H7. Journal of Food Engineering 191: 10–18.

Takeshita, K., Shibato, J., Sameshima, T., Fukunaga, S., Isobe, S., Arihara, K. and Itoh, M. 2003. Damage of yeast cells induced by pulsed light irradiation. International Journal of Food Microbiology 85(1-2): 151–158.

Takeuchi, K., Hassan, A. N. and Frank, J. F. 2001. Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. Journal of Food Protection 64(11): 1820–1823.

Vandamm, J. P., Li, D., Harris, L. J., Schaffner, D. W. and Danyluk, M. D. 2013. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. Food Microbiology 34(1): 151–157.

Veerendra Kumar, M. H. and Gupta, Y. K. 2003. Effect of *Centella asiatica* on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats. Clinical and Experimental Pharmacology and Physiology 30(5-6): 336–342.

Wachtel, M. R., Whitehand, L. C. and Mandrell, R. E. 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. Journal of Food Protection 65(1): 18–25.

Wekhof, A. 2000. Disinfection with flash lamps. PDA Journal of Pharmaceutical Science and Technology 54(3): 264–276.

Zheng, C. J. and Qin, L. P. 2007. Chemical components of *Centella asiatica* and their bioactivities. Journal of Chinese Integrative Medicine 5(3): 348–351.