EFFECTS OF PULSED-LIGHT TREATMENTS ON SHELF-LIFE AND DECONTAMINATION OF STRAWBERRIES AND BLUEBERRIES

by

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TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Strawberries and blueberries are highly perishable fruits with short shelf-life. They are also occasionally contaminated with pathogens such as *Salmonella* and human norovirus. In this study, we explored the potential of pulsed light (PL) for shelf-life extension and decontamination of these two fruits. Screening studies were conducted to identify effective PL treatments which were further evaluated. Three dry PL treatments (samples were exposed to PL directly), low intensity PL with a dose of 3 J/cm^2 (L3), medium intensity PL with a dose of 3 J/cm^2 (M3), and medium intensity PL with a dose of 5 J/cm^2 (M5), were identified for strawberries. These 3 PL treatments along with untreated control were applied to strawberries and strawberries dip- and spot-inoculated with a cocktail of *Salmonella*. Strawberries were then stored at refrigerated storage and analyses were conducted at selected time intervals. All three PL treatments showed delayed fungal development, slowed down overall spoilage, better preserved surface glossiness, and delayed anthocyanin accumulation. Among the three treatments, M3 showed best shelf-life extension effect, while L3 and M5 achieved slightly better decontamination effect as well as slightly enhanced surface redness. No significant difference in other quality attributes, including weight loss, firmness, acid or sugar contents were observed between the control and PL-treated strawberries. However, although not obviously reflected in shelf-life, a tendency of accelerated quality degradation was discerned in M5 samples, suggesting the use of high dose PL treatments should be cautiously executed or discouraged. For blueberries, two PL treatments, dry low intensity PL with a dose of 6 J/cm^2 (L6) and water-assisted low intensity PL with a dose of 9 J/cm^2 (wL9) (samples were exposed to PL while being washed in agitated water) were identified.

These 2 PL treatments along with untreated control were applied to blueberries and blueberries dip- and spot-inoculated with a cocktail of *Salmonella*. Blueberries were then stored at room temperature (~22°C) or 5℃ and analyses were conducted at selected time intervals. Neither dry nor water-assisted PL treatments showed benefits in extending the shelf-life of blueberries regardless the storage temperature. However, the two PL treatments significantly reduced the *Salmonella* population immediately after treatments and after 7 days of cold storage. Overall, the water-assisted PL treatment had better decontamination effect than the dry PL treatment. Surface lightness was directly reduced after both treatments. Reduced firmness was detected in water-assisted PL samples after 7 days of cold storage, while increased weight loss was detected during storage after dry PL treatment. The increased weight loss and decreased surface lightness were the major restrictions of dry PL treatment. Due to the limitation of our water-assisted PL system, blueberries were in contact with water much longer than necessary and our system could not provide a rapid drying and cooling process. In summary, dry PL treatment showed promise in extending shelf-life and decontamination of strawberries. Although water-assisted PL treatment could effectively inactivate *Salmonella* on blueberries, equipment need to be carefully designed to minimize the time blueberries are in contact with water and provide rapid drying and cooling of blueberries after water-assisted PL treatment.

Chapter 1

INTRODUCTION

Consumption of fresh produce is becoming more popular in these few decades since it has been linked to reduced risks of chronic diseases. Specifically, fresh berries are showing continuous growing markets due to their high nutritional values, tasty flavors and convenience for fresh consumption, especially for strawberries and blueberries.

Unfortunately, both berries are highly perishable with short shelf-life. On the one hand, fruit diseases may cause serious post-harvest loss from farmland to market. On the other hand, people are requiring higher berry qualities, making consumer intolerance another large contributor to high post-harvest loss. In both aspects, keeping high quality is a critical concern for fresh berry industries.

There are three major processes, respectively natural senescence and water loss, mechanical damage, as well as fungal decay that can lead to quick quality loss. For strawberries, fungal decay is the most serious problem (Mitcham, 2014), in which green mold induced by *Botrytus cinerea* is most commonly seen (Ceponis et al. 1987), while other diseases such as anthracnose rot and leather rot, caused respectively by *Colletotrichum acutatum* and *Phytophthora cactorum*, are also frequently detected. As for blueberries, the first restricting factor in shelf-life is natural senescence rather than microbial spoilage, although they are still susceptible to fungal spoilage similarly caused by *Colletotrichum acutatum* and *B.cinerea* (Nunes et al., 2004; Sanford et al., 1991; Woodruff et al.,1959). Chemical fungicides were widely used for many fresh

fruits, but are becoming unacceptable to consumers due to health concerns. Because of the complexity of quality deterioration, current food processing techniques being investigated as fungicide alternatives cannot effectively promote berry shelf-life without increasing processing cost or causing other side effects on berry sensory qualities (Hernández-Muñoz et al., 2006; Vicente et al., 2002). Apart from short shelf-life, fresh berries are also common vehicles of bacteria pathogens such as *Escherichia.coli* and *Salmonella spp.,* raising further safety concerns. An *E.coli* O157:H7 outbreak was reported to be linked to strawberries in 2011, causing 7 hospitalized and 1 death, while another *Salmonella* outbreak was linked to blueberries in 2009, resulting in 14 illness (Laidler et al., 2013; CDC database, 2009). Therefore, a safe, efficient, economic, and effective technique to prolong berry shelf-life as well as enhance safety is still in demand for industries.

Pulsed-light (PL) is a newly developed decontaminating technique which uses short time, intense, broad spectrum (200-1100nm) yet rich in UV-C light pulses to inactivate microorganisms. The disinfection efficacy of PL has been widely proved against various bacteria, yeasts, as well as fungal spores *in vitro (*Anderson et al., 2000; MacGregor et al., 1998*)*. However, reduced effects were shown *in vivo* due to 'shadow effects' of complex food surface structures (Gomez-Lopez et al., 2005). Although much work has been done focusing on microbial inactivation efficacy of PL, studies on shelf-life extension has been relatively scarce. Apart from microbial inactivation, other possible effects, such as influences on water loss, fruit appearance, texture firmness and nutritional values have been reported after UV-C treatments on berries as well as on other fresh produces. (Baka et al., 1999; Mercier et al., 1993; Mercier et al., 2001; Pombo et al., 2009). Since UV-C is considered as the major

functional wavelength of PL, similar effects yet with reduced treatment duration may be expected using PL. Therefore, in this study, we investigated the potential of PL in strawberry and blueberry shelf-life extension and decontamination to seek possible applications in fresh berry industries.

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Chapter 2

LITERATURE REVIEW

2.1 The Growing Fresh Berry Market in the US

Chronic diseases, such as heart diseases, stroke and diabetes, are troubling half of all American adults today (Ward, 2014), and many cases have been attributed to unhealthy personal lifestyles, especially poor eating patterns. To alleviate this problem, a healthier daily dietary composed of a higher percentage of fresh produce is recommended. According to the Dietary Guidelines for Americans (2015-2020), consistent intake of fresh produce is related with reduced risks of chronic diseases, and at least 2.5 cups of vegetables and 2 cups of fruits are suggested to be consumed daily at the 2000-calorie level to meet the health requirement for adults (DeSalvo et al., 2016).

Fresh produce is becoming popular due to at least two dietary concerns. For one thing, when compared with heavily processed food, fresh produce contains no added flavors and preservatives which are not favored by customers today. For another thing, fresh produce can provide not only various essential nutrients such as vitamin C and dietary fiber, but also more non-essential health-promoting phytochemicals than other food groups. Because many types of fresh produce are eaten *'as is'* without further processing, these nutrients can be better preserved. Such functional nutrients include anthocyanins, flavonoids and polyphenolic compounds, many have been proved to be anti-carcinogenic or anti-aging. Both aspects have contributed to the prosperity of the fresh market.

Specifically, U.S. consumers are increasingly turning to fresh berries from traditionally more favored fruits such as apples and bananas. Berry fruits contain high antioxidant contents, and more basically, they are favorable for their distinct sweet-sour flavors and pleasant aromas, and are especially suitable for consumption in fresh form. Strawberry and blueberry are the winner and 1st runner up in the berry market for consumer favorability, which is shown in the following USDA statistics. For strawberry, the per capita consumption in the U.S. has increased from 4.86 to 7.22 pounds per person in the first decade of the 21st century, showing near 50% increase. The total value of utilized production for strawberry in the U.S. market has been growing as well since 1990. Noticeably, before 2000, strawberry consumption ranked 3rd with less than half the consumption of that of apples, while in 2010, strawberry outcompeted apple for the first time and became the most valued fresh fruit in the U.S. market, and has remained competitive ever since. Although both fresh and processed markets for strawberries are increasing, the fresh market has been prospering more rapidly than the processed market, taking up 80% of the total in 2010 which is 17% more than that in 1970. As for blueberry, consumption per capita has more than tripled since 2005, showing an even faster growth than strawberry (Ferreira and Perez, 2016).

2.2 Strawberry and Blueberry Shelf-life Extension

Be it for organoleptic, nutrient or food safety concerns, the 'freshness' of vegetables and fruits is always foremost for consumers. According to FAO statistics, food waste caused by consumer intolerance of reduced produce quality contributed a large percentage of total post-harvest food loss in developed countries (Aulakh and Regmi, 2013). Therefore, keeping the high quality of fresh produce from farm to plate is undoubtedly a critical economic concern for the food industry. Moreover, the post-

harvest loss of fruits and vegetables was nearly 30% in the US in 2010 (Buzby et al., 2014). This number for developing countries can be even higher, reaching 100% in some extreme occasions, and the great loss has led to serious waste of global resources. Therefore, finding new approaches to extend shelf-life of fresh produce and to reduce post-harvest loss is a concern in the light of current global food shortage situations. Unfortunately, for strawberries and blueberries are highly perishable fruits with short shelf-life. Strawberry generally has a shelf-life of 5-10 days under ideal storage conditions, while blueberries may last longer for up to two weeks. Therefore, studies focusing on berry shelf-life extension are necessary.

2.2.1 Shelf-life judging criteria for strawberry and blueberry

Some of the most important quality attributes that will influence consumer purchase behaviors are appearance, texture and aroma/flavor (Gallagher et al., 2011). These properties will be discussed in detail for strawberries and blueberries.

2.2.1.1 Appearance

Consumers will expect a near perfect appearance for berries. The most prominent defect is visible fungal decay. Almost any sign of microbial growth, such as mycelium and spores, will make berry commodities unacceptable. Other appearance defects include loss of glossiness, dryness, and a darkened, spotted or dented surface. Noticeably for blueberries, the waxy, white bloom on the skin which gives the fruit a lighter blue color is also an important trait to be evaluated (Saftner et al., 2008).

2.2.1.2 Texture

Loss of firm texture is also unfavorable for both berry fruits. Although with enclosed packaging in retail markets, firmness is not usually judged at purchase and

thus may not directly influence customer selection, the texture properties evaluated while eating will significantly influence repeat purchase. Also, texture qualities are related with some appearance defects and can be roughly judged via visual evaluations. For example, surface leakage and shrinkage can both signify softened berry texture.

2.2.1.3 Aroma

Keeping a natural, pleasant aroma is very important for aromatic fruits such as strawberries. As for blueberries, although they don't give out much fragrance at early shelf-life stages, unfavorable smells will appear as a sign of senescence near the end of shelf-life which will then work as a negative factor to influence customer selection.

2.2.2 Main processes lead to berry shelf-life loss

During berry shelf-life, there are three major processes leading to quick quality loss, natural senescence and water loss, mechanical damage, and fungal decay.

2.2.2.1 Natural senescence and water loss

Berries are living tissues that naturally senesce. The fruit respiration process in which organic matter such as glucose, cellulose, citric acid and many other phytochemicals decompose, gradually changes berry color, firmness, aroma, as well as nutrient contents, leading to various signs of freshness loss. Their high water-content also makes them sensitive to water loss, which is caused by respiration as well as mechanical transpiration.

2.2.2.2 Mechanical damage

Both berries are extremely soft and are thus sensitive to mechanical damages such as bruising and pressing, as well as stem scar damage specifically for blueberries. Mechanical damage IS hard to completely avoid during harvest, post-harvest handling and transportation.

2.2.2.3 Fungal decay

Due to their rich nutrient content and high water activity, strawberry and blueberry are feasible for yeast and mold growth (but not for most bacteria as a benefit of low pH). Although post-harvest decay usually happens during transportation or storage, berries can be latently infected during fruit development at the farm, long before the appearance of visible fungi. During the long pre-harvest and post-harvest periods, there are various situations providing numerous infection opportunities. Once visible fungi occur on a single fruit, the spoilage organisms can spread easily due to the other berries arranged in a same container. Fungal spoilage types and severity vary among cultivars and are highly dependent on climate, harvesting and processing chains as well as storage conditions. It can cause serious loss especially during wet seasons and under excessive relative humidity storage conditions.

The most common molds isolated from strawberries and blueberries are *Botrytis cinerea*, followed by *Alternaria*, *Fusarium*, and *Penicillium*. *Rhizopous* is also a common problem specifically for strawberries (Tournas and Katsoudas, 2005).

2.2.3 Major difficulties in berry shelf-life extension studies

Although plenty of work has already been conducted, shelf-life determination can still be a difficult. It should be noted that the final shelf-life is determined by a ‘barrel effect’. That is, the worst character of all judging criteria sets the endpoint of

shelf-life. The large variations between single fruits, and the complicated interactions between spoilage processes make the final spoilage point hard to predict. For example, genetic differences between cultivars can lead to differences in their sensitivity toward different spoilage factors. Even for the same cultivar, single fruits experience unique local environments in growing, transportation and storage, such as differences in light, moisture and microbial exposure, which may result in large variances in their spoilage processes. Additionally, the three major spoilage processes, namely the natural senescence, mechanical damage and fungal decay, usually interact with each other, both antagonistically and synergistically. For example, loss of moisture contents may lead to surface shrinkage and thus decreased blueberry shelf-life, but some extend of surface dryness may inhibit rapid growth of spoilage microorganisms. Synergistic effects exist when physiological decomposition of polysaccharides produce more favorable oligosaccharides for microbial growth which in turn promotes nutrient decomposition, and finally visible fungal decay or firmness loss may decide the endpoint.

With current processing techniques, it is difficult to extend shelf-life because of the need to target several quality loss processes simultaneously and synergistically without compromising overall quality. For example, heat treatment primarily aimed at microbial inactivation will cause extra weight loss during treatment. It may extend the shelf-life of wet season berries stored under high RH conditions but may reduce product quality in dry environments where fruit shrinkage would happen long before visible fungi develops. This complexity may lead to contradictory study outcomes and even inconsistency within the same study between different batches of samples. Therefore, treatment advantages and disadvantages should be considered and weighed

based on specific seasons and storage conditions so as to attain credible shelf-life extension effects.

2.2.4 Strategies applied and investigated to extend berry shelf-life

2.2.4.1 Proper storage environment and packaging techniques to reduce natural senescence and water loss

Many strategies have been tested and applied to prolong berry shelf-life, but keeping proper storage conditions with suitably controlled temperatures, humidity and atmosphere compositions is most important. Low temperature will slow down yeast and mold spoilage, since microbial growth and propagation are not favored. More importantly, the fruit respiration rate can also be significantly reduced, thus natural senescence is retarded. A proper relative humidity will help preserve water content by reducing the driving force of moisture transpiration, which is the water vapor pressure difference between fruit surfaces and the atmosphere (Tano et al., 2005). Therefore, a storage condition of near 0 ℃ and 90-95% RH is recommended for strawberry and blueberry preservation, and a pre-cooling process should be applied as soon as possible after harvest to quickly remove field heat (Mitcham and Perkins-Veazie, 2014).

As for gas composition, since elevated CO_2 and reduced O_2 levels can also inhibit fruit respiration, controlled atmosphere (CA) storage can also slow down berry deterioration. A composition of 5-10% O_2, 15-20% CO_2 and 2-5% O_2, 15-20% CO_2 are respectively recommended for strawberry and blueberry fruits (Kadar, 2001).

Modified atmosphere packaging (MAP) is a packaging method based on the idea of replacing air with a gas mixture more suitable for preservation. However, low oxygen levels can cause off-flavors due to anaerobic fermentation. Nielsen and

Leufven, reported improved weight and appearance maintenance of strawberry using MAP without causing off-flavor problems, though they denoted possible variances between cultivars (Nielsen and Leufven, 2008). The interactions between temperature, gas composition as well as fruit respiration were also studied in detail for modified atmosphere packaged blueberries by Beaudry and Cameron et al (Beaudry et al., 1992; Cameron et al., 1994).

Based on MAP, active packaging technology which uses oxygen, carbon-dioxide or ethylene scavengers in the package to provide a more equilibrated, finer-tuned local atmosphere, has also been studied. Some of the gas scavengers were preliminarily effective in preserving strawberry quality (Aday et al., 2011). However, a proper gas equilibrium may still be difficult to maintain, and more research should be conducted before commercial application of these new packaging techniques.

2.2.4.2 Special handling skills and containers to reduce mechanical damage

To avoid mechanical injuries, berry handlers must operate with extra care. Using specially designed containers and proper stacking strategies can significantly reduce bumping and stacking damage during transportation and storage.

2.2.4.3 Processing techniques to reduce fungal decay

To reduce the initial load of yeast and molds and to inhibit their propagation are also important areas to work on. Chemical fungicides were widely used for many fresh fruits, but they are becoming more unacceptable to consumers today due to health concerns. Many natural bioactive molecules have been investigated as alternatives. Edible coating with chitosan, for example, has been effective in reducing strawberry fungal decay, weight loss, firmness loss and color change without

significantly influencing nutrient contents (Hernández-Muñoz et al., 2006; El Ghaouth et al., 1991). Other bio-chemicals such as carvacrol, methyl cinnamate and hexanal were either applied in edible films or fumed as vapor during storage. However, edible coating can be both costly and troublesome. Also, since the coating material will finally be consumed with fruits, extra attention should be paid to the possible allergic reaction induced by these materials. Vapor treatment may raise less health concerns than by edible coating, but requires long treatment period in storage, thus it may not benefit the transportation process during which serious spoilage may have already occurred.

Heat treatment by dipping strawberries for 15 minutes in 45℃ water can reduce post-harvest decay and weight loss. The sugar content of the berries was not significantly influenced after cold and room temperature storage, while loss of skin brightness and calyx color were shown right after treatment (Garcia, 1995). Similar results were also shown after air heating for 3h at the same temperature, indicating that heat treatment may reduce fungal decay but may diminish berry sensory qualities (Vicente, 2002).

The effects of other non-thermal processing techniques were also investigated. For blueberries, significant reduction in fungi proliferation was found after e-beam irradiation at a dose of 0.5kGy after both room temperature (28-30 ℃) and cold (4 ℃) storage, while other important chemical and nutritional properties were not significantly changed (Kong et al., 2014). The effects of UV-C irradiations at 1, 2 and 4 kJ/m^2 were also evaluated by Perkins-Veazie et al. The samples treated at 2 J/m^2 showed the lowest ripe-rot occurrence, and the antioxidant contents in some but not all treated cultivars were stimulated (Perkins-Veazie, 2008). Other techniques such as

high pressure processing (Tadapaneni et al., 2014) and ultrasound (Aday et al., 2013) have also been studied, all showing some positive effects in reducing fungal decay. However, the reduced decay may not necessarily result in shelf-life extension. Some work didn't give out convincing quality evaluations, while others indicated that treatment-induced side effects may negate the advantage of microbial inactivation. Therefore, a convenient, economic and effective method to prolong berry shelf-life is still in demand.

2.3 Pulsed-light as a New Food Decontaminating Technique

Pulsed-light (PL) is a decontaminating technique which uses short time, intense, broad spectrum (200-1100nm) yet UV-C rich light pulses to inactivate microorganisms (Gomez-Lopez et al., 2007). This technique first originated in Japan for the sterilization of medical devices and pharmaceuticals, and was then approved by the FDA in 1996 as a food processing technique. Since pulsed-light is partly based on the continuous wave ultraviolet (CW-UV) treatment, a brief introduction to UV technology is first given below before a more detailed discussion on pulsed-light disinfection effects, mechanisms as well as the factors influencing its efficiency. The applications of pulsed-light in decontamination and some reported effects on fresh produce shelf-life are reviewed last.

2.3.1 UV treatment in the food industry

Ultraviolet light has been widely used for food disinfection since its germicidal effect was first found in 1878. The UV spectrum ranges from 200 to 400nm. The most germicidal part resides in the shortwave UV-C range (200-280nm) with disinfection efficiency peak at 254nm. The medium wave UV-B (280-320nm) and long wave UV-

A (320-400nm) are also bioactive, but they are far less lethal than UV-C. The germicidal effect has been mainly ascribed to the high UV-absorbing character of DNA molecules. Direct exposure to UV-C light can induce DNA photoproducts that destroy DNA structure and further impede DNA replication, and finally microbial propagation is inhibited. Since DNA is the genetic material of almost all living organisms, UV-C should inhibit most if not all pathogenic and spoilage microbial growth, provided that a sufficient dose of energy is applied to cause lethal damage. Apart from the influences on DNA, ultraviolet light can also affect other functional molecules such as proteins and lipids. Proteins, for example, may undergo photo-oxidation induced by reactive oxygen species generated by UV and unfold, aggregat or fragment (Manzocco, 2015). Loss of protein functions may result in cell growth deficiency, although this phenomenon is far less detrimental than DNA damage induced by UV-C (Gayán et al., 2014).

Due to low required capital cost as well as simple treatment operation, UV treatment can be economic, convenient and applicable as part of processing chains in the food industry. However, there are several reasons restricting its further application. One problem is that there are DNA repair mechanisms which can restore UV induced damage in most microorganisms. The repair process can be activated by visible light exposure after UV treatment and thus may impair its disinfection efficiency. This problem may be overcome by avoiding visible light exposure after UV treatment. Another disadvantage is that UV light cannot penetrate opaque surfaces below a depth of around 0.2mm (Manzocco et al., 2014). As a result, UV treatment has been restricted to the decontamination of air, liquid and surfaces. Since only direct exposure

contribute to effective inactivation, strategies to enhance target cell exposure may potentially promote ultraviolet light decontamination efficiency.

There are two major types of UV lamps currently used in application. One is the low-pressure mercury-vapor lamp which generates monochromatic light at 254nm, and the other is the medium-pressure mercury-vapor lamp that produce a broader light spectrum from 200-600nm.

2.3.2 Characteristics of pulsed-light when compared with CW-UV

As an upgraded version, PL retains the advantages of CW-UV as an applicable food processing technique. It is different from CW-UV, however, mainly in the following two aspects. One is that PL equipment generates high intensity light pulses instead of continuous radiation, usually by Xenon lamps. The power of each light pulse can be magnified manifold by releasing the long-stored (in milliseconds) electricity in a relatively short time (in microseconds) (Dunn et al., 1997). The increased energy density results in reduced processing time as well as enhanced decontamination efficiency. Rice and Ewell reported that it takes PL only 40s to deliver the same total fluence of $1 J/cm^2$ which CW-UV generates in 3 hours (Rice and Ewell, 2001). This improvement can be exceptionally valuable for fresh produce since the processing time after harvest should be as short as possible. PL is supposed to have slightly better penetration on opaque surfaces also due to its high energy-density**.** In addition, it was reported that no enzymatic repair occurred in PL-radiated microorganisms. This is possibly another benefit due to the high enough intensity which can destroy DNA repair systems (Gomez-Lopez et al., 2007)**.** The other difference resides in the much broader spectrum generated by PL lamps, which covers ultraviolet, visible (400-700nm) as well as short infrared wavelengths (700-1100nm).

Due to possible bioactivities of other wavelengths, this difference may also produce extra effects in microbial decontamination as well as in shelf-life extension.

2.3.3 Disinfection effect of PL

The disinfection effect of PL has been widely studied. *In vivo* PL treatment can effectively work against various microorganisms, including pathogenic and spoilage bacteria, yeasts, as well as fungal spores. Experiments carried out on agar plates or in buffer solutions were conducted by several research groups (Gomez-Lopez et al., 2005(a); Rowan et al., 1999; Krishnamurthy et al., 2004). Bacteria log reductions differed by species and ranged from 2.5 for *Lactobacillus sake* to >5.2 for *Alicyclobacillus acidoterrestris* after a same PL treatment of 7J per pulse. The same treatment also resulted in less than 3 log reduction of yeast (*Candida lambica*) and fungal spores (*Aspergillus flavus* and *Botrytis cinerea*). Reduced effects were shown when decontaminating food surfaces, possibly due to the shadow effect caused by complex food surface structures (Sharma and Demirci, 2003).

2.3.4 Disinfection mechanisms of pulsed-light

2.3.4.1 Photochemical effect

Although pulsed light exhibits a broad range of wavelengths, almost no log reduction can be achieved independently by >300nm wavelengths, and UV-C accounts for half the germicidal effect within the UV spectrum (Wang et al., 2005). Therefore, the major disinfection effect of PL has also been ascribed to DNA damage caused by UV-C exposure, and thus a light source with higher UV content will show higher disinfection efficiency (Rowan et al., 1999).

2.3.4.2 Photothermal effect

Apart from the photochemical effect, a photothermal mechanism was proposed by Wekhof to account for the disinfection effects which cannot be fully explained by a photochemical mechanism (Wekhof, 2000). The photothermal effect is caused by high peak power (which means a large amount of energy is delivered in a short time) of pulsed-light treatment with fluence exceeding $0.5 J/cm^2$. The high intensity light pulses can cause instant, regional overheating due to the large difference in energy absorbed by treated microorganisms and their surrounding media. Abrupt temperature rise will lead to water vaporization inside cells, and the steam produced may disrupt cell membrane and cause death. This mechanism was further supported by the fact that cell deformation happened to *A.niger* only after PL treatment but not after CW-UV treatment (Wekhof, 2001). Further evidence of the photothermal effect was provided by Takeshita et al.(2003) who showed that although similar levels of DNA damage were caused by CW-UV and PL, leakage of cellular protein was only seen in PL treated yeast cultures.

2.3.5 Factors influencing pulsed-light microbial inactivation efficacy

It is reasonable to assume that the effectiveness of pulsed light treatment may depend on the actual energy received by the key molecular machinery of the target microorganisms. Therefore, we may classify the influencing factors into three categories: the total dose of energy generated, the percentage of energy absorbed by effective molecules, as well as the required inactivation threshold that will finally decide the effectiveness of the received dose.

For pulsed-light treatment, influencing factors include pulsed-light intensity and total dose. The light intensity stated here refers to the actual energy level reaching

the sample surface per unit time and area. For a certain PL device, by increasing the distance from the light source, the measured light intensity first increases to a maximum and then attenuates quickly. Since the intensity peak at a distance too close (1 inch for Steri-Pulse XL 3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA)) to the lamp would not be encountered in practice, we may simply consider a negative correlation between intensity and distance. Then with fixed intensity, the accumulation of treatment time (or equivalently number of pulses) will decide the total energy dose at the sample surface.

The accuracy of key PL parameters provided in some reports, such as fluence (the total dose of energy per unit area, measured in J/cm^2), and peak power (energy per pulse divided by pulse duration, measured in W/cm2) is not always assured (Gomez-Lopez et al., 2007) due to difficulties in measuring those parameters at irregularly shaped food surfaces. Even with identical light intensity and total dose, different devices may generate diverse spectra which lead to different disinfection efficacy. Therefore, more characterization work should be done before comparable results are to be generated under different PL device settings.

For target microorganisms, genetic differences in DNA repair abilities may influence the 'threshold' of effective treatment dose. Also, due to the poor penetration of UV light, differences in cell appearance, structure and molecular composition will influence PL efficiency. For example, it is proposed that gram-negative bacteria are generally more sensitive than gram-positives (Rowan et al., 1999), possibly because they lack the protection by the thick peptidoglycan structure which can absorb part of the light energy. Similar inactivation deficiency may also happen when dark pigments in spores take up large portion of the light energy thereby protecting other key

molecules (Anderson et al., 2000). Also, a high culture concentration will cause 'shadow effect' and reduce PL efficiency, since only the microorganisms at the surface layer are exposed and inactivated.

Other factors, such as media composition and physical conditions including temperature, humidity and pH, will also influence energy delivery or microbial sensitivity. Gomez-Lopez et al. (2005b) found that supplemented protein and oil significantly decreased PL decontaminating efficiency while starch and water didn't show much influence. Furthermore, a synergic effect of mild heat and PL was found by Marquenie et al., (2003) probably due to DNA repair inhibition by thermal treatment, suggesting that PL may work better with other techniques as part of the hurdle technology.

2.3.6 Possible effects of pulsed-light on fresh produce shelf-life

Although much work has been done focusing on microbial inactivation effects of PL, studies on its shelf-life extension proficiency has been relatively scarce. As stated above, we may not anticipate shelf-life promotion simply by the decontamination effect, because fresh produce contains living tissues with similar molecular machineries that may be influenced by light treatment as well. Therefore, apart from microbial inactivation, it is necessary to consider other possible effects of PL on fresh produces. Since our objective is shelf-life extension, our discussion will focus on possible effects on the three shelf-life influencing processes, natural senescence and water loss, mechanical damage and fungal decay.

2.3.6.1 Effects on natural senescence and water loss

The respiration rate of fresh produces can be influenced by PL. For example, the respiration rate of fresh-cut lettuce increased after pulsed light treatment (Gomez-Lopez, 2005b). This can be a regional effect caused by direct influences on key enzyme, or it can be a global influence on gene expression profiles. Since plant metabolism reacts sensitively to environmental factors such as light and temperature, the high intensity, broad spectrum light generated by PL lamps may also work as such a signal. Actually, many studies have focused on metabolic as well as nutritional changes caused by UV-C. Lower respiration rates of strawberry, white asparagus spears, broccoli heads and Chinese kale were reported after UV-C treatment. The changes in metabolism may bring about changes in color, aroma and flavor which may influence shelf-life and customer preference (Baka et al., 1999; Huyskens-Keil et al., 2011; Costa et al., 2006; Chairat et al., 2013).

Since vitamins and other functional phytochemicals also participate in metabolic reactions, they are also likely to be influenced by light treatments. A dramatic enhancement in vitamin D contents from 0 to 824 % daily value per serving was reported in mushrooms after one second PL treatment (Kalaras and Beelman, 2008). Changes in benefitial phytochemicals have also been reported in fruits such as strawberries (Baka et al., 1999), oranges (D'hallewin et al., 1999), apples (hu Dong et al., 1995), and grapes (Cantos et al., 2002) after UV-C radiation. Therefore, sensory enhancement and nutritional promotion may possibly be some bonuses brought by pulsed-light treatment.

The high energy-density may also cause problems on treated samples, influencing their natural senescence process and water loss. Although it was claimed in some reports that the treated fruits did not show global temperature damage,

overheating may still happen during short periods and cause regional damage. Loss of quality attributes induced by high dose treatment was found by Lagunas-Solar et al. (2006) who indicated that energy as high as 2 J/cm^2 can cause injury to apples, oranges and peaches. Direct long time exposure to high intensity PL is therefore not recommended according to published papers as well as our own previous work. PL treatment may also increase water loss due to direct heating evaporation as well as the accelerated transpiration rate during cooling after treatment.

2.3.6.2 Effects on mechanical damage

Although light treatment cannot directly reduce the occurrence of post-harvest mechanical injury, some molecular changes resulting in better resistance toward mechanical damage may be induced. The accumulation of cell wall degrading enzymes can be slowed down after UV-C treatment in tomatoes, and thus product firmness was better preserved (Barka et al., 2000). Increase in tissue toughness was also reported by Huyskens-Keil et al. (2012) in asparagus. This enhancement in firmness can be of extra value for berry fruits that are soft and sensitive to mechanical damage.

2.3.6.3 Effects on microbial decay

The direct microbial inactivation mechanism of PL has already been elaborated above. Inactivation of both inoculated and natural bacteria, yeast and molds has been studied in detail. Noticeably for fungi, Lagunas-Solar et al. (2006) reported that PL treatment at as low a dose as $0.5 J/cm^2$ can effectively control most fungi on fruit surfaces. Furthermore, indirect microbial inhibition may also be induced in some types of fresh produces, since many phytoalexins that work as natural fungicide can be

promoted by UV-C, and the natural resistance of fruits toward yeasts and molds can then be enhanced (Ben-Yehoshua et al., 1992; Mercier et al., 1993; Mercier et al., 2001; Gomez-Lopez et al., 2007).

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Chapter 3

EFFECTS OF PL IN STRAWBERRY SHELF-LIFE EXTENSION

Abstract

In purpose of strawberry shelf-life extension, pulsed-light treatments of low intensity $3J/cm^2$ total dose (L3), medium intensity $3J/cm^2$ total dose (M3) and medium intensity $5J/cm^2$ total dose (M5) were applied to strawberry samples before cold storage. All three PL treatments delayed visible fungal development and overall spoilage, better preserved strawberry surface glossiness and retarded anthocyanin accumulation during storage. Among the three treatments, M3 showed better shelf-life extension effects, although L3 and M5 achieved better decontamination effects and slightly enhanced surface redness right after treatment. No noticeable difference in firmness, pH, titratable acidity and total soluble solid was significantly induced. However, although not obviously reflected in final shelf-life, a tendency of accelerated quality degradation was discerned in the samples treated with higher total dose PL, suggesting cautious use of high total dose treatments in future applications.

3.1 Introduction

Strawberry is worldly favored for its sweet and sour flavor, appealing fragrance as well as its high contents of health promoting compounds such as anthocyanin and phenolic contents. The strawberry market in US has been increasing continuously in the past decades, encouraging more planting in native farmlands in California, Florida, Oregon and so on, as well as more import from worldwide (USDA ERS, 2013). Unfortunately, strawberries are highly perishable and have a relatively short shelf-life. The most severe factor to cause strawberry post-harvest loss is fungal decay, with grey mold spoilage caused by *Botrytis cinerea* being the leading disease

(Mitcham, 2014). Other quality loss such as mechanical injuries and loss of moisture content are also very common, respectively due to the soft flesh structure and lack of protective skin of strawberries. All above characteristics have made strawberry highly sensitive to handlings as well as atmospheric changes.

Due to their soft, irregular surface structure, chemical fungicide wash or spray which are commonly used in fresh produce industries can be highly unfavorable in strawberry productions, since the chemicals may easily adhere to strawberry surfaces and be absorbed, posing threat to consumer well-being. Various non-chemical wash food processing techniques such as heat treatment (Garcia et al., 1995; Civello et al., 1997), UV-C radiation (Baka et al., 1999, Nigro et al., 2000), edible coating (Del-Valle et al.,2005), vapor treatment (Hu et al., 2012) and combined treatments (Pan et al., 2004; Allende et al., 2007) have been studied, all showing fungal inhibition effects to some extends. However, either other side effects in sensory properties or excessive capital, material or time cost must be introduced, making industrialization less favorable. Therefore, alternative techniques that are effective, rapid and applicable in strawberry quality preservation is in great demand for strawberry industries.

Pulsed-light is a newly developed microbial decontamination technique, and has been proved to work effectively against various bacteria, fungi and viruses on agar plates or in culture liquids in previous researches. *In vitro* microbial inactivation effects of PL for food safety concerns have also been studied on minimally processed fresh produces (Gomez-Lopez et al., 2005). However, the inactivation efficacy of PL has been diminished due to the reported ‘shadow effect’ which is caused by poor light penetration. High intensity and high total dose PL radiations are usually applied in researches to enhance microbial inactivating. However, due to its high energy-density,

intense PL treatments may cause direct damage on sample surfaces and may induce surface heating, both of which are harmful to food quality. As a result, although PL has showed the potential to inhibit microbial development, PL studies aiming at fresh produce shelf-life extension has been relatively scarce.

In this study, we investigated the influence of PL treatments on strawberry shelf-life to look for possible applications in strawberry industries. Instead of using high intensity, high total dose PL treatments that are theoretically more effective in microbial inactivation, we adapted the commercial PL device with a self-designed large treatment chamber, so as to apply a milder PL treatment with reduced intensity. With the new device, we investigated the influence of low intensity 3J/cm^{2}otal dose (L3), medium intensity 3J/cm^{2}total dose (M3) and medium intensity 5J/cm^{2}total dose (M5), hope to achieve strawberry shelf-life extension without further diminish their sensory characteristic as well as nutritional values through storage.

3.2 Materials and Methods

3.2.1 Strawberries

Strawberry fruits used in this research were kindly provided by Dole (Dole Food Company, Inc., Thousand Oaks, CA). Strawberries were harvested and transported overnight from California or Florida farmlands, and were sorted immediately upon arrival. Samples with any sign of rot or diseases were discarded, and intact samples with uniform color and size were selected for treatment. Based on the average sample size of each batch, specific number of strawberries were allocated in each pint PET clamshell (4/6/8 fruits per package, Highland packaging solutions,

Plant City, FL). The packed samples were then separately placed in refrigerator at 2-4℃ to be cooled for two hours before treatment.

3.2.2 PL treatments

PL treatments were conducted with a reconstructed PL system, which consists of a commercial PL lamp with its controlling and cooling modules (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA), and a self-designed, enclosed treatment chamber (inner size 60cm×45cm×70 cm) connected with a high flow ozone destruct unit (Ozone Solutions Inc, Hull, IA). The commercial Xenon PL lamp is enclosed in a lamp housing, which is mounted at the top of the chamber. The lamp generates 180-1100nm wavelength with pulse width of 360μs and pulse rate at 3 pulses / sec and according to previous researches, 40% of its energy generated is within the UV spectrum (Hsu and Morazu,2011).

To be treated, strawberries were placed separately on a height-adjustable meshed metal shelf inside the chamber. PL intensities received by samples were adjusted by changing the height of the shelf to alter sample distance from the PL lamp. Three PL intensities were used in this research, respectively the high intensity (0.21 J J/cm^2 per pulse, metal shelf at ~15cm from the quartz window of the lamp housing), medium intensity (0.10 J/cm^2 per pulse, ~35cm from quartz window) and low intensity (0.05 J/cm^2 per pulse, ~55cm from quartz window) treatments. All treatments were applied inside the chamber with door fully closed, and each treatment was always followed by an ozone discharge process to avoid ozone overflow. After being treated one side, the strawberries were manually flipped on the shelf to treat other side, with half the total dose applied to each side. Samples were placed back into the original clamshells after both sides being treated.

3.2.2.1 Measurement of PL parameters

The PL intensity was measured using a Vega laser power meter (Ophir Optronics Inc., Wilmington MA) with a connected pyroelectric energy sensor (PE-50C, Ophir Optronics). Parameters were set at 300nm wavelength and 500μs pulse width. The sensor was placed at the center of the metal shelf at each height measured, and a 10s treatment was used for a stable intensity reading. The total doses were calculated by multiplying the number of pulses with the measured intensity.

3.2.2.2 PL parameter selection

A series of pre-experiments were performed to help select optimal PL parameters. Sorted strawberries were irradiated with high intensity 3 or 6 J/cm^2 total dose, medium intensity 1.5, 3, 5, 6, 9 J/cm^2 total dose or low intensity 1.5, 3, 6 total dose PL. At least 3 clamshells (24 strawberries) were used for each tested treatment, and were observed daily to monitor weight loss, molds development and visual deterioration. Three parameters of L3 (low intensity, 3 J/cm^2 total dose, 10s treatment time), M3 (medium intensity, 3 J/cm^2 total dose, 5s treatment time), M5 (medium intensity, 5 J/cm^2 total dose, 8s treatment time) were selected for further studies.

3.2.3 Experiment design

Experiments were performed in 5 batches. Strawberry fungal development and weight loss were first studied in a same batch. The spoilage index of individually packed samples was evaluated in a second, and the sensory properties, nutrient contents, and natural yeast and mold analysis were studied together in a third batch. The decontamination of spot- and dip- inoculated *Salmonella* were each performed in a single batch. Samples in a same clamshell, usually containing 4-8 berries, were always treated together as a unit, while different clamshells were treated one by one in

random sequences. Different numbers of clamshells may be treated in different batches of experiments to fit in specific sample sizes of different evaluations. Except for the fungal decay evaluations that have been repeated four times, all experiments were repeated three times.

3.2.4 Strawberry packaging and storage conditions

Except for the strawberries used for spoilage index studies, all other samples were packed in clamshells, and the clamshells were further arranged into corrugated boxes (12 clamshells in each box) to be stacked for cold storage at 2-4 ℃, 30-40% RH, in order to assimilate commercial packaging and storage conditions. Samples for spoilage index studies were individually packaged in 16.8 x14.9cm sandwich zipper bags (Glad Products Company, Oakland, CA) and stored at 5 ℃, 85-95% RH for the convenience of individual observations and gradings.

3.2.5 Strawberry fungal decay and weight loss

3.2.5.1 Fungal decay

Five clamshells of strawberries were separately treated for each of the four treatments. Fungal development was checked in each clamshell, and the accumulative number of clamshells showing visible fungal decay was recorded for each of the control or PL treatments every two days until all clamshells became spoiled. Once with any sign of fungi, the whole clamshell of strawberries was discarded for fear of cross-contamination between different clamshells.

3.2.5.2 Weight loss

Weights of empty clamshell containers were recorded as W_C before strawberry samples were packed, and the total weight of each clamshell with strawberry samples was measured right before treatments as the initial weight (W_{d0}), then followed every two days during storage (W_{dn}, n representing the number of days after treatment at weight measurement) until the first appearance of fungal decay. Clamshell positioning inside a corrugated box was randomly shuffled after weight measurements to reduce variations caused by uneven air circulating. Weight loss was indicated by the percent loss of initial weight, which was calculated using the following formula:

$$\%\ \text{weight loss at day n} = \frac{(W_{d0} - W_C) - (W_{dn} - W_C)}{(W_{d0} - W_C)} \times 100\%$$

3.2.6 Spoilage index evaluation

48 strawberries were used for each treatment, and the strawberries were individually sealed in sandwich bags with punctured holes right after treatments then stored at 5℃, 85-95% RH for 10 days. During storage, samples were visually evaluated every two days and were sorted into 5 grades from 0 to 4, according to a judging criteria chart provided by Dole Company. The number of samples assorted into each grade was recorded as N_0 to N_4accordingly, and a spoilage index was calculated for each treatment using the following formula,

$$\text{Spoilage Index (\%)} = \frac{0\times N_0 + 1\times N_1 + 2\times N_2 + 3\times N_3 + 4\times N_4}{4\times 48} \times 100\%$$

This experiment was repeated three times.

3.2.7 Total yeast and mold, quality attribute and nutrient content analyses

Analyses of natural yeasts and molds, sensory quality attributes as well as nutrient contents of control and treated strawberries were conducted using a same

batch of samples. All evaluations were performed right after treatment, and then at day 10 and day 20 during storage. 6 clamshells of strawberries were treated for each of the Ctrl, L3, M3 and M5 treatments, and 2 out of the 6 were used at each sampling day for all the above analyses.

3.2.7.1 Natural yeast and mold count

2 strawberries were randomly selected from the two clamshells and were put into a 24oz. filter bag (Whirl-Pak, Nasco, USA). Dey-Engley (D/E) neutralizing broth was added at a 4:1 v/w ratio before samples were homogenized for 2min at 260rpm using a stomacher (Seward 400, Seward, London, U.K.). The homogenate was then 10×serial diluted in 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD) and spread plated on potato dextrose agar (Difco) to be further incubated at room temperature for 5 days. Plates with countable colony numbers between 10 to 150 were counted. Plating were done in triplicates.

3.2.7.2 Quality attributes

Strawberry surface color, firmness, as well as acid and sugar contents were also evaluated. For fruit surface color, the Hunter L, a*, b* values were measured at 5 random spots on a single fruit using a chrome meter (Minolta CR-400, Minolta, Osaka, Japan). 5 fruits were used for each treatment to give 25 reads at each sampling day. After color measurements, the same fruits were cut in halves for firmness analysis. Strawberry firmness was evaluated by a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming UK) with 5kg load cell. A 1/4-inch diameter cylinder flat probe (TA-24, Stable Micro Systems) was used to puncture 5mm into the flesh. The test speed used was 1.0mm/s, and the pre- and post- test speeds are 5 and 8mm/s

respectively. Maximum force to break strawberry surface was recorded and shown in Newton (N). Two spots around the equatorial area were measured for each strawberry half so as to give 20 reads using the same 5 berries.

Another 5 strawberries that were not used for color and firmness tests were minced and then blended with blender for 1 min to make strawberry puree. 45mL puree was centrifuged (Centra CL2, Centrifuge, Thermo Scientific, Waltham, MA) at 5000rpm for 10min in a 50mL centrifuge tube (Falcon, BD Biosciences, San Jose, CA) and the supernatant was collected for further evaluations. The pH value of the supernatant was read by a pH meter (pHTestr20, Oakton Instruments, Vernon Hills, IL), and total soluble solid (Brix°) was measured using a refractometer (Reichert Abbe Mark II, Reichert Inc., Depew NY). Both measurements were made in triplicates. For titratable acidity, 5g juice was weighed in beaker and then diluted with 50mL DI water. The diluted juice was then titrated with 0.1M NaOH (Fisher Scientific, Hampton, NH) to pH 8.1-8.2 measured with the same pH meter. Titration were made in duplicates and the titratable acidity was described as mg citric acid/g strawberry juice.

3.2.7.3 Nutrient contents

3.2.7.3.1 Extraction

Same puree made for acid and sugar content evaluations was also extracted to be used in nutritional analyses. 2 g puree was accurately weighed in a 15mL centrifuge tube (Falcon, BD Biosciences, San Jose, CA) and 10mL acidified methanol (Sigma–Aldrich, Inc., St. Louis, MO, USA, with 0.1% HCl, Fisher) was added. The tubes were vigorously shaken for 1min and then stored under 4 ℃ for 2 hours. Mixtures were

centrifuged at 5000rpm for 10min. The methanol supernatant was collected in a new 50mL centrifuge tube, and the fruit sediment was extracted for a second round as described above using another 10mL acidified methanol. The second extract was centrifuged and collected together with the first extract. Extraction was done in duplicate tubes for each puree, and triplicate reactions and plate reads were further made in 2mL micro-centrifuge tubes (Seal-Rite, USA Scientific, Ocala, FL) for each extract in all three analyses.

3.2.7.3.2 Anthocyanin

The total monomeric anthocyanin contents of strawberry were measured using the pH differential method introduced by Glusti and Wrolstad. 100μL methanol extract was diluted in 500μL pH 1.0 0.025M potassium chloride (Fisher) and pH 4.5 500μL 0.4M sodium acetate (Fisher) buffer. The absorbance readings of both dilutions were measured at 508nm and 700nm. The absorbance of the sample diluent (A) was then calculated as

$$A = (A_{508} - A_{700})_{pH\ 1.0} - (A_{508} - A_{700})_{pH\ 4.5}$$

and the anthocyanin concentration in the extract was calculated as μg Pg-3-glu per milliliter by the following formula

$$\text{Pg-3-glu (μg/mL)} = (\text{A} \times 433.2 \times 6 \times 1000)/17300$$

in which 433.2 is the molecular weight of Pg-3-glu, 6 is the dilution factor of the sample diluents, and 17300 is the molar absorptivity of Pg-3-glu in 0.1% HCL acidified methanol which is reported by Giusti et al. The anthocyanin contents of fresh strawberry samples were then calculated and presented as μg Pg-3-glu /g fresh weight.

3.2.7.3.3 Total phenolic content

The total phenolic content was determined using Folin-Ciocalteau method (Singleton et al., 1999). To make a final 2mL reaction system, 50μL of strawberry extract was added to 1650μL water, then 100μL 0.25M Folin-Ciocalteau reagent (Sigma–Aldrich) was mixed and let stand for 3 minutes before 200μL 1N sodium carbonate (Fisher) was added to stop the reaction. The reaction tubes were then incubated in dark at 37 ℃ for 45min for complete color development before absorbance reading at 765nm. The total phenolic content was then expressed as gallic acid equivalents by fitting the absorbance into a standard curve predetermined by standardized gallic acid (Sigma–Aldrich) solutions.

3.2.7.3.4 Total antioxidant activity

The total antioxidant activity of strawberry extract was determined by the DPPH method reported by Brand-Williams et al. with modifications. 40μL extract and 60μL 2.5mM DPPH solution (Sigma–Aldrich) was mixed in 1900μL methanol to make a 2mL 75uM DPPH reaction system. The reaction tube was sit in dark under room temperature for 15min before absorbance reading at 515nm. The total antioxidant activity was then expressed as Trolox equivalents by fitting the absorbance into a standard curve predetermined by standardized Trolox (Sigma–Aldrich) solutions.

3.2.7.3.5 Absorbance reading

Absorbance readings were conducted together after all reactions finish. 200μL reaction solution in each 2mL reaction tube was added to a single well of flat bottom 96-well plate (Falcon, BD Biosciences, San Jose, CA) and then read at specific

wavelengths by a multi-mode reader (Synergy 2; Biotek Instruments, Winooski, VT, USA).

3.2.8 Decontamination of spot- and dip-inoculated *Salmonella*

Decontamination effects of PL against spot- and dip-inoculated *Salmonella* were conducted separately in two batches. 5 clamshells of strawberries were used for each treatment to be analyzed at day 0, 5, 10, 15 and 20 during storage respectively.

3.2.8.1 Inoculum preparation and strawberry inoculation

3.2.8.1.1 Bacteria strains

Four *Salmonella enterica* serotypes of Newport, Montevideo, St. Paul and Stanley that were previously adapted to be nalidixic-acid resistant (Huang et al., 2013) were used in this study. The four strains were retained on tryptic soy agar (Difco) supplemented with 0.6% yeast extract (Bacto) and 50μg/mL nalidixic-acid (Fisher) at 2-4 ℃. All culture tubes and plates were incubated at 37℃.

3.2.8.1.2 Spot inoculation

Four serotypes were individually incubated in 10mL tryptic soy broth (Bacto) tubes supplemented with 0.6% yeast extract and 50μg/mL nalidixic-acid (TSBYE-NA) for 24h at 37℃ and then each transferred to 2 tubes of 10mL TSBYE-NA for another 24-hour incubation so as to obtain a final concentration of ~10^9CFU/mL. All 8 tubes of *Salmonella* culture were mixed and centrifuged in two 50 mL centrifuge tubes at 4000g for 10 minutes. The media supernatant was discarded, and the bacteria sediment was re-suspended with 20mL 0.1% peptone water to obtain a 4x concentrated *Salmonella* inoculum. Strawberry samples were assorted in clamshells

beforehand and placed in biosafety hood for inoculation. Total 1 mL of inoculum was inoculated on the surfaces of 6 berries in each clamshell and was operated carefully to avoid calyx inoculation. The inoculated berries were left with clamshell lid open in the biosafety hood for two hours and then stored in refrigerator overnight before PL treatment.

3.2.8.1.3 Dip inoculation

Similarly, the four strains were individually incubated in 10mL TSBYE-NA tubes for 24 hours and were each transferred to two 200mL TSBYE-NA media in 800mL Erlenmeyer flasks (Corning Inc., Corning, NY) for another 24-hour incubation. All 1600mL culture of the four strains was mixed and centrifuged to discard media supernatant, and then re-suspended with total 3200mL of peptone water in a bucket as dip inoculum. 150 assorted strawberries were then dipped in the inoculum with sufficient stir for 2 minutes. The strawberries were drained with strainer and then separated into 20 clamshells (6 strawberries per clamshell) to keep each clamshell in similar total weight. The dip-inoculated berries were also left with clamshell lid open in the biosafety hood for two hours and then stored in refrigerator overnight before PL treatment.

3.2.8.2 Microbial analyses

For each treatment and at each sampling point, 6 strawberries in a clamshell were separated in 3 sterile 24 oz. filter bag for triplicate plating. Net weight of strawberries was weighed for each filter bag, and D/E neutralizing broth was added at a v/w of 4:1 for homogenization. The homogenate was then 10×serial diluted in 0.1% peptone water and spread-plated on TSAYE-NA to be further incubated under

37℃ for 48 hours. Plates with countable colony numbers between 30 to 300 were counted.

3.2.9 Statistical analyses

Analyses of variance (ANOVA) were performed using JMP Pro 13 within a same sampling day taking 'treatment' as source of variance, and time of repeats as blocks. Tukey's HSP test was used to detect significant differences at $\alpha = 0.05$ level between all four treatments.

3.3 Results and Discussion

3.3.1 PL parameter selection

A series of pre-tests were conducted to select the PL parameters for further systematic experiments. Results showed that no matter the intensity, high total dose treatments ($\geq$6J/cm^2) didn't show consistent shelf-life enhancing effects in different replicates. Medium doses of 3 and 5J/cm^{2}delayed fungal decay more effectively than low dose (1.5J/cm^2). In addition, specifically for high intensity treatments, although fungi inhibition effect was also displayed after a medium (3J/cm^2) total dose radiation, significant increase in sample weight loss was also detected at the meantime. Considering less of radiation uniformity and other inclined side effects on samples, no high intensity treatment was selected. The medium intensity 3J/cm^2, 5J/cm^2, as well as low intensity 3J/cm^{2}treatments were used for further studies.

3.3.2 Effects of PL on strawberry shelf-life

3.3.2.1 Visible fungal decay

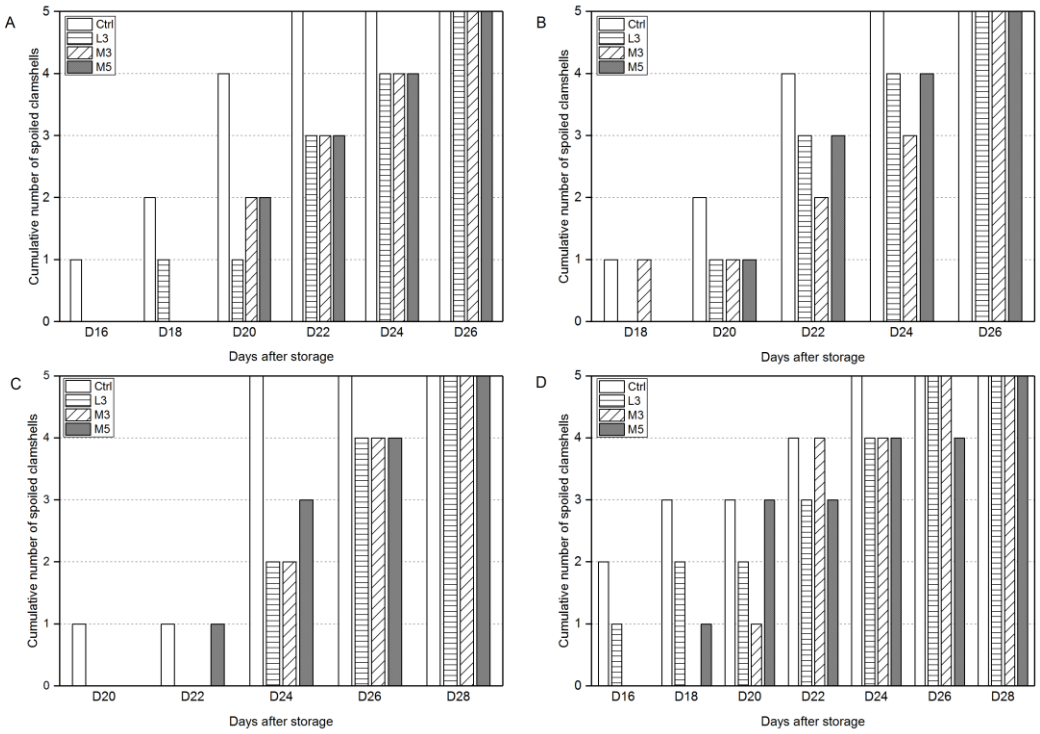

Figure 3.1 Accumulative numbers of strawberry clamshells showing visible fungal decay after Ctrl, L3, M3 and M5 treatments during storage.

Note: Data were collected every two days after treatment until all clamshells spoiled. The four figures labeled as A, B, C and D show results of four independent replicates respectively.

Due to low RH used in our research, visible fungi appeared relatively late at 16 - 20 days after treatment. Two most common signs of decay were green and white molds. Most clamshells showed spoilage during a short period from day 20 to 28. Sporadically earlier spoilage almost always displayed in control samples during day 16 to day 20. In PL treated samples, spoilage appeared two to four days later than control in most cases, with exceptions of the 2nd replicate M3 and the 4th replicate L3 treatments which showed visible fungi on the same day as control.

Besides later fungal development, PL treated samples also showed later total spoilage of all 5 clamshells than control, indicating an overall delay in fungal development. Complete spoilage of PL treated samples generally happened between day 26 to day 28, which was also 2-4 days later than that of control. Comparing the three PL treatments, M3 performed slightly better than L3 and M5 by showing latest first fungal appearance in 3 out of the 4 replicates. More obvious difference between the three treatments was not discernable in this experiment. Therefore, PL delayed visible fungal development on clamshell stored strawberries, with M3 treatment showing slightly later development than L3 and M5.

3.3.2.2 Spoilage index

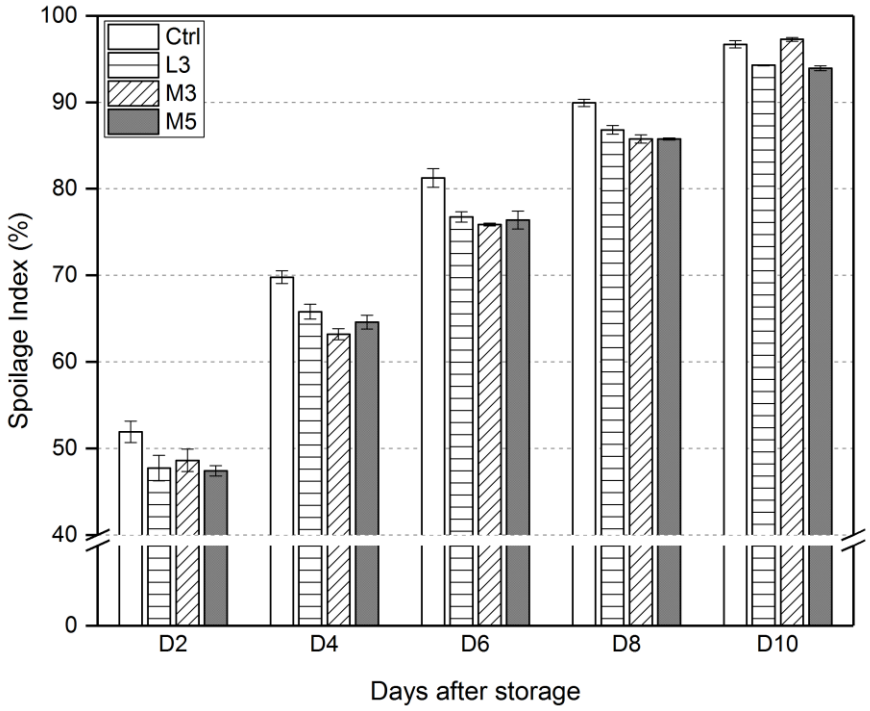

Figure 3.2 Spoilage index (%) of individually packed Ctrl, L3, M3 and M5 treated strawberry samples during 10 days of storage at 5℃.

Note: Data were collected every two days after treatment until day 10. Error bars represent ±SE of three replicates.

The main purpose of using PL in shelf-life extension is to inhibit fruit decay caused by microbial growth, and this effects has been proved in 3.3.2.1. Although fungal decay is the most contributor to strawberry post-harvest lost, we found that many strawberry samples had become unacceptable for consumption before visible fungi developed, indicating that other spoilage processes still play much importance in determining strawberry shelf-life. The spoilage index evaluation was designed to give a comprehensive evaluation of sample acceptance. As shown in Figure 3.2, for all strawberry samples, rather high spoilage indexes of over 40% were presented only two days after treatment. This was mostly contributed by surface injuries or leakages caused by mechanical compressions, possibly due to unavoidable handlings during treatment. While after day 2, other phenomenon such as loss of glossiness and signs of microbial decay including leather rot and black sunken spots became more obvious, which further increased the spoilage index. Visible mycelium of grey or white molds may appear from day 4 to 6 onwards on a few samples. The time of visible mold development is much earlier than that of clamshell samples, possibly due to high local relative humidity inside individual packages. When comparing PL treatments with control, the PL radiated samples still showed reduced spoilage index by day 8, although the effect was not distinctly indicated in values. Relatively more obvious effects of PL were shown before day 6, and the largest reduction in spoilage index of 6.6% was shown by M3 at day 4. As a conclusion, PL has also showed positive effects in delaying overall quality loss of strawberries during cold storage, yet noticeable difference between the three PL treatments was not clearly indicated.

3.3.3 Weight loss

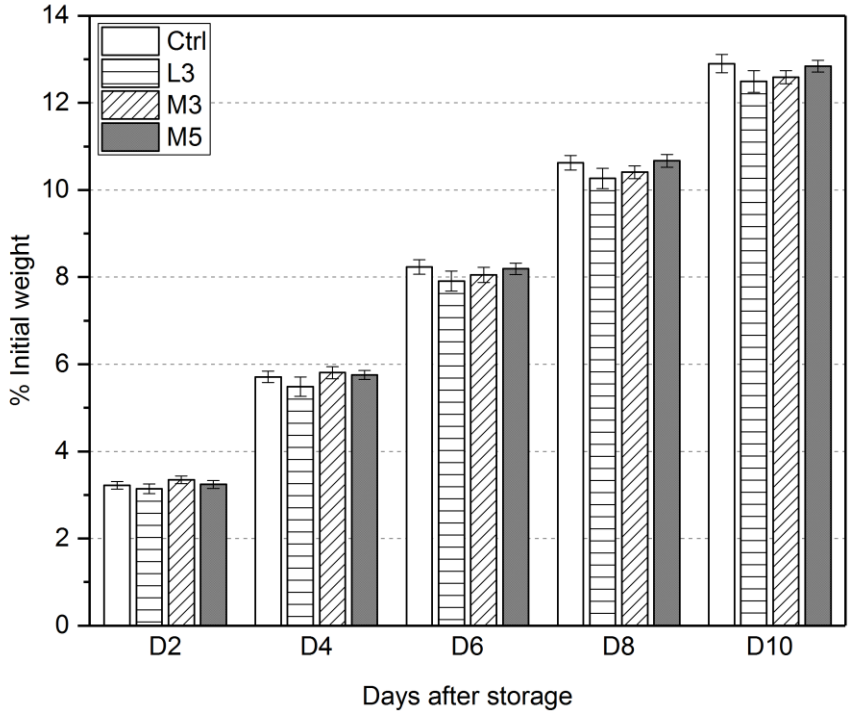

Figure 3.3 Weight loss (%) of Ctrl, L3, M3 and M5 treated strawberry samples during 10-day storage.

Note: Data were collected every two days after treatment until day 10 and were shown as percentage loss of initial weight. Error bars represent ±SE of three replicates. No significant difference between treatments was detected at any sampling day and thus was not labeled in the figure.

In our experiment, strawberries showed relatively high weight loss during storage due to low storage RH. For both control and PL treated samples, moisture loss of over 3% was detected at day 2, and a >10% loss was achieved at day 8. Significant differences between treatments were not detected on any of the sampling days. However, it may still be worth noting that slightly reduced moisture loss was detected after L3 treatment all through the 10-day storage. Meanwhile, M3 showed slightly higher moisture loss when compared with control by day 4, but was surpassed by control as well as M5 at day 6 and afterwards. At the very least, although weight loss enhancement can be a reasonably anticipated side effect of PL treatments (as have been detected in high intensity PL treated samples in pre-experiments), none of the three selected PL parameters promoted strawberry weight loss.

3.3.4 Natural yeast and mold growth

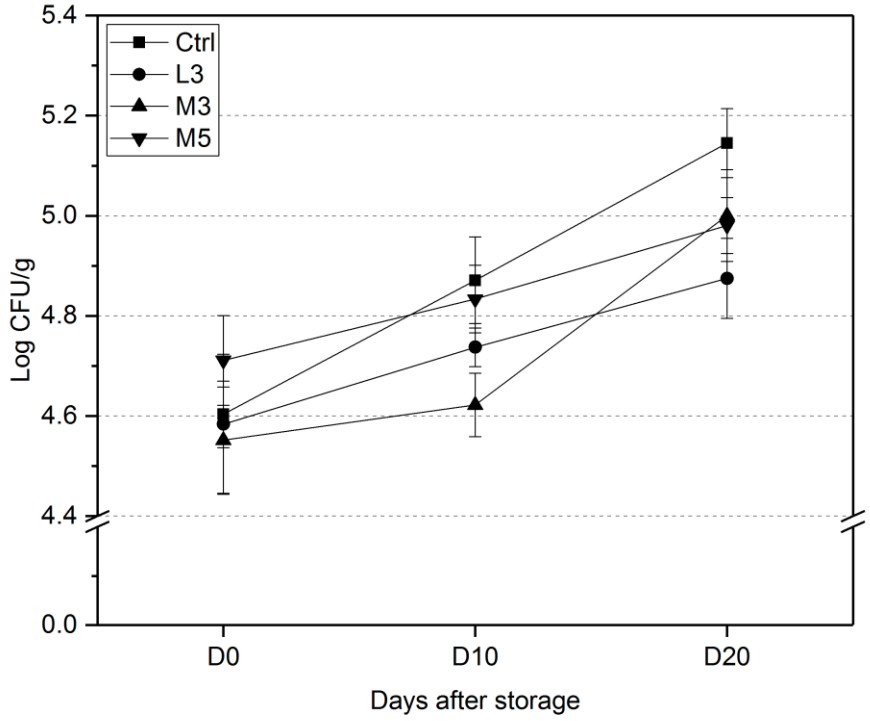

Figure 3.4 Total yeast and mold count of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20.

Note: Data are shown as Log CFU/g strawberry fresh weight. Error bars represent ±SE of three replicates.

Analysis of natural yeasts and molds on strawberries was also conducted. During cold storage, unsurprisingly, the total count for all samples gradually increased by 0.54 for control, and by an average of 0.34 for PL treated samples. Although this increase was numerically low, it should be noted that visible molds had already developed at the end of storage on day 20. This can possibly be explained as that local enrichment in microbiota on strawberry surfaces may have been compromised by the large total mass of whole fruits after homogenization.

Right after treatment, no significant reduction was detected**.** However, at d10, the log CFU concentration of control increased by 0.26, which more than doubled the average increase of 0.12 log CFU/g for PL treated samples. To be more specific, L3 treatment showed most obvious increase of 0.15 CFU/g in the three treatments, while M3 showed least growth of 0.07 CFU/g by day 10. M3 also showed the lowest count which is statistically lower than control at day 10. Therefore, yeasts and molds growth was inhibited after PL treatments during early and mid-storage.

While at day 20, control still showed highest count in all four treatments, yet L3 instead of M3 showed the lowest count, which may indicate that the advantage of M3 treatment found in the above shelf-life studies may be more obvious during early and middle stages of storage. This is agreeable with the phenomenon that M3 showed most delayed first fungi appearance but not the time of total spoilage, as well as the spoilage index which was mostly inhibited by M3 during early storage.

Effect of PL against yeast and molds have been proved *in vitro*. Marquenie et al (Marquenie et al, 2003) has reported 3 log reductions of *B.cinerea* as well as *Monilia fructigena* conidia using PL treatments of over 150s. The low or even none direct log reduction detected after treatment in our study was possibly due to low

intensity and total dose. The PL parameters used in Marquenie's research were not reported. However, a much shorter treatment time was used in our research (no longer than 20s), suggesting a possible difference in total dose. In fact, less than 0.3 log reduction was achieved by using treatments of ~20s in the above research. Also, for *in vivo* inactivation, part of natural microbiota may reside in fleshes or under calyx that PL can hardly reach. Since samples were treated in suspension in the above research, it was likely to show better inactivation effects.

However, in our research, yeast and mold growth in PL treated samples were still delayed during storage when compared with control. This is an indication that other mechanisms may have been induced to resist microbial development. The yeasts and molds that had been exposed to but not completely inactivated by PL may became injured and developed slowly at low temperature. Another mechanism proposed is that PL may have induced antifungal phytoalexins synthesis, which has been reported in carrots and tomatoes against *B. cinerea* (Mercier et al., 1993; Charles et al.,2008). In sum, the result of total yeast and mold count supported the shelf-life studies in 3.3.2.1 and demonstrated the effect of PL in delaying fungal decay.

3.3.5 Quality attributes

3.3.5.1 Surface color

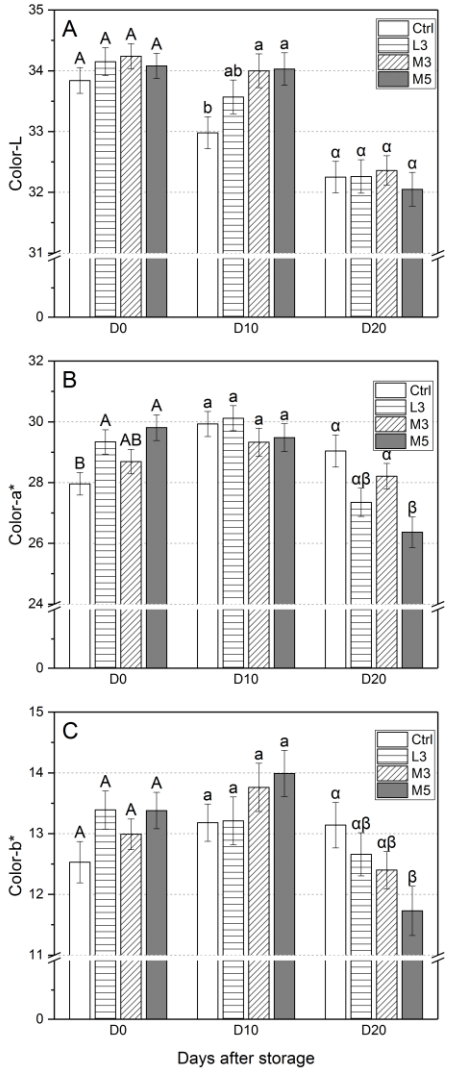

Figure 3.5 Color L (figure A), a* (B), b* (C) values of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20.

Note: Error bars represent ±SE of three replicates. Treatments labeled with same alphabetic letter at each sampling day are not significantly different (α= 0.05).

The surface color L, a*, b* values, indicating surface lightness, red and blue color respectively, are presented in figure 3.5. No significant instant effect on L value was detected after any of the three PL treatments. During storage, all samples showed reduced L value as expected, in parallel with the glossiness loss during storage. However, at day 10, both treatments using medium PL intensity (M3 and M5) showed significantly higher L values than control, and the low intensity L3 treatment also presented higher L value though without statistical significance. This delayed decrease in L value can be an indicator of better preserved surface glossiness after treatment. However, significant differences among treatments were no longer detected at day 20. L3 and M3 samples still showed higher L value, yet the samples treated by higher total dose M5 dropped to a lower level than that of control, possibly indicating an accelerated quality loss of M5 treated samples during late storage.

The red color of strawberry, reflected by a measured a*value, is also an important parameter to influence strawberry appearance and customer preference. As shown in Figure 3.5, the a* values of strawberries were promoted instantly after all PL treatments, although significance was only detected in L3 and M5. The overall a* value of stored strawberries was slightly increased at day 10, but then more obviously dropped at day 20. No obvious difference among treatments was detected at day 10, while at day 20, the higher dose M5 treatment showed significantly lower a* level than control. Again, this possibly indicates an accelerated red pigment degradation after high dose PL treatment during late storage stages.

The b* value, although influences overall surface color display, was not as important an evaluation factor as L and a* for strawberries. Significant difference

between treated and control samples was not discerned right after treatment or at day 10, yet a lower b* value was shown by the high dose M5 treatment at day 20.

In sum, PL showed both instant and continuous influences on strawberry color parameters. All three treatments slightly enhanced strawberry red color right after treatment, better preserved surface lightness during storage. However, the samples treated with medium intensity, $5J/cm^2$PL showed slightly accelerated color loss at the end of shelf-life, while the lower total dose L3 and M3 samples didn't show significant color loss when compared with control at day 20.

3.3.5.2 Firmness

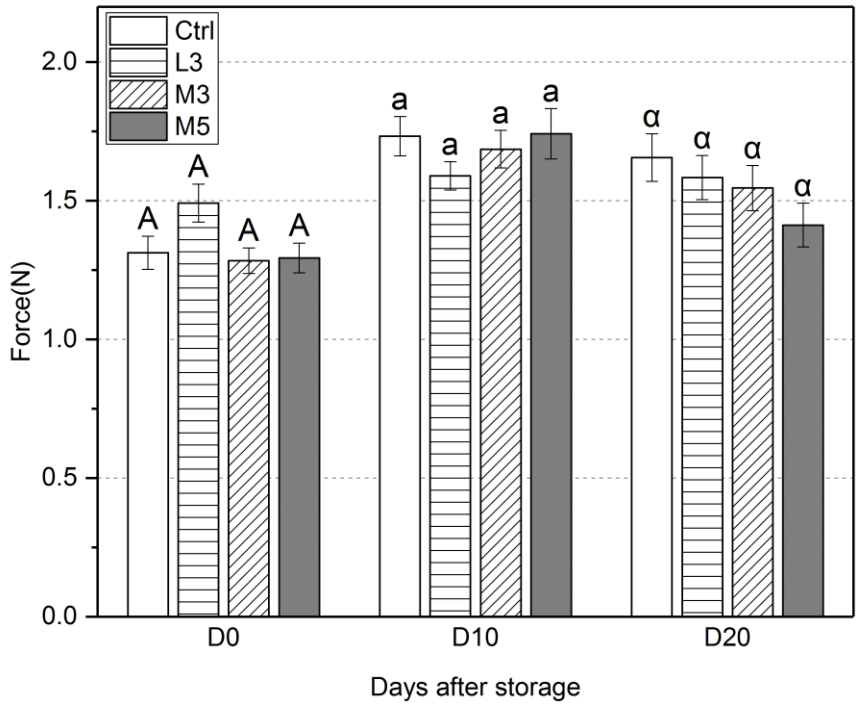

Figure 3.6 Firmness of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20.

Note: Data are shown as maximum surface breaking force in Newton (N). Error bars represent ±SE of three replicates. Treatments labeled with same alphabetic letter at each sampling day are not significantly different (α= 0.05).

Strawberries have very soft texture which makes them sensitive to mechanical stress, thus firmer texture which makes them more resistant to mechanical injuries is highly favorable, and has become a most concerned factor in cultivation. In our experiment, the overall strawberry firmness first increased at day 10, then dropped a bit to a level still higher than that of day 0. To be more specific, control, M3 and M5 samples showed significantly increased firmness values at d10 when compared with d0, while L3 samples showed slightly enhanced firmness value right after treatment which was kept rather stable until the end of storage. No significant difference between treatments was detected before, during and after storage, and the large natural variations between individual samples may account for this lack of significance.

The influences of PL on strawberry firmness in our research may be hard to interpret. Although softening has been found in some of the cultivars during storage, many have reported strawberry hardening as in this experiment, making firmness increase not necessarily favorable. Another problem that may complicate the interpretation is that surface irregularity happens extensively in strawberries. Local softening and hardening may happen on a single fruit at different spots, introducing larger variations to the result. Therefore, a promotion or reduction in firmness value during storage after PL radiation may be hard to judge as advantageous or not, and should be considered together with other attributes.

3.3.6 Nutrient contents

3.3.6.1 Total anthocyanin

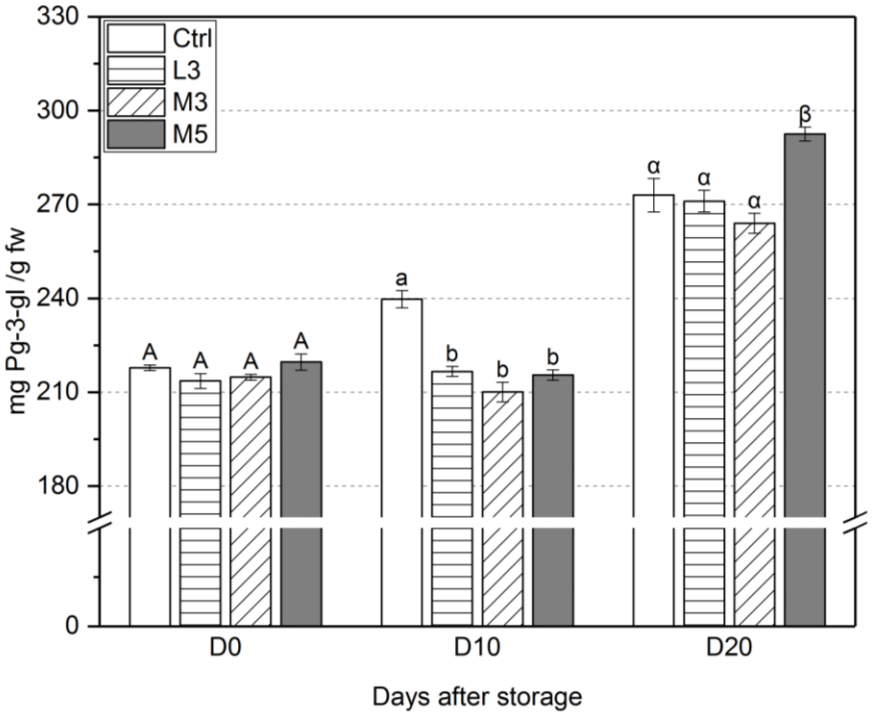

Figure 3.7 Total anthocyanin concentration of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20.

Note: Data are shown as mg Pg-3-gl per gram of strawberry fresh weight. Error bars represent ±SE of three replicates. Treatments labeled with same alphabetic letter at each sampling day are not significantly different ($\alpha = 0.05$)

As have been mentioned, fresh berries became popular in global market due to their health promoting benefits, especially those contents related with high antioxidant activities, including anthocyanins and phenolic contents. Anthocyanin contents are the major contributors of strawberry red color. In production, to better preserve qualities during transportation, strawberries are usually harvested before fully ripe, and color development continues at surface as well as in inner fleshes due to continuous anthocyanin synthesis during post-harvest storage. In our research, an overall increase in anthocyanin contents has been found during 20 days of storage. No significant effect on anthocyanin level was induced instantly after treatment. However, a noticeable difference between control and all PL treated samples was shown at day 10, when control showed significant anthocyanin promotion compared with d0 while the anthocyanin contents of all PL treated samples were kept similarly as their initial levels. This can be an indication of delayed ripening after PL treatment. However, the difference between control and the two low total dose treatments L3 and M3, became statistically insignificant, while samples treated with M5 even reached a significantly higher level than control. This may be another indication of accelerated quality change as an influence of high dose PL treatment during late storage. Inhibited anthocyanin accumulation during storage after UV-C treatment has also been reported in other literatures, (Vicente et al., 2002; Erkan et al., 2008) and the effect of PL may possibly induced by its UV-C spectrum.

It should be noted that although anthocyanins are the major source of red color, the measured anthocyanin contents were not positively correlated with the red color reading a* shown in Figure 3.5. The instant change in a* value shown in L3 and M5 may be a combined result of surface structure change or a local modification of

anthocyanin molecules in the superficial layer. Another possible explanation may be that the measured value of anthocyanin content, indicated as the concentration of a single anthocyanin element of pelagonidin-3-glucoside, may not fully reflect the transitions in anthocyanin constituents, usually from pelagonidin-3-glucoside to cyanidin-3-glucoside and the consequent darkening in color. Anyway, although no health beneficial promotion in anthocyanin level was induced by PL, inhibition of anthocyanin synthesis during storage may be an indication of ripening delay after PL treatment.

3.3.6.2 Total phenolic contents

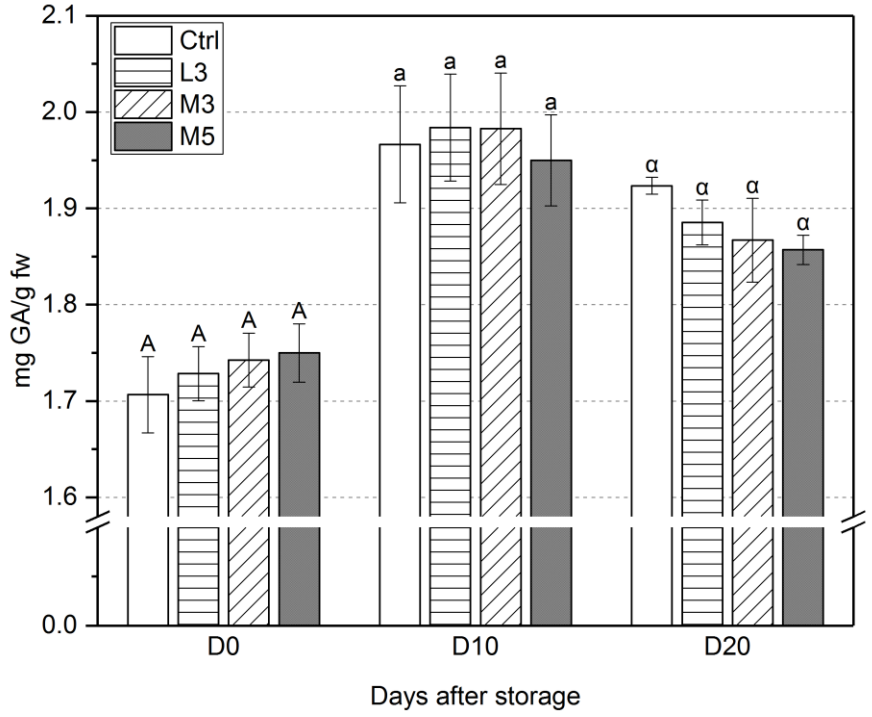

Figure 3.8 Total phenolic contents of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20.

Note: Data are shown as mg gallic acid equivalent per gram of strawberry fresh weight. Error bars represent ±SE of three replicates. Treatments labelled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

The phenolic contents of strawberries increased during the first 10 days and then dropped a bit to a still higher level than that of D0. No significant difference was shown at d0, d10 or d20 between treatments, indicating that PL parameters used in this study didn't play discernable effect on strawberry phenolic contents. However, although without any significance, all treatments showed slightly higher concentration at day 0 while lower level at day 20 when compared with control. Interestingly, this first-increased-then-decreased pattern was also detected in strawberry color a* and b* values, with M5 showing the most influenced changes in all measured parameters, indicating that a higher total dose treatment may play more influences on strawberries and should be carefully evaluated before use.

3.3.6.3 Total antioxidant activity

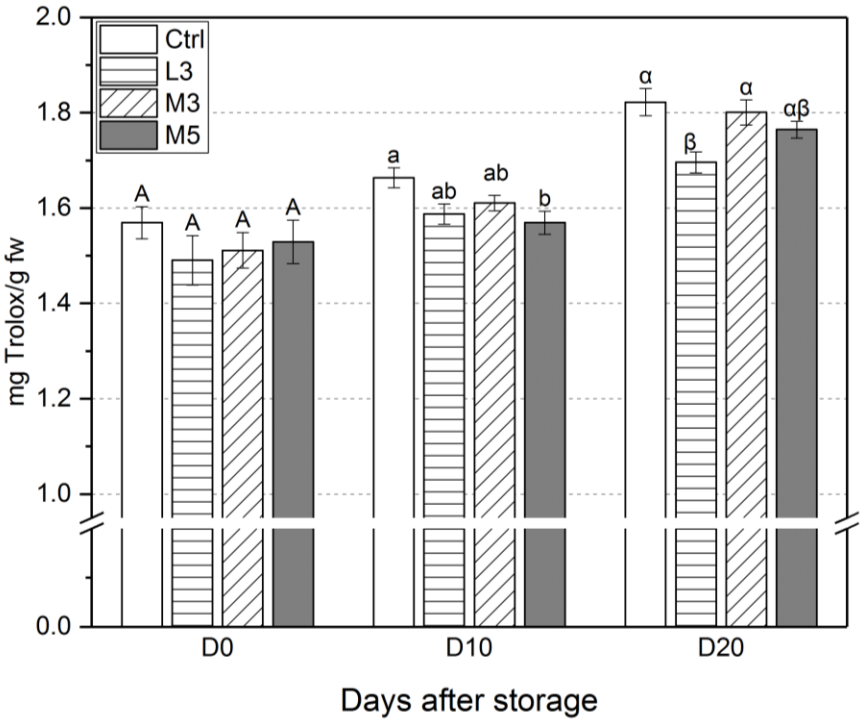

Figure 3.9 Antioxidant activity of Ctrl, L3, M3 and M5 treated strawberry samples at day 0 (after treatment), day 10 and day 20, tested by DPPH method.

Note: Data are shown as mg Trolox equivalent per gram of strawberry fresh weight. Error bars represent ±SE of three replicates. Treatments labelled with a same alphabetic letter at each sampling day are not significantly different ($\alpha = 0.05$).

The antioxidant activity of strawberries gradually increased during storage, as shown in both treated and control samples. Similar as the anthocyanin content, no significant changes in the antioxidant activity of strawberries was shown right after treatment, though slight decreases were shown in all PL treated samples insignificantly. Lowered antioxidant activities were sustained till the end of storage, with M5 and L3 respectively showing significantly lower antioxidant activity than control at D10 and D20. M3 treatment didn't show significant difference compared with control at any sampling days. Increased antioxidant activity tested in ORAC values have been reported after UV-C treatment of 1, 5 and 10min by Erkan et al, (Erkan et al., 2008) which is not in accord with the results shown in our experiment, indicating that other mechanisms may have influenced the effects of PL on strawberry antioxidant activities.

The effects of PL on the health promoting components of strawberries has also been studied by Luksiene et al. No significant difference in total anthocyanin, total phenolic contents and total antioxidant activities between PL and control samples was reported either in this research, but the changes of these compounds during storage was not reported. It should be noted that all nutritional contents were valued based on strawberry fresh weight, thus changes in strawberry total weight during storage should be considered when evaluating the overall changes in nutrient concentration. However, since weight loss was not influenced by PL treatments, comparisons between treatments at each sampling day should still reflect the differences induced by PL treatments.

In sum, based on our research as well as previous studies, PL didn't show discernable side effects on the anthocyanin, total phenolic contents or antioxidant

activities of strawberries right after treatment. However, it is also unlikely that PL may induce dramatic enhancement in these health-promoting molecules. Therefore, the value of PL to be used for nutritional promotion may be limited.

3.3.7 Effect of PL treatments on inactivation of *Salmonella* spot- or dip-inoculated on strawberries

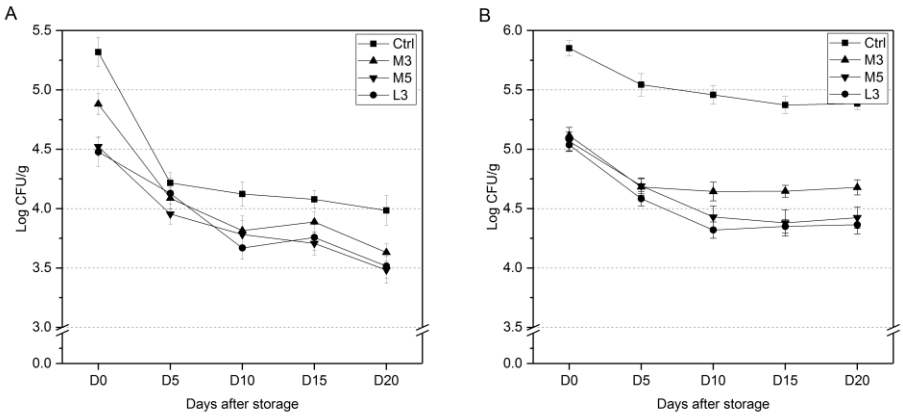

Figure 3.10 Changes in: A. dip-inoculated and B. spot-inoculated *Salmonella* count of Ctrl, L3, M3 and M5 treated strawberry samples from day 0 (after treatment) till day 20.

Note: Data are shown as Log CFU per gram of strawberry fresh weight. Error bars represent ±SE of three replicates.

For both dip and spot inoculated samples, the *Salmonella* population showed continuous decrease during the first 10 days of storage in all samples, which may be an effect of surface drying. Then the *Salmonella* count stayed at a relatively stable level during d10 to d20. All treated samples had reduced total count than control, while M3 showed slightly higher level than the other two treatments at most of the sampling points.

Direct inactivation effects were achieved after all treatments. For dip inoculation, M5 and L3 showed similar reduction of 0.8 log, while M3 had relatively reduced effect of 0.4 log. The better effects of M5 over M3 can be reasonably attributed to its higher total dose, while the advantage of L3 over M3 was possibly due to more effective and uniform exposure when samples were treated at a larger distance from the light source. Enhanced decontamination efficacy was shown in spot inoculated samples. L3 and M5 still showed ~0.8 log reduction, and this effect was slightly extended to ~1 log during d10-d20, indicating continuous inhibition after PL exposure. M3 showed somewhat higher reduction of 0.7 log when compared with its 0.4 log in dip inoculated samples. Similar as the results in dip inoculation, L3 and M5 showed very similar plate count values from day 10 to day 20, both lower than M3. The total count of spot inoculated samples stayed at a higher stable level of around 4.5 for all PL treated samples and around 5.4 for ctrl during storage, when compared with dip-inoculated samples (4.0 for PL treated and 4.5 for control). One possible reason may be ascribed to the difference in initial load between two inoculation methods, and a more likely reason may be in *Salmonella* distribution. Since large portion of bacteria may reside in the calyx that can became easily dried and thus may not support

Salmonella growth after dip-inoculation, higher storage death can be reasonable for dip-inoculated samples.

This part of study can provide a straight-forward evidence of the decontamination efficacy of the three treatments. As have stated above, L3 and M5 showed better effects than that of M3, but this is not in accord with the total yeasts and molds analysis when M3 showed most reduced total count during storage, which may be an indicate that effects of PL against bacteria and fungi may work differently during storage.

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Chapter 4

EFFECTS OF PL IN BLUEBERRY SHELF-LIFE EXTENSION

Abstract

To investigate the impacts of PL on blueberry shelf-life, a low intensity, 6 J/cm^2 total dose dry PL treatment (L6) and a low intensity, 9 J/cm^{2}total dose water-assisted wet treatments were applied to blueberries before they were separately stored for 3 days under room temperature or 7 days at 5℃. Evaluation of sample spoilage showed neither dry nor wet treatments benefited blueberry storage, even though direct inactivation of natural yeasts and molds were achieved. Surface lightness was instantly reduced after both dry and wet treatments. Reduced firmness was detected in wet PL treated samples after 7 days of cold storage, while weight loss was significantly lifted by dry treatment under both storage conditions. Delayed anthocyanin accumulation and reduced antioxidant activity were also induced by both treatments under room temperature at the end of storage, while slight enhancement in total phenolic contents was instantly induced by wet treatment. For safety concerns, the dry treatment achieved 0.9 and 0.7 log reduction against spot and dip inoculated samples right after treatment, but the differences between dry treatment and control became obscured after storage. The wet PL worked effectively against spot inoculated *Salmonella* and achieved 4.4 log reduction right after treatment, and the inhibition effect was continued till the end of storage under 5℃ with nearly no detectable *Salmonella* count at day 7, indicating enhanced PL decontamination efficacy when combined with cold storage. In short, although the selected PL treatments, especially the wet treatment, may help reduce total yeast and mold count as well as improve blueberry safety, PL parameters and treatment methodology should be modified in further studies before possible application of PL in blueberry industries.

4.1 Introduction

Modern people have become more concerned about dietary health due to the prevalence of chronic diseases. Blueberries, as a widely-known good source of antioxidants, has become increasingly popular, showing fast-growing markets in these few decades (Ferreira and Perez, 2016). USA is currently the largest blueberry producer in the world with total production double its near competitors. However, like strawberries, blueberries also have a short shelf-life due to soft textures especially when they are fully ripe, and are likely to present reduced shelf-life if not properly handled and stored, causing high post-harvest loss. Blueberries can be susceptible to fungal infection during wet seasons, and are most likely to show ripe rot, *alternaria* rot as well as green molds induced respectively by *Colletotrichum acutatum*, *Alternaria spp*. and *Botrytis cinerea*. Nonetheless, in most cases, mechanical damage and physiological senescence can be more important reasons to cause blueberry post-harvest quality loss than fungal decay (Nunes et al., 2004; Sanford et al., 1991; Woodruff et al.,1959).

In production, blueberries are usually not washed for fear of possible fungal development, and in general, no additional processing technique is used for shelf-life's concern besides sufficient pre-cooling and cold storage. Current lab-scale researches in purpose of shelf-life promotion have mostly focused on controlled atmospheric storage (Beaudry et al., 1993; Schotsmans et al., 2007), vapor treatments (Chiabrando et al., 2011) as well as edible coating (Duan et al., 2011). Reduced fungal decay and cultivar-dependent antioxidant promotion were also detected after UV treatments (Perkins-Veazie,2008).

As have been elaborated in Chapter 3, PL treatments showed positive effects in strawberry shelf-life extension. Therefore, its applicability in blueberry industries has

been of interest for our further studies. Also, in our previous work, a water-assisted PL system was developed to achieve higher anti-pathogenic efficacy as well as to avoid possible surface heating caused by dry PL radiation (Huang et al., 2014). To further investigate the influence of dry and water-assisted PL treatments on blueberry quality attributes and shelf-life, in this study, we thoroughly evaluated the spoilage percentage, weight loss, sensory properties as well as some major health-benefit compounds of blueberries after treatment and at the end of cold or room temperature storage to look for possible application strategies of PL in blueberry industries.

4.2 Materials and Methods

4.2.1 Blueberries

Blueberries were purchased from local wholesale stores and stored at 5℃ in a refrigerator until use. Samples without any sign of rot or damage were selected and sorted based on firmness, color and size uniformity.

4.2.2 PL treatments and storage conditions

Around 110 g of sorted blueberries were weighed and placed in each 4.4 oz blueberry clamshell (Highland packaging solutions, Plant City, FL) and then refrigerated for 2 h at 5℃ before PL treatment. PL treatments were performed using the PL equipment described in chapter 3. Two PL processing methods, dry (samples were exposed to PL directly) and water-assisted (samples were washed in agitated water while being exposed to PL) treatments, were used. Two untreated controls were included. The dry control consisted of blueberries that were not subjected to any treatments. The water-assisted control consisted of blueberries washed in water, but were not exposed to PL. A low PL intensity of 0.066 J/cm^{2}per pulse was selected for

both dry and water-assisted PL treatments. PL doses of 6 J/cm^2 (30-s PL processing time) and 9 J/cm^2 (45-s PL processing time) were used for the dry and water-assisted PL treatments, respectively.

For dry PL treatment, blueberries were removed from a clamshell and shaken on a round plate wrapped with a piece of aluminum foil at 180 rpm using an orbital shaker (Orbi-Shaker JR., Benchmark Scientific, Inc. PO Box 709 Edison, NJ) while being exposed to PL for 30 s. The shaker allowed all the surfaces of blueberries to be exposed to PL and aluminum foil could effectively reflect PL. After treatment, the shaker was turned off and the ozone discharger unit was turned on for 150 s to remove ozone from the PL chamber. The treated blueberries were then placed into 4.4 the original clamshell. Dry control samples were kept in their original clamshells with lid open at room temperature for 180 s. For water-assisted PL treatment, blueberries were removed from a clamshell and placed in 500 mL water in an 8'' square baking dish (Pyrex, Corning Inc., Corning, NY) containing a stirrer bar. The inside of the dish was wrapped with a piece of aluminum-foil to reflect PL. A magnetic stirrer (Fisher Scientific, Hampton, NH) underneath the dish was used to agitate the water in the dish to create turbulent flow so that random rotation and movement of food samples could be achieved during PL treatment. The blueberries were washed using this water-assisted PL system for 45 s followed by turning on the ozone discharger unit for 135 s. The water-assisted control samples were washed using the same washing system for 180 s without being exposed to PL. After treatment, the water-assisted PL-treated and un-treated control samples were poured on a stainless-steel strainer to remove water and then put into 18 oz PET clamshells without lids. The bottoms of the clamshells were lined with paper towels to remove residual moisture. The clamshells were placed

in the refrigerator at 5℃. A 5" portable fan (O2Cool, Chicago, IL) at high speed mode was placed inside the refrigerator to enhance air circulation. Samples were gently shaken by hand every 10 min to facilitate drying. After 40 min of cooling and drying, blueberries were placed into the original 4.4 oz. clamshells and cooled inside the refrigerator for 80 min to fully cool the berries before storage.

The PL-treated (dry and water-assisted) and untreated controls (dry and water-assisted) in clamshells with lids closed were placed in corrugated boxes and stored at either 5℃ with 85-95% RH or room temperature (~22°C) with 60-70% RH for 7 or 3 days, respectively. The spoilage evaluation was conducted at the end of storage (3 days at room temperature and 7 days of at 5℃), the weight loss analysis on daily basis, and other analyses on day 0 and at the end of storage.

4.2.3 Spoilage evaluation

After storage, blueberries in each clamshell were individually evaluated based on its overall acceptance. Those with visible microbial growth, obvious sign of surface dryness, shrinkage, and leakage, or with extra-soft and easily-crumbled texture were judged as spoiled. The number of spoiled blueberry fruits were counted and spoilage percentage was calculated by dividing the number of spoiled samples by the total number of blueberry fruits in each clamshell. For each of the 4 treatments, 3 clamshells were used for each storage condition.

4.2.4 Weight loss

Weights of empty clamshells were recorded as W_C and the total initial weight of each clamshell with blueberry samples was measured right before any treatments were applied (W_{d0}). The weights of the packaged samples were measured daily during

storage (W_{dn}, n represents the number of days after treatment). Clamshells inside a corrugated box was randomly shuffled after each weight measurement to reduce variations caused by uneven air circulation. Weight loss representing the percent loss of initial weight of blueberries was calculated using the following formula:

$$\%\ \text{weight loss on day n} = \frac{(W_{d0} - W_C) - (W_{dn} - W_C)}{(W_{d0} - W_C)} \times 100\%$$

4.2.5 Quality attributes

One day 0 and at the end of storage, a clamshell from each of the 4 treatments were removed for analyses. For surface color measurements, 25 fruits were randomly removed from a clamshell and the Hunter L, a*, b* values of samples were measured at the top of the blueberries by a chrome meter (Minolta CR-400, Minolta, Osaka, Japan). After color measurements, the same blueberries were used for firmness measurement using a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming UK) with 5 kg load cell. A 2-inch diameter cylinder flat probe was used to test the compression force when blueberry samples were deformed by 3 mm. The test speed of the probe was 1.0 mm/s with pre- and post- test speeds of 5 and 8 mm/s, respectively.

The remaining blueberries in the clamshell were homogenized for 1 min using a blender to make blueberry puree. Puree (45 mL) was collected in a 50mL centrifuge tube (Falcon, BD Biosciences, San Jose, CA) and then centrifuged at 5000 rpm for 5 min (Centra CL2, Centrifuge, Thermo Scientific). The supernatant was re-mixed with the sediment using a stir stick. This centrifugation-re-mix procedure was repeated once before another 10min centrifugation to obtain clear blueberry juice, or the supernatant. The pH value of the supernatant was measured using a pH meter (pHTestr20, Oakton Instruments, Vernon Hills, IL) and total soluble solid (Brix°) was

measured using a refractometer (Reichert Abbe Mark II, Reichert Inc., Depew NY). Both measurements were made in triplicates. For titratable acidity, 5 g of supernatant were diluted with 50 mL of DI water and then titrated with 0.1 M NaOH (Fisher Scientific, Hampton, NH) to pH 8.1-8.2. Titration were made in duplicates.

4.2.6 Natural yeasts and molds and nutrient contents

4.2.6.1 Natural yeasts and molds

One day 0 and at the end of storage, a clamshell from each of the 4 treatments were removed for analyses. Twenty grams of berries in a clamshell were weighed and then homogenized with 180 mL of 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD) in a 24 oz. filter bag (Whirl-Pak, Nasco, USA) for 2 min at 260 rpm using a stomacher (Seward 400, Seward, London, U.K.). The homogenate was then serially diluted 10 folds in 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD) and spread plated onto potato dextrose agar (Difco). The plates were incubated at room temperature for 5 days before colonies were counted.

4.2.6.2 Nutrient contents

4.2.6.2.1 Extraction

The remaining blueberries in the clamshell were pureed as described above. One g puree was accurately weighed in a 15 mL centrifuge tube and 4.5 mL acidified methanol (Sigma–Aldrich, Inc., St. Louis, MO, USA, with 0.1% HCl, Fisher) was added. The tubes were vigorously shaken for 1 min and then stored at 5℃ for 2 h. Mixtures were centrifuged at 5000 rpm for 10 min. The methanol extracted supernatant was collected in a new 15 mL tube and the fruit sediment was extracted

for a second round using another 4.5 mL of acidified methanol. The second extract was collected together with the first one and made to a total of 10 mL for anthocyanin, total phenolic contents and total antioxidant capacity analyses. The extraction was done in duplicate tubes for each pureed sample, and triplicate reactions and plate reading s were further made in 1.5-mL micro-centrifuge tubes (Seal-Rite, USA Scientific, Ocala, FL) for each extract in all three analyses described below.

4.2.6.2.2 Total anthocyanin

The total monomeric anthocyanin contents of blueberries were measured using the pH differential method described by Glusti and Wrolstad (2001). Blueberry extract (100 µL) was diluted in 500 µL of 0.025 M potassium chloride with pH 1.0 (Fisher) and 500 µL of 0.4M sodium acetate buffer with pH 4.5 (Fisher). The absorbance readings of both dilutions were measured at 508 nm and 700 nm. A volume of 200−µL reaction solution in a 1.5-mL reaction tube was added to a single well of flat bottom 96-well plate (Falcon, BD Biosciences, San Jose, CA) and then read at specific wavelengths by a multi-mode reader (Synergy 2; Biotek Instruments, Winooski, VT, USA).

The absorbance of the sample diluent (A) was then calculated as

$$A = (A_{508} - A_{700})_{pH\ 1.0} - (A_{508} - A_{700})_{pH\ 4.5}$$

and the anthocyanin concentration in the extract was calculated as µg Pg-3-glu per mL by the following formula

$$\text{Pg-3-glu (µg/mL)} = (\text{A} \times 433.2 \times 6 \times 1000)/17300$$

in which 433.2 is the molecular weight of Pg-3-glu, 6 is the dilution factor of the sample diluents, and 17300 is the molar absorptivity of Pg-3-glu in 0.1% HCL

acidified methanol (Giusti et al. 1999). The anthocyanin contents of fresh strawberry samples were then calculated and presented as μg Pg-3-glu /g fresh weight.

4.2.6.2.3 Total phenolic contents

The total phenolic contents were determined using the Folin-Ciocalteau method (Singleton et al., 1999). To make a final 1.5-mL reaction system, 30 μl blueberry extract was added to 1245 μl DI water, then 75 μl of 0.25M Folin-Ciocalteau reagent (Sigma–Aldrich) was mixed in. The reaction carried on for 3 min before 150 μl of 1N sodium carbonate (Fisher) was added to stop the reaction. The reaction tubes were then incubated in the dark at 37℃ for 45 min for complete color development before absorbance reading at 765 nm. The total phenolic contents were calculated as gallic acid equivalents per gram fresh weight by fitting the absorbance into a standard curve predetermined by standardized gallic acid (Sigma–Aldrich) solutions.

4.2.6.2.4 Total antioxidant activity

The total antioxidant activities of blueberry extracts were determined by the DPPH method reported by Brand-Williams et al. (1995) with modifications. Thirty μL of extract and 45 μL of 2.5mM DPPH (Sigma–Aldrich) were mixed in 1470 μL methanol to make a 1.5-mL 75 uM DPPH reaction system. The reaction tubes sit in the dark at room temperature for 15 min before absorbance reading at 515 nm. The absorbance of the reaction solution in each tube was blanked with a sample diluent made by adding 30 μL of blueberry extract to 1470 μL methanol. The total antioxidant activity was then expressed as Trolox equivalents per gram fresh weight, calculated by

fitting the absorbance into a standard curve predetermined by standardized Trolox (Sigma–Aldrich) solutions.

4.2.7 Effect of PL treatments on inactivation of *Salmonella* spot- or dip-inoculated on blueberries

4.2.7.1 Inoculum preparation and blueberry inoculation

Four *Salmonella enterica* serotypes of Newport, Montevideo, St. Paul and Stanley that were previously adapted to be nalidixic-acid resistant (Huang et al., 2013) were used in this study. The four strains were maintained on tryptic soy agar (Difco) supplemented with 0.6% yeast extract (Bacto) and 50 µg/mL nalidixic-acid (Fisher) at 2-4 ℃ (TSAYE-N). Individual strains were grown in 10 mL of tryptic soy broth (Difco) supplemented with 0.6% yeast extract and 50 µg/mL of nalidixic acid (TSBYE-N) overnight at 35 °C. For spot inoculation, each *Salmonella* culture was transferred into 2 new tubes each containing 10 mL of TSBYE-N. The tubes were incubated for 24 h at 35 °C. Each culture was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 × g for 10 min (Sorvall ST16 R, Thermo Scientific) at 20 °C. The pellet was re-suspended in 6.5 mL of sterile 0.1% peptone water. Five hundred mL of the *Salmonella* cocktail was inoculated on the surfaces of 100-g blueberries in an 18 oz PET clamshell. The spot-inoculated berries were dried in a biological safety hood for 2 h at room temperature with clamshell lid open. Blueberries were placed into 4.4 oz. clamshells and then stored overnight at ~4 °C with clamshell lid closed to facilitate bacterial attachment. For dip inoculation, each *Salmonella* culture was transferred to 2 flasks containing 200 mL of TSBYE-N. The flasks were incubated, mixed and centrifuged as described above. The pellet was re-suspended in 1600 mL of 0.1% peptone water. Blueberries

(1500 g) were then dipped in the *Salmonella* cocktail and gently stirred for 2 min. The blueberries were drained with a strainer and spread on trays lined with paper towel to be air dried for 2 h. The inoculated samples were then placed into 12 4.4 oz blueberry clamshells (Highland packaging solutions, Plant City, FL) (110 g blueberries/clamshell) which were stored overnight at ~4 °C with clamshell lid closed.

4.2.7.2 PL treatments

The inoculated blueberries were removed from the clamshells and irradiated using the dry and water-assisted PL treatments. The PL-treated and the water-assisted control blueberries were placed back into new clamshells. The clamshells were placed into corrugated boxes and stored as described above. *Salmonella* counts were determined on day 0 and after 3 and 7 days of storage at room temperature and 5℃, respectively.

4.2.7.3 Microbial analyses

For each treatment and at each sampling point, 20 g of blueberries in a clamshell were weighed and then homogenized with 180 mL of Dey-Engley neutralizing broth in a 24oz. filter bag (Whirl-Pak, Nasco, USA) using a stomacher (Seward 400, Seward, London, U.K.) for 2min at 260 rpm. The homogenate was then serially diluted 10 folds in 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD) and spread plated onto TSAYE-N. Plates were incubated at 35℃ for 48 hours before colonies were counted.

4.2.8 Statistical analyses

Three independent trials were conducted. Analyses of variance (ANOVA) were performed using JMP Pro 13 within a same sampling day. ‘Treatment’ was taken

as source of variance, and time of repeats was taken as blocks. Tukey's HSP test was used to detect significant differences at $\alpha = 0.05$ level between all four treatments.

4.3 Results and Discussion

4.3.1 Effects of PL on blueberry shelf-life

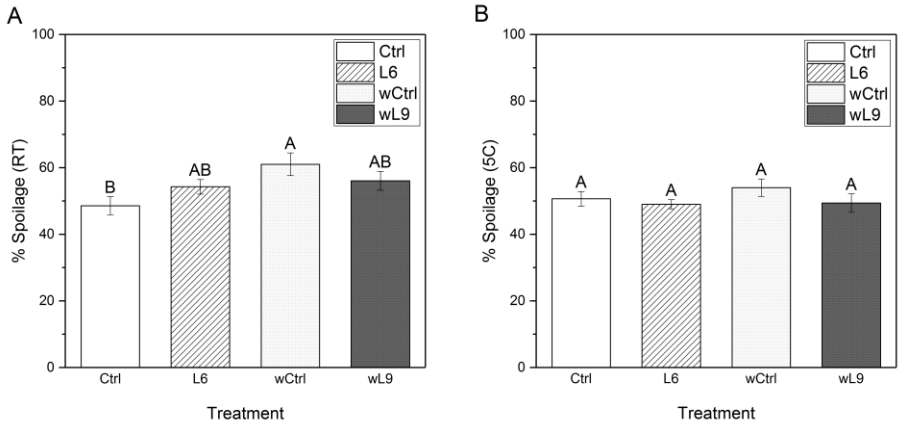

Figure 4.1 Percent spoilage (%) of Ctrl, L6, wCtrl and wL9 treated blueberry samples after: A. 3 days of storage at room temperature, B. 7 days of storage at 5℃.

Note: Error bar represents ±SE of three replicates.

Spoilage percentages of room temperature and cold stored blueberries after treatments are presented in Figure 4.1. After 3 days of storage under room temperature, all treated samples showed higher total spoilage than Ctrl, and a significant lift was shown in wCtrl samples. Although wL9 treatment showed less spoilage than wCtrl which indicated the effect of PL radiation, the overall quality loss was still more severe than non-treated dry control. Therefore, both dry and wet treatments used in our research are not recommended for blueberries stored under room temperature. As for 5℃ storage, slightly lowered spoilage indexes were shown after both PL treatments, though the effect was insignificant. Still more severe spoilage was induced in wCtrl after washing, suggesting that washing may not be practical for blueberries under both storage conditions.

Looking further into the spoilage process, some mostly observed quality defects include leakage (splitting), softening, shrinkage, followed by visible green and white molds. For room temperature samples, leakage is more commonly seen in both wCtrl and wL9 than in L6, possibly being an influence of sample washing, while shrinkage at the stem scar indicating excessive moisture loss can be discerned in both dry and wet PL treated samples. As for cold stored blueberries, splitting and shrinkage became less prevalent, similar as reported by Sanford et al (Sanford et al., 1991), but still many samples became unacceptable due to softened texture. Noticeably, occurrence of visible fungi varied dramatically between experiments. For all blueberry samples used in this project (not restricted to the samples used for spoilage evaluations), according to visual observations, some may show 10-20% visible fungal decay while others may not develop any fungi. Since fungal spoilage can be greatly

influenced by seasons, fields and cultivars and thus can be highly unstable, evaluations based merely on fungal development were not conducted.

The fact that fungal development showed less importance in determining blueberry shelf-life has been reported in various literatures (Woodruff et al., 1959; Sanford et al., 1991, Nunes et al., 2004). As have been stated in Chapter 3, our main purpose of using PL treatment was to inhibit microbial decay for shelf-life extension. Since fungal decay is less decisive, inhibition of microbial growth may not lead to shelf-life promotion, which makes PL treatment less effective for blueberry shelf-life. The washing process, on the other hand, may not be favorable in two ways. The extra moisture may facilitate fungal growth, which was stated as the main reason a washing process was not recommended for blueberries in many literatures. Moreover, even with fungal decay not being a most serious problem in our research, washing still played other side effects and resulted in higher spoilage. In fact, blueberry splitting induced by rainfall near harvest has been widely found in cultivation, and more severe influences were shown on fully ripe samples. This influence can be simply simulated by water-soaking in lab-scale researches (Marshall et al., 2006), which suggested that a post-harvest water treatment can be highly unfavorable, especially for some splitting sensitive cultivars. Reduced side effects may be shown if a reduced washing time was combined with more effective drying and cooling processes.

4.3.2 Weight loss

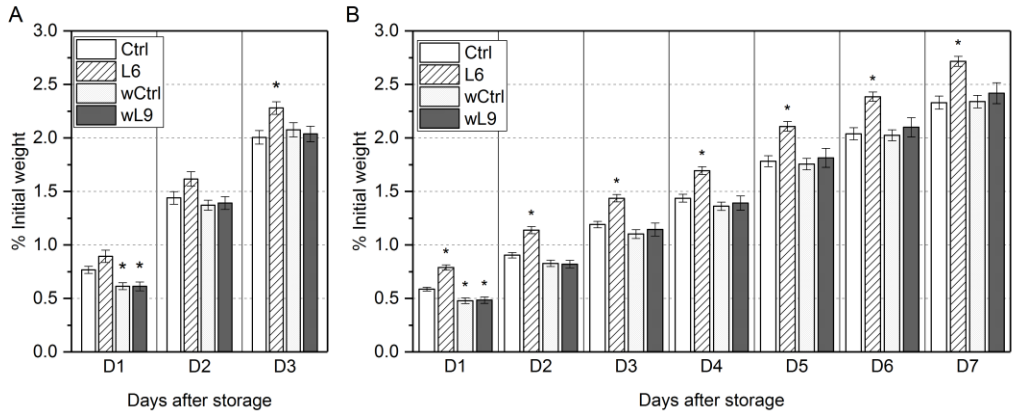

Figure 4.2 Weight loss (%) of Ctrl, L6, wCtrl and wL9 treated blueberry samples stored at: A. room temperature and B. 5℃ during storage.

Note: Data were collected every day after treatment until the end of storage, and are shown as percentage of initial weight. Error bars represent ±SE of three replicates. Treatment labeled with asterisk showed significant difference when compared with control using Dunnett' s test (α= 0.05).

Not surprisingly, blueberries stored at room temperature showed higher daily weight loss than those stored at 5℃. An average daily loss of 0.36 % was detected at 5℃,while under room temperature, the value almost doubled to 0.7%. Physiological and mechanical weight loss can both vary under different temperatures and RH. However, similar weight loss patterns were still shown by RT and cold stored samples. Under both environments, dry PL lifted sample weight loss. This is in accord with the phenomenon that dry treated samples showed more stem scar shrinkage which indicated higher moisture loss in our spoilage study. For RT samples, the increase was shown from day 1, though statistical significance was only detected at day 3. While at 5℃, significance was detected from the first day after treatment and was kept all through storage.

It is presumable that extra handlings and temperature increases during treatment can lead to higher weight loss. However, the difference between control and dry treatment became more significant rather than vague during storage, suggesting that some other influences were kept through storage and contributed to larger difference. Lifted respiration rate may be induced, and mechanical injuries on skins which lead to higher moisture transpiration is also possible after PL radiation. As for wet samples, during early storage, reduced weight loss in both wCtrl and wL9 samples indicated that extra water may have been absorbed, as have been similarly reported in many blueberry splitting researches, though invisible residue moisture may also be left on sample surfaces, due to the restrictions of our drying process. However, this advantage was not kept till the end of storage.

Blueberry weight loss is, in many aspects, an important parameter related to shelf-life. For producers and retailers, reduced fresh weight will directly result in loss

of total income. Moreover, high moisture loss of over 2% has been proved as the main reason to cause blueberry firmness loss (Paniagua et al., 2013). Therefore, the enhancement in weight loss may be a highly unfavorable effect caused by the selected PL radiation that lead to higher spoilage.

4.3.3 Natural yeasts and molds

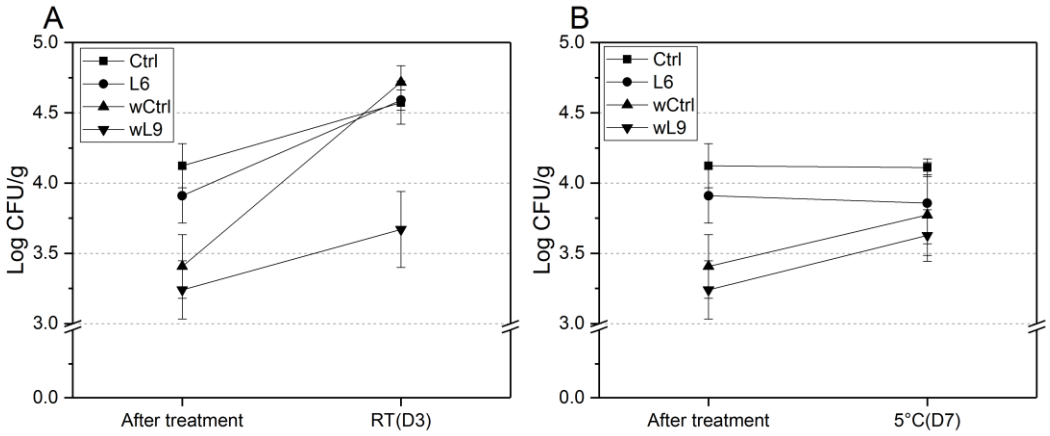

Figure 4.3 Total yeasts and molds count of Ctrl, L6, wCtrl and wL9 treated blueberry samples after A: 3 days of storage at room temperature, B. 7 days of storage at 5℃.

Note: Data are shown as Log CFU/g blueberry fresh weight. Error bars represent ±SE of three replicates.

To further explain the effects of PL treatments on the spoilage process of blueberries, analyses of natural yeasts and molds were also conducted. Considering direct decontamination effects, L6 reduced the natural Y/M by 0.2 log. Water circulating directly rinsed off 0.7 log CFU/g, and the 9 J/cm^2 PL radiation further contributed 0.2 log reduction and resulted in 0.9 log CFU/g total reduction after wL9 treatments.

At room temperature, yeasts and molds development was rather active. After 3 days of storage, the total counts of room temperature samples increased 0.7 log CFU/g on average. To be more specific, Ctrl showed 0.5 log total growth, while wCtrl showed highest growth of 1.3 log among all treatments. As a result, the total count reduced by rinsing was completely compensated by the out-growth at room temperature. L6 also grew faster when compared with Ctrl and showed similar final yeasts and molds count as Ctrl, suggesting possible surface injuries and leakages that further facilitated fungal growth, while the wL9 samples only developed 0.4 log CFU/g and was still 0.9 log lower than Ctrl samples.

As for cold stored samples, Ctrl and L6 didn't show obvious change in total count after 7 days of storage, and L6 even showed slightly decrease, indicating continuous inhibition under cold temperature, which was also reported in our strawberry researches. While the wCtrl and wL9 samples showed more obvious growth, though the final counts were both lower than Ctrl, showing that water rinsing still favored yeast and mold growth under cold storage. Anyway, due to the instant decontamination effect, wL9 still showed reduced total yeast and mold count at the end of storage at both conditions, although the reduced total yeast and mold loads didn't result in reduced spoilage, as was shown in Figure 4.1.

4.3.4 Quality attributes

4.3.4.1 Surface color

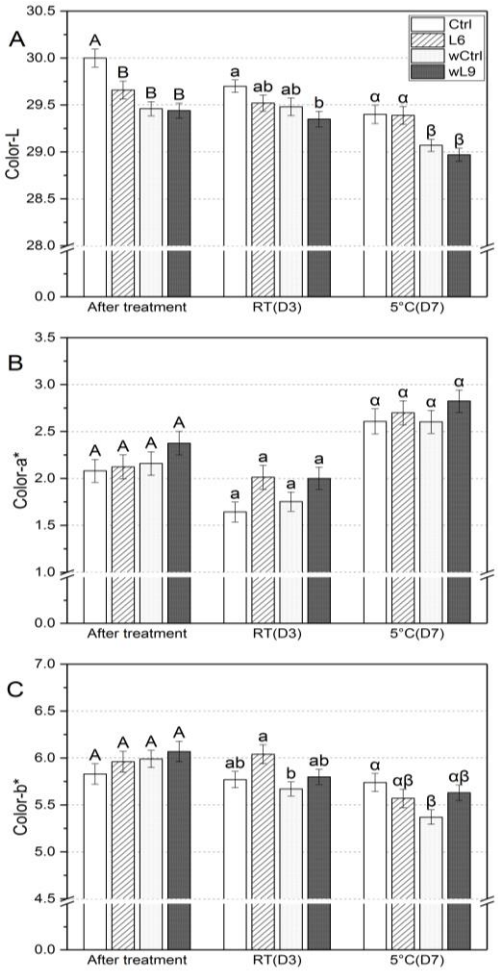

Figure 4.4 Color L (A), a* (B), b* (C) values of Ctrl, L6, wCtrl and wL9 treated blueberry samples after 3 days of storage at room temperature and 7 days of storage at 5℃.

Note: Error bars represent ±SE of three replicates. Treatments labeled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

Similar as strawberries, the overall L value of blueberries decreased during storage, which is in accord with observed loss of surface glossiness and white bloom during storage. As for the influence of PL treatments, decreased L value was significantly shown right after L6 and wL9 treatments as well as wCtrl. The lowered L values were kept till the end of storage at both storage conditions, and the wCtrl and wL9 samples seemed to show more severe changes than L6.

Surface darkening is also highly unfavorable for blueberries. According to Nunes et al, L can be a good indicator of surface brightness and is more important than the other two parameters of a* and b* in monitoring blueberry color change. In previous work of our lab, the side effects of high intensity PL on blueberry surface lightness has also been found. An obvious burn-like darkening sign was shown on the surface area exposed to high intensity and/or high total dose PL, and 2 pulses of high intensity PL can already induce discernable surface darkening. This may impede PL application in blueberry industries. Therefore, the wet treatment was designed to protect samples from direct heating by PL. However, a more obvious reduction in L value was shown after wL9 treatment. Washing alone seemed to play adverse influence on surface lightness, possibly due to structures changes after water-soaking, and the bloom may have been 'washed up' by water circulation. Since wL9 showed most reduced L value at all three sampling days, it was likely that washing and the high dose PL radiation played synergic effect.

As for a* and b* values, no significant influence was induced after PL treatments. In fact, PL may have slightly influenced redness since all PL treated samples showed higher a* than the two controls at the end of storage. As for b* value, the wCtrl samples seemed to be more influenced than the two treated samples,

suggesting that the b* readings may be easily influenced by or may be an indication of the overall spoilage of the samples.

4.3.4.2 Firmness

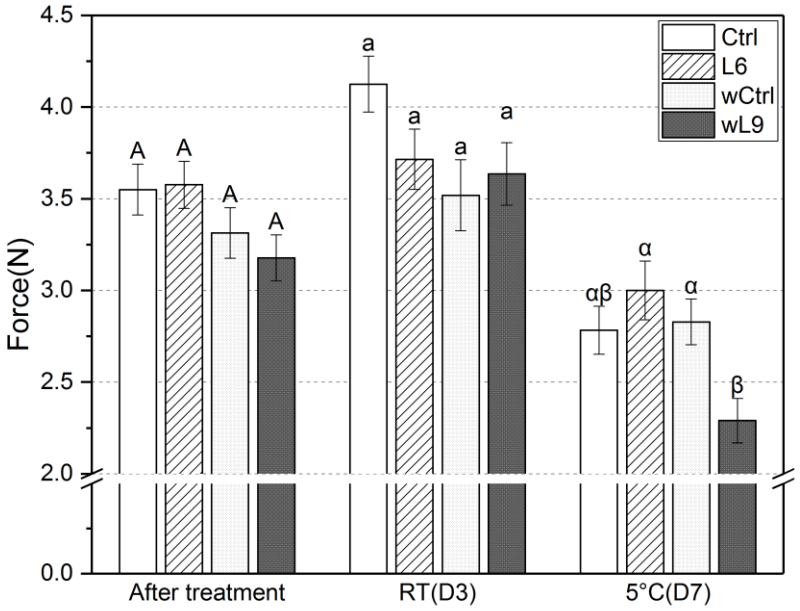

Figure 4.5 Firmness of Ctrl, L6, wCtrl and wL9 treated blueberry samples after 3 days of storage at room temperature and 7 days of storage at 5℃.

Note: Data are shown as compression force in Newton (N)**.** Error bars represent ±SE of three replicates. Treatments labeled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

The overall firmness of blueberries slightly increased after room temperature storage while decreased after cold storage. This is in accord with the result reported by Deng et al. (Deng et al.,2013) At all three sampling points, no significant difference between PL treated and control samples was detected, possibly due to large variations between individual samples. However, lowered firmness was consistently shown in wCtrl and wL9 samples when compared with Ctrl. The only significant difference detected was shown between L6 and wL9 at the end of cold storage, indicating that PL may show different influences on sample firmness when applied in different methodology.

As stated above, the firmness of blueberry is highly related to weight loss. Although specific correlations may differ between cultivars, a trend has been found that the weight loss below a threshold, usually at around 2%, will first enhance firmness, while any further weight loss will lead to reduced firmness (Paniagua et al., 2013). In our experiments, both room temperature and cold stored strawberries lost around 2.5% total weight at the end of their shelf-life, with cold stored samples showing slightly higher weight loss than that of room temperature samples. When referring to sample firmness, however, contrary changes were shown by the two sample groups. Since weight loss was caused by moisture loss as well as nutrient decomposition, although similar total weight loss levels were shown in value, the relative contributions made by mechanical or physiological loss may result in different firmness readings. Therefore, interpretation of firmness values may be difficult and tricky and should be combined with other spoilage observations.

4.3.5 Nutrient contents

4.3.5.1 Total anthocyanin

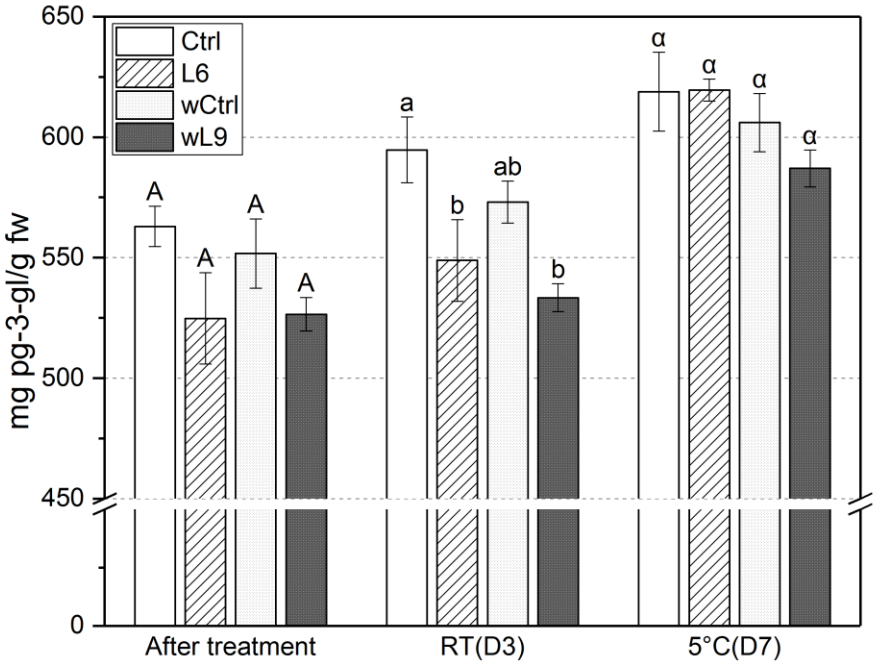

Figure 4.6 Anthocyanin concentration of Ctrl, L6, wCtrl and wL9 treated blueberry samples after 3 days of storage at room temperature and 7 days of storage at 5℃.

Note: Data are shown as in mg Pg-3-gl per gram of blueberry fresh weight. Error bars represent ±SE of three replicates. Treatments labeled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

As of strawberries, the total anthocyanin contents of blueberries increased after storage, and showed highest level after 7-day cold storage at 5℃ for all treatments. wCtrl showed comparative anthocyanin contents as Ctrl at all three sampling points. Both PL treatments reduced anthocyanin content right after treatment, though insignificantly, and the difference compared with Ctrl became more discernable and significant after room temperature storage, showing a retarded anthocyanin accumulation process in PL treated samples. After 7 days of cold storage, however, the difference between all treatments were again not significant as day 0. Similar delay of anthocyanin accumulation was also detected in our strawberry research. Since water circulating didn't show much influence, the 10% reduction in anthocyanin at the end of room temperature storage were more likely to be ascribed to PL radiation.

4.3.5.2 Total phenolic contents

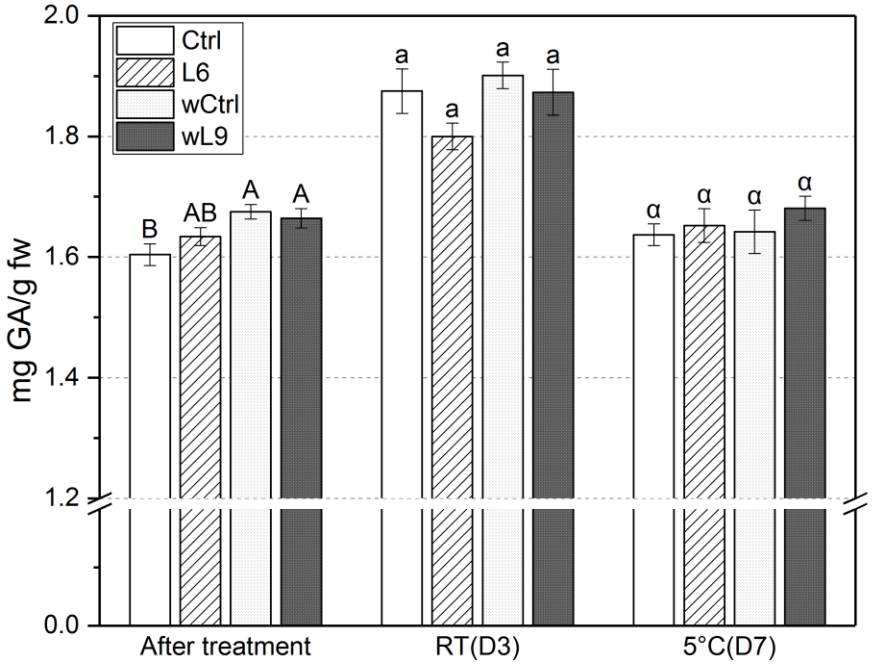

Figure 4.7 Total phenolic contents of Ctrl, L6, wCtrl and wL9 treated blueberry samples after 3 days of storage at room temperature and 7 days of storage at 5℃.

Note: Data are shown as mg gallic acid equivalent per gram of blueberry fresh weight. Error bars represent ±SE of three replicates. Treatments labeled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

As for phenolic contents, although the two wCtrl and wL9 showed significantly enhanced total phenolic contents right after treatment, a ~5% lift most obviously shown in wCtrl samples may not be of much importance and may not be directly related with PL radiation. No other obvious difference or noticeable pattern was shown after both storage conditions, and the difference at d0 caused by water circulating may be an influence caused by bloated skin structure which further eased skin cell breaking and thus enhanced higher phenolic extraction efficacy.

4.3.5.3 Total antioxidant activity

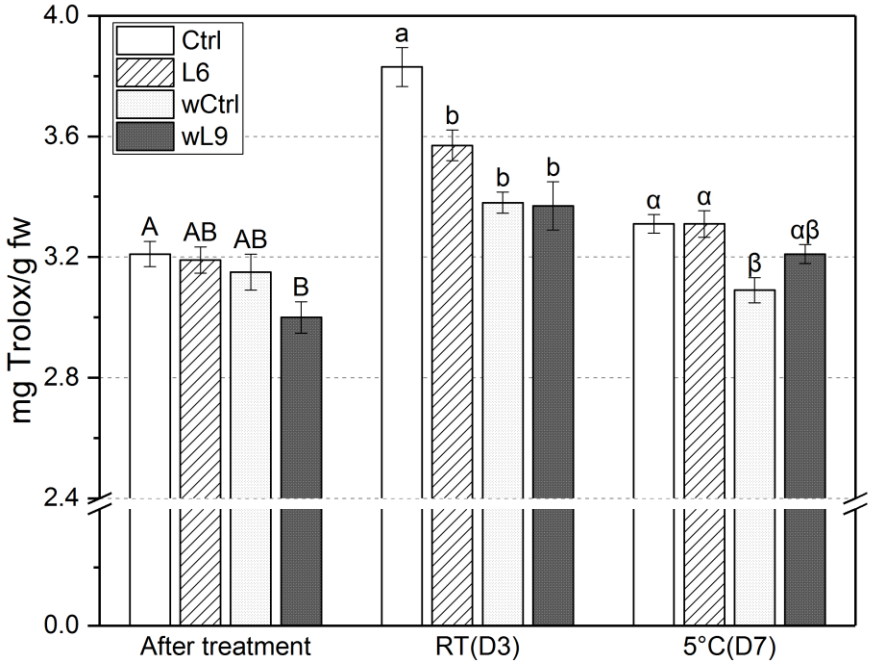

Figure 4.8 Antioxidant activity of Ctrl, L6, wCtrl and WL9 treated blueberry samples after 3 days of storage at room temperature and 7 days of storage at 5℃, tested by DPPH method.

Note: Data are shown as mg Trolox equivalent per gram of blueberry fresh weight. Error bars represent ±SE of three replicates. Treatments labeled with a same alphabetic letter at each sampling day are not significantly different (α= 0.05).

The overall total antioxidant activity level increased significantly after 3-day storage under room temperature, while it showed a comparative level as day 0 after 7 days under 5℃. As Connor et al. have reported, blueberry antioxidant activity first increases during early storage but then gradually drops. This process can go rather slowly at low temperature, which is in accord with our result.

At all three sampling points, Ctrl showed highest antioxidant capacity among four treatments. A most discernable phenomenon is that at day 3, all treated samples showed significantly lower antioxidant activity than Ctrl while at day 7, and the wCtrl was the only treatment that showed significant difference when compared with Ctrl. Overall, more discernable differences were detected in the wCtrl and wL9 treatments than in L6 treatment, suggesting that the antioxidant activity may be a more spoilage-related parameter, and is more influenced by the spoilage process than by the PL treatment.

Significant increase in anthocyanin, antioxidant contents as well as total phenolic contents after UV-C radiation has been reported in blueberries by Wang et al. (Wang et al., 2009) In our research, however, except for the total phenolic contents that have been slightly enhanced after PL treatments, both anthocyanin and total antioxidant activity of blueberry fruits showed a tendency to be decreased rather than increased after PL radiation. It is possible that other wavelength in PL spectrum showed adverse influences, but a more likely explanation is that the reductions were caused by high intensity and total dose used in our research. In fact, optimal UV-C total dose of 0.215-0.43 J/cm^2that showed most obvious anthocyanin, antioxidant contents and total phenolic promotion effects was found in Wang's research. A higher dose of 0.645 J/$cm^2$was proved to suppress further increase, and similar results have also been reported in strawberries by Baka,1999. Although it is difficult to calculate how much total dose of radiation was exactly applied in UV-C spectrum in our treatments, it is likely that an excessive oxidative stress was applied, and even if further synthesis of these antioxidative compounds was induced, a large amount of the molecules may have been used to work against the high oxidative stress caused by PL and finally presented a reduced total concentration. Also, the concentration of antioxidant compounds may also be highly related with overall senescence, indicating an inner state of chemical transition and degradation. Since wCtrl has significantly influenced overall sample spoilage, it may still be reasonable to see changes after merely water circulating treatments.

4.3.6 Effect of PL treatments on inactivation of *Salmonella* spot- or dip-inoculated on blueberries

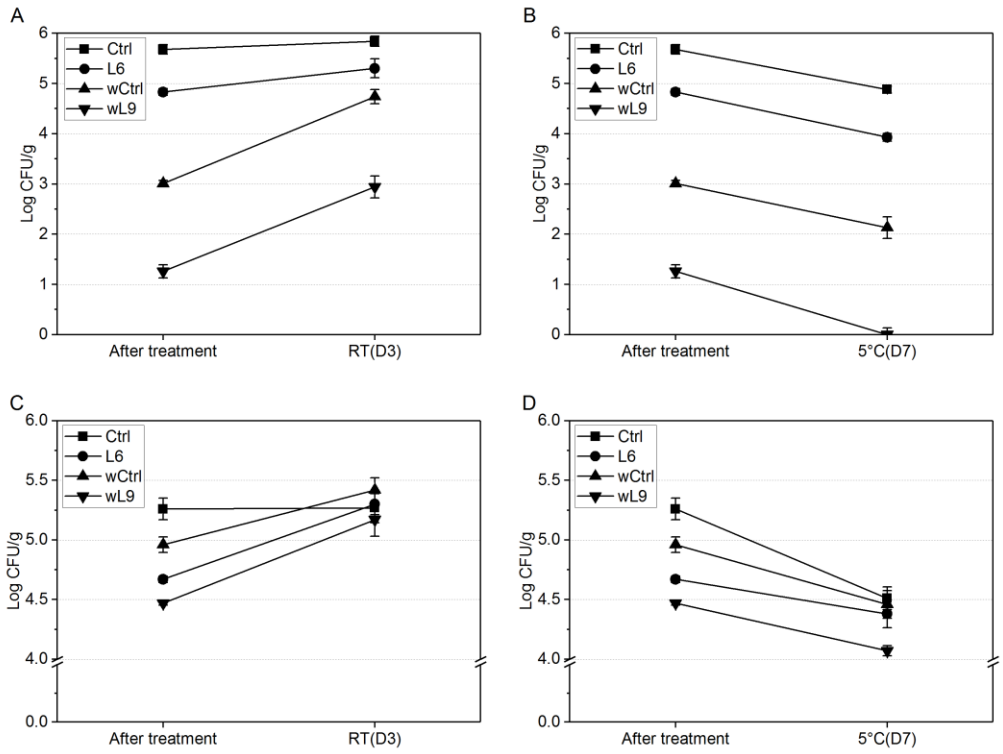

Figure 4.9 Changes in total *Salmonella* count of spot-inoculated *Salmonella* after Ctrl, L6, wCtrl and wL9 treatments during low and room temperature storage.

Note: A. 3 days of storage at room temperature, B. 7 days of storage at 5℃ and dip-inoculated s *Salmonella* after: C. 3 days of storage at room temperature, D. 7 days of storage at 5℃ for Ctrl, L6, wCtrl and wL9 samples. Data are shown as Log CFU per gram of blueberry fresh weight. Error bars represent ±SE of three replicates.

The PL inactivation effects on dip and spot inoculated *Salmonella* strains were shown above. For both dip and spot inoculated samples, at room temperature, *Salmonella* kept actively propagating and showed higher total count than day 0, while when kept under 5℃, the bacteria was gradually dying and showed lower count after storage.

For spot-inoculated blueberries, L6 treatment reduced total *Salmonella* count by 0.9 log. Washing process alone achieved 2.7 log reduction while another 1.7 log was further attained when combined with PL treatment and the total log reduction for wL9 was 4.4. Therefore, water stirring enhanced the inactivation efficacy of PL on spot inoculated *Salmonella*. After three days of storage under room temperature, Ctrl and L6 treated samples still showed higher total count than wet treated samples, but both wCtrl and wL9 samples showed obviously faster growth than L6 and Ctrl samples. To be more specific, Ctrl only showed less than 0.2 log increase while L6 increased 0.5 log. Both wCtrl and wL9 samples showed 1.7 log increase in *Salmonella* total count, possibly due to the higher moisture content that facilitated bacteria growth. For samples stored under 5℃, further after-treatment reduction of 0.8-0.9 log was detected in Ctrl, L6 and wCtrl samples. The wL9 treated samples showed a slightly higher reduction of 1.3 and the total count became undetectable, indicating a more serious injury caused by the high dose wet treatment.

As for dip inoculated berries, all treatments showed less inactivation efficacy than on spot inoculated samples as expected. Washing only reduced 0.3 log total count since the bacteria are more firmly attached around the blueberry surface, and may be absorbed in the stem scar or reside underneath complex structure of calyx. Dry and wet PL treatments achieved log reduction of 0.6 and 0.8 respectively. Possibly due to

the reason that less cells were washed up into the water, the combing PL with washing didn't further improve inactivation efficacy as on spot inoculated samples.

At the end of room temperature storage, all groups showed similar total count and the effects of treatments became undetectable. The wCtrl samples even showed higher total count than Ctrl. For cold stored samples, the L6 and wCtrl groups presented very similar *Salmonella* count of 4.4-4.5, while only wL9 treatment showed lower count of 4.0 log CFU/g.

In strawberries, 0.8 reduction for dip and spot inoculated samples were achieved by a dry, low intensity 3 J/cm^2treatment. Decontamination on blueberry surfaces should be more effective due to reduced shadow effect, as a benefit of its smooth surface structure. However, in this study, the higher total does L6 treatment didn't show any enhanced decontamination effects. Since strawberries were manually flipped over so that both sides are effectively exposed to PL yet the blueberries were treated with machine shaking, this can be an evidence that the blueberries were not effectively exposed to PL radiation.

In conclusion, as for *Salmonella* inactivation, the wet PL showed higher efficacy than dry PL for both dry and spot inoculated samples. However, extra moisture residue seemed to have side effect for samples under room temperature and may result in faster pathogen propagation. The water content didn't seem to do harm under cold storage, and better inactivation effect can be achieved when wet treatment was combined with cold storage.

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Chapter 5

DISCUSSION AND FUTURE WORK

5.1 Pulsed-light Showed Potential Use in Strawberry Industries

As stated in chapter 2, pulsed-light has been proved to be an applicable food processing technique, showing high efficiency and efficacy in microbial inactivation. Previous studies have indicated less effectiveness of PL on high-fat and high-protein food, thus it may be more applicable in fresh produces (Gomez-Lopez, 2005b). In our strawberry research, PL treatments delayed visible fungal decay and overall spoilage without noticeably influencing weight loss, firmness and flavor attributes. Since strawberries are highly susceptible to fungal decay, delay in visible fungal development can be of vital importance in shelf-life extension. PL treatments also slightly enhanced strawberry redness and better preserved its surface glossiness during storage. Pathogen inactivation effects were also obtained by PL to slightly enhance strawberry safety. In sum, the selected PL treatments enhanced strawberry shelf-life and indicated potential use of PL in strawberry industries.

In fact, more enhanced shelf-life promotion may be expected in industries than in lab-scale treatments for strawberries. Since a flash lamp is the only required equipment, industrialized PL treatment can be easily combined with pre-cooling, which is a commonly used process in fresh industries before cold storage. By this means, the treatment heat introduced by PL radiation can be directly removed. As stated before, one of the biggest advantages of PL treatment is its high treatment efficiency, and an effective treatment may only take seconds. In this case, neither extra processing time nor independent equipment would be required, and the equipment adaptation should be easy. Moreover, mechanical injures caused by handlings in our

lab-scale research can be avoided in industry, and the time-consuming ozone discharging process can also be combined with cooling in industry. In short, combining a brief PL treatment with pre-cooling and ozone discharging processes in an automated manner may more effectively prolong strawberry shelf-life in industry.

5.2 Future Directions of Pulsed-light Research in Blueberry Industries

On the other hand, however, neither dry nor wet PL treated blueberry samples showed discernable shelf-life promotion after cold storage, and they even induced higher spoilage under room temperature. The side effect which requires most attention is the enhanced weight loss, since weight loss is highly related with blueberry surface and inner structure change and may result in loss of firmness and nutrient contents, let alone that weight loss will directly reduce marketing income. There is no other evidence to clarify whether the weight loss was influenced by mechanical injury and/or physiological changes in blueberries and thus requires further exploration. Some other side effects observed in this experiment were more likely caused by improper treatment methods rather than by PL radiation. Splitting caused by water-soaking partly contributed to the high spoilage percentage of wet treated blueberries, and the high-moisture may have also enhanced microbial development and exacerbated sample decay, indicating that blueberries may not be suitable for wet treatments. The inoculation studies, which showed a reduced *Salmonella* inactivation efficacy of PL on blueberries than on strawberries, has strongly suggested that the shaking device used in blueberry research didn't effectively enhance PL exposure. Mechanical collisions introduced by shaking may have compensated the microbial inhibition effects in determining final shelf-life.

Two characteristics of blueberries may also be responsible for the unsatisfying result. One is its dark, purplish skin color that easily absorbs light energy and accumulates heat. Fine and Gervais have detected more rapid sample heating in dark colored food than in light colored food after PL radiation (Fine and Gervais 2004). What's worse, the blueberry surface bloom was fragile and can be easily removed by heating, which further makes blueberry appearance sensitive to sample heating possibly caused by PL. The other character is that visible yeasts and molds is a less severe problem for blueberries than for strawberries. Therefore, fungi inhibition may not necessarily lead to shelf-life promotion for blueberries. More encouraging results shall be expected if modified devices and treatment methods were to be used to avoid the above side effects, and for PL radiation, a milder parameter using lower total dose may still be worth trying in future studies.

5.3 PL Pros and Cons in Fresh Produce Shelf-life Extension

As stated before, the biggest disadvantage of PL is its poor penetration, which has restricted its decontamination efficacy in many produces. In terms of microbial inactivation, effective exposure was of same importance as sufficient total PL dose to achieve higher log reduction. As a result, *in vitro* decontamination of fresh produces with complex or large shaded surfaces may be difficult, which have been reported in many researches (Lagunas-Solar et al., 2006; Ozer and Demirci, 2006). In our research, unsurprisingly, no notable log reduction in total yeast and mold count was achieved on strawberries using a low total dose treatment. However, during cold storage, treated strawberries showed retarded fungi development at day 10, indicating that other fungal inhibition mechanisms may exist. Continued growing inhibition under cold environment may be ascribed to cell injuries, while other reasons such as

induced phytoalexin synthesis has also been proposed. Therefore, despite the limited decontamination effects, PL still showed possible shelf-life extension effects in fresh strawberries, suggesting that although the poor penetration of PL may be a limitation for direct decontamination efficacy, any indirect effect should not be neglected in shelf-life studies.

Another possible side effect that may be caused by PL is surface heating or injury. The high energy was considered as an advantage of PL over UV-C to enhance microbial inactivation efficacy. However, in shelf-life studies, a high intensity treatment which may cause surface heating or injury should be avoided. Fresh produces are sensitive to high energy treatments in two ways. On the one hand, their natural metabolism, especially respiration, is highly sensitive to temperature change. Therefore, remarkable regional heating on surface may still result in regional respiration promotion, although the heating effect may be compromised by the large total mess of the fruit and may not result in overall temperature increase. On the other hand, high intensity treatment may also damage surface cell structure, leading to indiscernible cell leaking, loss of surface firmness, or making tiny surface holes that facilitate moisture transpiration. All mentioned aspects are highly unfavorable and may reduce produce shelf-life. For example, in our study, although all selected PL treatments extended strawberry shelf-life, the high total dose M5 seemed to have slightly accelerated strawberry deterioration during late storage, which was indicated in many characters, such as the significantly reduced color a* and b* as well as higher anthocyanin accumulation and more degraded phenolic contents. Significant increase in weight loss was also shown in high intensity PL treated samples in our pre-experiments. Therefore, in parameter selection, usage of intense treatments should be

carefully pondered. Due to highly variated sensitivity toward PL treatments between produces, PL parameter optimization should be performed prudently and specifically for certain target produce, and even for specific cultivars and farms of a same produce.

Influence of PL on health promoting compounds such as anthocyanin and phenolic contents may be reasonably anticipated, since the intense PL which covers the active UV spectrum can work as oxidative stress and induce further changes in antioxidant molecules. However, for anthocyanin and phenolic contents, as well as total antioxidant activity, it is not likely that nutritionally valuable changes would be induced after PL treatment. The total phenolic contents showed slight enhancement of less than 5% for both berries, while anthocyanin contents and total antioxidant capacity were slightly reduced rather than promoted after treatment. Therefore, dramatic nutritional promotions as reported for the vitamin D level in mushrooms may not be expected. However, since these compounds are highly related with metabolism, monitoring the changes of bioactive molecules during storage may give hint to elucidating the mechanisms PL treatments on fresh produces.

5.4 Conclusion

To sum up, PL may be an applicable processing technique in strawberry industries for shelf-life promotion and fungal control. Though the tested parameters and methods didn't show much positive effects of PL in blueberries, further investigations with modified methodology may still be worth trying.

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