

EFFECTS OF PULSED ULTRAVIOLET LIGHT ON THE ANTIOXIDANTS,
PHYTOCHEMICALS, AND MICROFLORA INACTIVATION OF FRESH BLUEBERRIES

By

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To my family and friends

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LIST OF ABBREVIATIONS

μL	Microliter(s)
μmole	Micromole(s)
AAPH	2,2' azobis (2-amidinopropane) dihydrochloride
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
AOS	Active oxygen species
C	Carbon
°C	Celcius
CFU	Colony forming unit
cm	Centimeter
DNA	Deoxyribonucleic acid
DPPH	1,1-dipheyl-2-picryl-hydrazil
FRAP	Ferric Reducing Antioxidant Power
Ft	Feet
g	Centrifugal force
g	Gram
GPx	Glutathione peroxidase
h	Hour(s)
H ₂ O ₂	Hydrogen peroxide
J	Joule
Kg	Kilogram
kJ	Kilojoule
LDL	Low density lipoprotein
Log	Logarithmic

NADPH	Nicotinamide adenine dinucleotide phosphate
m	Meter(s)
M	Molar
mg	Milligram(s)
mL	Milliliter(s)
mm	Millimeter(s)
mM	Millimolar
mmole	Millimole(s)
N	Newton(s)
nm	Nanometer(s)
nM	Nanomolar
O ₂	Superoxide
ORAC	Oxygen Radical Absorbance Capacity
PUV	Pulsed Ultraviolet Light
ROS	Reactive oxygen species
rpm	Revolutions per minute
s	Second(s)
SOD	Superoxide dismutase
TAPC	Total aerobic plate count
UV	Ultraviolet light
v/v	Volume to volume

Abstract of Thesis Presented to the Graduate School
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Blueberries have been drawn more attention in recent years for their antioxidant capacities and proposed health benefits. Pulsed ultraviolet (PUV) light has been well known for its disinfection effects on surface of fresh fruits. However, little is known about its effects on the antioxidant capacity and quality characteristics of fresh small fruits, such as blueberry, raspberry, and strawberry. Fresh blueberries, gathered from a local farm in Gainesville, were treated with PUV for 30, 60, 90, and 120 s. The objective of this study was to examine the effects of PUV light on antioxidant capacity, phytochemicals, physiochemical attributes, inactivation of natural flora, and antioxidant enzymes.

Results show that PUV treatment enhanced the antioxidant capacity of fresh blueberries when assayed with ORAC and FRAP. However, no significant difference ($P \leq 0.05$) was observed with DPPH assay. There was no significant difference ($P \leq 0.05$) in the soluble solids, pH, titratable acidity, firmness or color of fresh blueberries within 120 s of PUV exposure. The PUV light also significantly increased the total phenolic, total flavonoid and total anthocyanin contents. Log reductions were observed in TAPC and total yeast and mold count, 1.97 and 1.27 respectively. The activity of antioxidant

enzymes, SOD and GPx, showed different responses to PUV exposure. The SOD activity showed no difference with PUV treatment, whereas the GPx activity increased by 50% with PUV treatment for 120 s.

In conclusion, PUV light illumination enhanced antioxidant capacity, phytochemicals and antioxidant enzyme activity and decreased microorganisms of fresh blueberries while maintaining other quality characteristics.

CHAPTER 1 BACKGROUND

Introduction

Blueberry (*Vaccinium corymbosum*) is one of the few fruits native to North America belonging to the *Vaccinium* genus, which consists of three major species with commercial and practical use. These include *V. corymbosum* (northern highbush), *V. ashei* (southern rabbiteye), and *V. angustifolium* (low bush or wild blueberry). *Vaccinium corymbosum* is the primary species used for modern cultivated blueberry industry. Significant differences exist in the phenolic content and antioxidant capacity among these species and within other *Vaccinium* species (Trehane 2004).

Blueberries are a rich source of phenolic compounds, naturally occurring secondary metabolites in fruit and vegetables and are appreciated for their high antioxidant activity scores. These compounds include quercetin, kaempferol, myricetin, chlorogenic acid, and procyanidins (Sellappan and others 2002). Another group of distinct phenolic compounds are the flavonoids (flavones, isoflavones, flavonones, anthocyanins, and catechin). Flavonoids are considered biologically active compounds, and exhibit a wide range of health benefits, including antioxidant (Cao and others 1996), antifungal (Benkeblia 2004), and anti-carcinogenic properties (Ames 1983).

Anthocyanins are one of the major water-soluble flavonoids in blueberries and give red, purple, and blue color to other fruits and vegetables (Sapers and others 1983). These pigments (red, blue, purple, pink, and orange) are dispersed within the vascular sap of the epidermal tissues in the skin of some fruits, with the exception of certain red fruits in which the pigments are found both in the skin and flesh (Manach and others 2004). Pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin are among

the most commonly occurring anthocyanins in foods. Differences in the degree of methylation or hydroxylation of these anthocyanins, result in varying shades of blue or red in fruits (Scalbert and Williamson 2000). Anthocyanins are stabilized by conjugating with other flavonoids (copigmentation), making them resistant to light, pH, and oxidation within the plant tissue (Scalbert and Williamson 2000).

It is well established that free radicals play an important role causing various diseases, such as cancer, vascular, and neurodegenerative disease (Jacob 1995). Phenolic compounds can act as antioxidants by scavenging free radicals, absorbing oxygen radicals, and chelating metal ions (Halliwell 1995). Espín and others (2007) reported that anthocyanins and anthocyanin-rich berries or plant extracts exhibited a wide range of potential health benefits in both human and animal studies. These health benefits are attributed to anthocyanins free radical scavenging properties (Groot and Rauen 1998).

The UV light has been used for several decades in food industry to inactivate microorganisms, especially pathogens. However, there is a little information, UV light affects some secondary metabolites in plants. Based on a theory (Lindo and Caldwell 1978; Chalker-Scott 1999) regarding far-red and UV-B radiation independent actions on anthocyanin formation in patience dock (“garden patience”) (*Rumex patientia*), it is believed that UV light may be implicated in plant phenol and anthocyanin biosynthesis. According to Mohr and Drumm-Herrel (1983), anthocyanin biosynthesis is described as a protective mechanism induced to protect the plant against sunlight and environmental stressors.

Pulsed UV light technology has been regarded an emerging technology, which has been applied to decontamination of food surfaces and packing materials. Other applications include the pasteurization of milk and mitigation of food allergens (Chung and others 2008; Demirci and Panico 2008). However, while product safety is an essential criterion for public health and safety, the quality and shelf life of food should not be compromised. Two important quality parameters for consumer acceptability and fresh-market value are texture and color, and these are mainly attributed to the eating quality of fresh fruits. Additionally, physiochemical properties, such as soluble solids and titratable acidity, also contribute to the eating quality of fruit, as they are primarily responsible for taste.

Blueberries: General Approach, Chemical and Health Properties

Vaccinium species, such as blueberries and cranberries, have been one of the main sources used in the human diet for thousand of years, often gathered in the wild. Blueberries and cranberries are two examples of commercially important fruits. Blueberries are a member of the *Ericaceae* family with other acid-loving species such as cranberry and lingonberry (Rowland and others 2012). Some of the oldest evidence of blueberry consumption, a 200-year-old sealskin pouch containing wild blueberries found in Northern Canada, exposes its importance to humans. Blueberries are also a good source of food for birds, such as various species of grouse, partridge, quail, American robins, European blackbirds, as well as for mammals. In North America, bears and chipmunks consume the berries. Also the leaves and young stems are an important part of the daily diet of deer, elk, rabbit, and hare (Trehane 2004).

Blueberries often grow in acidic and imperfectly drained soils, which otherwise would be unfit for agricultural production (Dhanaraj and others 2007). Commercial

blueberries are categorized into three groups based on their shrub heights: (1) the lowbush group species, of heights, not more than one meter (e.g. *V. myrtilloides*, *V. angustifolium*, and *V. lamarckii*.); (2) the half-high group, formed of a series of hybrids. (Representatives include *V. corymbosum* × *V. lamarckii*, *V. corymbosum* × *V. brittonii*, and *V. atrococcum* × *V. vacillans*); (3) the highbush group ranges from 1.5-7 m (including *V. elliotii*, *V. corymbosum*, and *V. simulatum*) (Eck and Childers 1966).

Highbush Blueberries

Highbush blueberries (*Vaccinium corymbosum*) are tetraploid members of the *Vaccinium* family. Shrub height varies typically between 4 to 6 ft. (1.2 to 1.8 m), and occasionally 10 to 12 ft. (3 to 3.7 m). The fruit characteristics are a blue to black color with 5-10 mm diameter, and good or excellent flavor. They are a many-seeded berry and mature two or three months after flowering. The growth areas of *V. corymbosum* range from Eastern North America and Nova Scotia and Quebec in Canada to as far west as northeastern Illinois, northern Indiana, and the more southern parts of Michigan are the growth area of *V. corymbosum* (Eck and Childers 1966; Trehane 2004).

Native Americans called blueberries 'starberries' since there is a star-shaped scar left on the fruit by the calyx. They were gathered, freshly consumed, and either dried in the sun or with smoke for winter. Later, early settlers noticed the usefulness of blueberries. They consume blueberries while fresh, stewed, with milk, or mixed with corn or wheat flour to make cakes or bread (Trehane 2004).

Chemical Properties of Blueberries

The phytochemical content of berries is affected by preharvest environmental conditions and the degree of maturity at harvest, as well as by genetic differences (Łata and others 2005). Blueberries have high antioxidant levels and are rich in anthocyanins

(Grace and others 2009; Papandreou and others 2009). Related studies proved that the accumulation of anthocyanins continues in overripe blueberries, as well as after harvest and during the storage period. The complex anthocyanin pattern and high total anthocyanin content of blueberries are well known among berries (Lohachoompol and others 2008). The quality assessment of blueberry products is related to the anthocyanins levels, since they are the major responsible ingredients (Sun and others 2012).

Health Benefits of Blueberries

Blueberries have drawn public attention for years as they have a broad spectrum of pharmacologic properties, including antioxidant and anti-carcinogenesis. Blueberries may also prevent the macular degeneration, reduce the risk of heart disease, aid in treatment of urinary tract disorders, improve night vision, and enhance memory (Rowland and others 2012; Sun and others 2012). Anthocyanins are considered the compound in blueberries responsible for improvement in night vision, prevention of macular degeneration, anti-cancer activity, and reduced risk of heart disease (Rowland and others 2012). The antioxidant capacity of blueberries is also affected by the total anthocyanin content, genotype, environmental variation, maturity, and postharvest storage circumstances (Lohachoompol and others 2008).

It is well known that blueberries are a good source of polyphenolic compounds and secondary plant metabolites, which have multiple hydroxyl groups attached to benzene rings (Hurst and others 2010; Vrhovsek and others 2012). Blueberries are thought to be one of the most desirable and nutritious fruits since they have a high antioxidant capacity, and a high concentration of anthocyanins and other phenolic compounds (Prior and others 1998). Other polyphenolic compounds that blueberries are good

sources of chlorogenic acid, quercetin, kaempferol, myricetin, procyanidins, catechin, epicatechin, resveratrol, and vitamin C. These compounds, along with anthocyanins, provide the antioxidant activity found in blueberries (You and others 2011). The phytochemical concentration of blueberries is related to the cultivar, state of maturity, geographic origin, growing season, horticultural practices, post harvest storage conditions, and processing procedures (Łata and others 2005).

Blueberries are believed to be potential substitutes for synthetic colorants and are used in dietary supplements due to their attractive, bright colours, water soluble nature, and positive therapeutic effects (Wang and others 2010).

Generation of Free Radicals

Oxygen is essential for the existence of aerobic organisms, such as human beings, animals, and plants. However, it participates in formation of toxic reactive oxygen species (ROS), known as free radicals. These free radicals include superoxide (O_2^-), and hydroxyl radicals, hydrogen peroxide (H_2O_2), and singlet oxygen (1O_2). The term reactive oxygen species includes oxygen-containing radicals with one or more unpaired electrons and reactive oxygen-containing compounds without unpaired electrons, such as H_2O_2 (Battin and Brumaghim 2009). These radicals, produced from partially reduced O_2 , are unavoidable consequences of aerobic respiration (Noctor and Foyer 1998; Lee and others 2003; Hayes and others 2005). Although oxidative phosphorylation is the primary source of free radicals, they can also arise through 5-lipoxygenase-, cyclooxygenase-, cytochrome P450-, and xanthine oxidase-catalyzed reactions (Hayes and others 2005). Hence, cellular ROS and oxidative stress generate by environmental agents, such as UV radiation, ozone (O_3), nitrogen dioxide (NO_2), tobacco smoke, carbon tetrachloride (CCl_4), paraquat, and chemotherapeutic drugs

(Battin and Brumaghim 2009). The hydroxyl radical and $^1\text{O}_2$ are generally desired to be present in low since they are very reactive (Noctor and Foyer 1998). The damage of free radicals is considered as an underlying cause of various diseases, including cancer, inflammatory, and neurodegenerative diseases (Battin and Brumaghim 2009).

Under basal conditions, ROS are produced during various cellular processes, but they can be minimized by well-integrated antioxidant systems, such as the enzymes superoxide dismutase, catalase, and glutathione peroxidase; the macromolecules albumin, ceruloplasmin, and ferritin; and low molecular weight antioxidant polyphenols, including ascorbic acid, α -tocopherol (vitamin E), β -carotene, reduced glutathione, uric acid, and bilirubin (Cao and others 1999). Superoxide and H_2O_2 can occur at very high rates even under optimal conditions in all major areas of aerobic biochemistry and are products of several enzyme systems (Noctor and Foyer 1998). There must be a balance between the production of ROS, such as H_2O_2 and superoxide anion, and their removal by antioxidant systems of plants (Jimenez and others 2002).

Antioxidant Capacity of Fruits and Vegetables

Oxidative stress may occur in all organisms in any environment with access to oxygen in all organisms. “Nutraceutical” is a term used for any nontoxic food extract supplement, which provides medical or health benefits, including the prevention and treatment of chronic diseases, including cardiovascular diseases, cancers, and neurodegenerative diseases (Lin and Weng 2006; You and others 2011). Dietary antioxidants are important as nutraceuticals since they protect the body from free radicals, ROS, and reactive nitrogen species, derived from normal metabolic processes or from external sources (Terpinc and others 2012). Some phytochemical compounds, found naturally in foods, possess a variety of antioxidant defense mechanisms. These

compounds increase the oxidative stability of foods with antioxidant mechanisms, in which free radicals, prooxidants, and oxidation intermediates are controlled (Fennema and others 2008). Free radicals are produced in high amounts in multitude of pathological conditions (Stratil and others 2007) and are thought to be responsible for many common chronic degenerative diseases, such as cancer, heart, vascular, and neurodegenerative diseases (Ehlenfeldt and Prior 2001; Stratil and others 2007; Patthamakanokporn and others, 2008). Free radicals are derived from two sources to be in the body: (1) endogenous sources, such as nutrient metabolism and the ageing process, and (2) exogenous sources, such as air pollution. Various substrates in the body can be attacked by the free radicals, which contribute to chronic disease development. As an example of these substrates, oxidatively modified LDL has been hypothesized to be a causative agent of cardiovascular disease (Patthamakanokporn and others 2008).

As proven by many research studies, the various components, existing in fruits and vegetables, are responsible for their beneficial health effects as the compounds have antioxidant activity. Vitamin C, vitamin E, carotenoids, and polyphenols, such as phenolic acids, catechins, flavonols, and anthocyanins, are some examples of beneficial compounds, found in foods, and possess strong antioxidant capacity (Ehlenfeldt and Prior 2001; Giovanelli and Buratti 2009). The species and variety of the fruit, the environmental and cultivation techniques and the cultivation conditions all have effects on the antioxidant properties of fruits (Koca and Karadeniz 2009).

Blueberries are well known for their strong antioxidant capacity, found to be more correlated to total phenolic content rather than anthocyanin concentration (Giovanelli

and Buratti 2009). Blueberries have appreciable levels of phenolic compounds, including anthocyanins, flavonols, chlorogenic acid, and procyanidins (Koca and Karadeniz 2009).

Chemical Structures and Attributes of Total Phenolic, Flavonoid, and Anthocyanin Compounds

Structure of Phenolic Compounds

Diets rich in fruits, vegetables, and derived beverages have protective effects obtained not only from fiber, vitamins and minerals, but also from the diversity of plant secondary metabolites, especially phenolic compounds and flavonoids (Crozier and others 2010). Phenolic compounds derived from the pentose phosphate, shikimate, and phenylpropanoid pathways in plants are one of the most widely occurring groups of phytochemicals with considerable physiological and morphological importance in plants (Balasundram and others 2006). They are a major class of antioxidants found almost in all plants, often at high concentrations in fruits and vegetables, and protect organisms against oxidative stress produced by free radicals (You and others 2011).

Phenolic compounds are an important group of natural antioxidants with possible beneficial effects on human health and are widely distributed in medicinal plants, spices, grains, pulses, and other seeds besides vegetables and fruits. Thus, phenolic compounds in foods may be found naturally occurring in the original plant or may be formed during processing. They can protect against harmful action of ROS, especially oxygen free radicals (Stratil and others 2007; Terpinic and others 2012). Besides improving the color and sensory characteristics of fruits and vegetables, phenolic compounds have an important role in growth and reproduction, protecting the organism against pathogens and predators (Balasundram and others 2006).

The structure of phenolic compounds consists of an aromatic ring, bearing one or more hydroxyl substituents. The group of compounds is generally named as polyphenols, despite the structural diversity occurring in hydroxyl substituents. Phenolic compounds range from simple phenolic molecules to highly polymerized compounds. Several classes can be categorized within phenolic compounds as a result of the structural diversity. Phenolic acids, flavonoids, and tannins are considered as the main dietary phenolic classes (Balasundram and others 2006).

Structure of Flavonoid Compounds

As a very large group of natural products with their conjugates, flavonoids are found in many plant tissues, inside the cells or on the surfaces of different plant organs (Stobiecki and Kachlicki 2006). They are the most common group of polyphenolic compounds in the human diet and there are more than 2000 individual flavonoids, known at present (Ramassamy 2006). Their major dietary sources are fruits, vegetables, cereals, tea, wine, and fruit juices (Spencer and others 2009). Thus, these metabolites are responsible for much of the red, blue, and purple pigmentation found in plants (Winkel 2006). Polyphenolic compounds have vital developmental roles in plant structural integrity, UV photoprotection, reproduction, defense against pathogens and pests, pollen fertility, pigmentation and internal regulation of plant cell physiology and signaling (Ferrer and others 2008; Guo and others 2008).

Flavonoids provide important biochemical and physiological attributes in the various cell types or organs (seed, root, green part, fruit) in which they accumulate. Both in biotic and abiotic conditions, numerous plant functions occur due to the different classes of flavonoid and their conjugates as the plant interacts with the environment. Flavonoids are very important components of human and animal diets because of their

abundant presence in plants and ability to induce human protective enzyme systems (Chang and others 2009). The regular consumption of plant secondary metabolites may have serious consequences for health, both positive and negative since the metabolites have different biological activities (Stobiecki and Kachlicki 2006).

In nature, flavonoids participate in many functions, such as providing pigmentation for flowers, fruits and seeds to attract pollinators and seed dispersers, protection against UV light, plant defense against pathogenic microorganisms, plant fertility and germination of pollen, and acting as signaling molecules in plant microbe interactions (Schijlen and others 2004; Chang and others 2009).

Flavonoids are low molecular weight phenolic compounds with fifteen carbon atoms, arranged in a C₆-C₃-C₆ configuration. Since they constitute over half of the 8000 naturally occurring phenolic compounds, flavonoids are the largest group of plant phenolics. As shown in Figure 1-2, there is the aromatic A ring which is derived from the acetate/malonate pathway, the B ring which is derived from phenylalanine through the shikimate pathway, and the C ring. The C ring is responsible of the major flavonoid classes with variations in substitution patterns. Flavonoid classes differ in the saturation of the heteroatomic C ring, in the placement of B ring at the C-2 or C-3 positions of C ring, and in the hydroxylation patterns (Balasundram and others 2006; Stobiecki and Kachlicki 2006).

The main dietary groups of flavonoids are: (1) flavonols (e.g. kaempferol, quercetin), found in onions, leeks, and broccoli; (2) flavones (e.g. apigenin, luteolin), found in parsley, and celery; (3) flavanones (e.g. hesperetin, naringenin), found in citrus fruit, and tomatoes; (4) flavanols (orcatechins) (e.g. (+)-catechin, (-)-epicatechin,

epigallocatechin, epigallocatechin gallate (EGCG), found in green tea, red wine, and chocolate; (5) isoflavones (e.g. daidzein, genistein), found in soy and soy products, and (6) anthocyanidins (e.g. pelargonidin, cyanidin, malvidin), found in red wine and berry fruits. Flavones and flavonols are the most structurally different flavonoids are the most widely found of the major flavonoid classes (Schijlen and others 2004; Balasundram and others 2006; Ramassamy and others 2006; Stobiecki and Kachlicki 2006; Spencer and others 2009).

The modification of flavonoids can be made by hydroxylation, methoxylation, or O-glycosylation of hydroxyl groups, as well as by C-glycosylation directly to the carbon atom of the flavonoid configuration (Stobiecki and Kachlicki 2006).

Structure of Anthocyanin Compounds

Anthocyanin is a term, derived from the Greek words, flower and blue (de Pascual-Teresa and Sanchez-Ballesta 2008). Anthocyanins are glycosides of anthocyanidins and are assumed to be the most important water-soluble natural pigment group in plants (He and Giusti 2010; Patras and others 2010; You and others 2011). They are one of the compound classes existing under the flavonoid group (Barnes and others 2009) and comprise the majority of flavonoids. Although there are up to 600 different species of anthocyanins, which have been reported (Barnes and others 2009), only a few anthocyanidins, including cyanidin (Cy), delphinidin (Dp), pelargonidin (Pg), peonidin (Pn), malvidin (Mv), and petunidin (Pt), are distributed in flowers, pulps, and skins of fruits, particularly in berries and vegetables, and approximately 95% of all anthocyanins are derivations of them. Anthocyanins are responsible for bright colors in orange, red, and blue due to the environmental pH values (He and Giusti 2010; You and others 2011).

Colored fruits such as berries, cherries, peaches, grapes, pomegranates, and plums, as well as many dark-colored vegetables such as black currant, red onion, red radish, black bean, eggplant, purple corn, red cabbage, and purple sweet potato, are sources of edible anthocyanins in nature (He and Giusti 2010). Anthocyanin levels are ranged between 0.25 mg/100 g fresh weight in the pear and 500 mg/100 g fresh weight (FW) in blueberries. Berries (deep purple or black) are the fruits richest in anthocyanins (>20 mg/100 mg FW) and are very strongly colored fruits (Prior 2003). Bilberries (*Vaccinium myrtillus* L.) and blueberries are believed to be good sources of anthocyanins with approximate anthocyanin concentrations of, 1210 mg/100 g and 212 mg/100 g fresh weight respectively (Bornsek and others 2012). Anthocyanins exist in blueberries in high quantities, mainly as glycosylated forms, flavonols (such as quercetin, kaempferol, and myricetin), catechins (such as (+) catechin, (-) catechin, and their oligomeric forms), and benzoic and cinnamic acids (Giovanelli and Buratti 2009).

The presence of different kinds of pigments from phenylpropanoid and terpenoid classes, three major groups of which are chlorophylls, carotenoids, and anthocyanins, provides the color of flowers and fruits (Gonzali and others 2009). Anthocyanins, as secondary plant metabolites, are considered to be responsible for the colors, purple, blue, and red, found in many plants (Prior 2003; Lohachoompol and others 2008; He and Giusti 2010; Patras and others 2010) and are believed to exist in the dissolved form as part of the cell sap in the vacuole of the plant's epidermis cells as dissolved form (Barnes and others 2009). Plants may be protected by anthocyanins from UV-induced damage since anthocyanins absorb light strongly (He and Giusti 2010).

Anthocyanins are considered as a key quality parameter of fruits and vegetables and impact consumer sensory acceptance as they provide characteristic color. Hence, anthocyanins are considered as an important factor for plants to attract animals and lead seed dispersal and pollination (He and Giusti 2010; Patras and others 2010).

Different colors can occur due to the anthocyanin species. Since anthocyanins are highly soluble in aqueous solutions, the acidity of the environment is effective, as well as the structure of anthocyanin and the presence of co-pigments, on the color of fruits. In aqueous solution, anthocyanins can be found in four main equilibrium species, which are the flavylium cation, the quinonoidal base, the carbinol or pseudobase, and the chalcone C. The relative amounts of each equilibrium form differ depending on the pH of the solution and the structure of the anthocyanin (de Pascual-Teresa and Sanchez-Ballesta 2008). A red color and the primary structure of the compound can occur when the pH of a solution is below 2.5 and the anthocyanin is in the predominant form of the low pH, flavylium state. Most of the flavylium ions transform to other anthocyanin forms, some of them quinonoidal forms (blue color) and other pseudobases and chalcones (colorless), as the pH of the medium increases. In solutions with pH values between 4 and 6, weakly acidic solutions, a secondary structure, a mixture of anhydrous and pseudobases, of the compound occurs. The purple anhydrobases occur first and later rapidly decolorize to colorless pseudobases. In the pH values above 8, a colorless chalcone structure occurs due to the pyran ring opening (de Pascual-Teresa and Sanchez-Ballesta 2008; Barnes and others 2009; He and Giusti 2010).

The core structure of the anthocyanin, a flavylium cation, is described as a C6-C3-C6 skeleton with a phenolic ring fused to a pyran and an additional phenolic ring

connected at the 2 position of the pyran (Barnes and others 2009; Patras and others 2010). A formal positive charge found on the oxygen of the pyran ring makes the different anthocyanins from the rest of the flavonoid group (Barnes and others 2009). The flavonols and flavandiols are formed as the hydroxyl groups are added to core flavonoid rings. They are the entry points of the formation of proanthocyanins and anthocyanins (Ferrer and others 2008). Anthocyanins are glycosides of 18 anthocyanidins. Anthocyanidins differ from each other by their degree of methoxylation and hydroxylation and only 6 of them are common in nature (Lohachompol and others 2008). Cyanidin is the most commonly occurring anthocyanidin in nature (Guo and others 2008). The difference between anthocyanin structures not only affects plant color, but also affects antioxidant capacities.

The positive therapeutic properties of anthocyanins, besides being nontoxic and nonmutagenic, have been explored in many studies (Wang and others 2010). According to many studies conducted on health, anthocyanins are powerful antioxidants, which protect against oxidative damage from reactive species by neutralization of free radicals via various mechanisms. Anthocyanins have a positive charge and aromatic hydroxyl group, which can easily release protons to the free radicals. Cells are protected against oxidative damage, which causes aging, various diseases, and mortality by cardiovascular disease and some types of cancer (Barnes and others 2009). Anthocyanins are also believed to contribute many health benefits such as prevention of heart disease, inhibition of carcinogenesis, diabetes and inflammatory activity in the brain (Lohachompol and others 2008; Patras and others 2010). The intake of

anthocyanins in humans has been reported to be approximately 180- 215 mg/day in the United States (Zafra-Stone and others 2007).

Anthocyanin compounds have many important roles, such as functions in flower pigmentation, counteracting the negative effects of nitrogen and reactive oxygen species, maintaining the redox homeostasis of biological fluids, contributing to defenses against phytopathogens, as well as protection against UV light, in plants (Guo and Wang 2010).

Anthocyanin synthesis in plant seedlings is a part of plant growth regulation and metabolism (Guo and Wang 2010). Anthocyanins are unstable and easily oxidized. Environmental factors, such as light, temperature, and altitude, agronomic factors, nutrient supply, species, and even cultivars are some of the factors, which affect anthocyanin content of fruits and vegetables (He and Giusti 2010; Patras and others 2010). The stability of anthocyanins are influenced by properties of the plant and environment, such as pH, storage temperature, chemical structure, concentration of anthocyanins present, light, UV radiation, oxygen, and presence of enzymes, proteins and metallic ions. The presence of some compounds, such as sulphur dioxide, ascorbic acid, and metal ions, in the medium also affects anthocyanins (de Pascual-Teresa and Sanchez-Ballesta 2008; Patras and others 2010).

Natural Microflora of Fresh Blueberries

Fresh fruits have an appropriate medium for growth of several types of fungi and bacteria. Fruits can be contaminated in the field, during harvesting, transport, marketing, or by the consumer (Tournas and Katsoudas 2005). Fresh fruit consumption may also cause foodborne illness. However, many diseases related to foodborne occur every year, and most of them are not reported properly. Despite of that fact, the proportion of

foodborne illness related to fresh products increased from 1% to 6% between the years 1970 to 1990. Each year, approximately 73,000 cases of *Escherichia coli* O157 infections and 2 million cases of salmonellosis infections are reported in the United States (Bialka and others 2008). Recent outbreaks of *E. coli* O158:H7 were linked to packaged baby spinach, *Salmonella saintpaul* to hot peppers and tomatoes, and *Salmonella poona* to imported cantaloupes in the United States (Lynch and others 2009).

Fresh fruits and vegetables are usually prepared in traditional ways, including washing, trimming, cutting, and peeling, right before consumption at home and restaurants. The food industry has begun to implement novel postharvest processes to please the consumer desire for fresh or fresh-like vegetables and prevent quality losses during traditionally process (Schenk and others 2011). Pulsed UV (PUV) light is an application of intense light pulses (ILP), a novel technology used for decontamination of food surfaces. Other application examples of this novel technology are pulsed white light and intense light pulses (Rajkovic and others 2010). Using short time high frequency pulses of an intense broad spectrum light is the approach adopted for killing microorganism by this novel technology. The effectiveness of these short intense light pulses is related to the structural changes in microbial DNA. Decontamination with PUV light also depends on the light absorption efficiency of microorganisms. Also, certain food components may absorb the effective wavelengths, therefore reducing the decontamination capability of this treatment (Gómez-López and others 2005).

Enzymatic Pathway of Antioxidant

Free radicals may cause serious physiological damages to the living organisms (Lee and others 2003). Fruits and vegetables expose their protective effect against free

radicals using their antioxidant components, including ascorbate, glutathione, carotenoids, tocopherols, and phenolics. Lipids, proteins, and nucleic acids are protected by these phytochemicals against oxidative damage caused by free radicals (Łata and others 2005). On the other hand, plants have antioxidant enzymes, including superoxide dismutase (SOD), peroxidase, and catalase, which play a major role in the selective detoxification of free radicals in living organisms (Lee and others 2003; Jaleel and others 2009). Glutathione and ascorbate exist in two forms, which are biologically active in their reduced and oxidized forms. Environmental stress generally increases the level of the latter one. The oxidized form of these antioxidant components generates harmful free radicals as plants mature. The enzymes ascorbate peroxidase and glutathione reductase catalyze the regeneration of glutathione and ascorbate, and indirectly remove free radicals. Free radicals can be directly detoxified by catalases (Łata and others 2005). Antioxidant enzymes can catalyze the quenching process of active oxygen species (AOS) preventing the formation of destructive radicals or being involved in the direct generation of AOS. These enzymes also interrupt the cascades of the uncontrolled oxidation (Noctor and Foyer 1998). There is a positive correlation between antioxidant activity and antioxidant enzyme activity. However, different species and genotypes vary in this correlation (Wang and others 2010).

In plants, high intra-cellular concentrations of AOS and reduced compounds, such as NADPH, can be induced by the photosynthetic process. An array of enzymes must supplement the protection process against these reactive compounds. Superoxide dismutase (SOD) is the first enzyme of the cellular line of defense against oxidative

stress, composed of several enzymes and antioxidants. They prevent membrane peroxidation and other injuries (Perl-Treves and Galun 1991; Lee and others 2003).

Superoxide dismutase (SOD) and catalase are involved in the destruction of superoxide and hydrogen peroxide H_2O_2 (Jaleel and others 2009). The SODs are metalloenzymes and are found in all aerobic organisms. They are considered to be essential components for function and survival/defense of aerobic organisms since they scavenge the toxic superoxide radicals, formed as by-products of aerobic respiration during many stress conditions (Perl-Treves and Galun 1991; Bowler and others 1992; Lee and others 2003; Jaleel and others 2009). The SODs are unique and ubiquitous enzymes and their activity determines the concentrations of substrates in the defense mechanism. Types of SODs are classified by the metal cofactor. The three known types of SODs are copper/zinc (Cu/ZnSOD), manganese (MnSOD), and iron (FeSOD) forms. Their sensitivity characteristics to inhibitors, KCN and H_2O_2 , differ by types. The Cu/ZnSOD is sensitive to both KCN and H_2O_2 , FeSOD is sensitive to only H_2O_2 , and MnSOD is resistant to both (Perl-Treves and Galun 1991; Bowler and others 1992).

Superoxide radical is generated upon reduction of O_2 and the generation is highly reactive in contrast to O_2 . The generation of superoxide anion may occur by natural sources, such as byproducts of metabolic activities and electron-transport chain. Also, it can be reduced by external agents, including ozone, UV-B, gamma rays, light-induced photo inhibitory conditions, and chemicals like paraquat or methyl viologen (Jaleel and others 2009). Superoxide dismutase rapidly catalyzes the dismutation of the superoxide anion and produces less reactive hydrogen peroxide and O_2 (Rabinowithc and Sklan 1980; Lee and others 2003):



Catalases and/or peroxidases remove H_2O_2 and reduce it to H_2O . If it is not removed quickly, hydrogen peroxide reacts with superoxide anion radicals and forms highly reactive hydroxyl radicals by the Haber-Weiss reaction. The SODs may be effective on the prevention and cure of many kinds of human diseases, including arthritis, rheumatism, ischemic heart disease, and illness caused by radiation. They protect the skin against UV-induced cutaneous damage when light is applied topically on skin (Reviewed by Lee and others 2003).

Glutathione (reduced form) is the preponderant non-protein thiol, regulator of sulfur uptake at root level and is involved in the reduction of disulfide proteins. It is a major component in cellular antioxidant systems since it acts as a detoxifying agent for endogenous radical species. It is also a cofactor for different detoxifying enzymes, such as glutathione peroxidases (GPx) and glutathione-S-transferases (Jimenez and others 2002; Battin and Brumaghim 2009; Jaleel and others 2009). Glutathione peroxide has an antioxidant function of reducing peroxides, such as H_2O_2 . Selenium-independent glutathione peroxide is present in the stem of higher plants, but is absent in leaves and roots (Arora and others 2002; Battin and Brumaghim 2009).

Senescence, an oxidative process, is the breakdown of cell wall components and membrane disruption, which causes the cellular decompartmentation and loss of tissue structure in higher plants (Lacan and Baccou 1998). The activity of all SOD enzymes with other oxygen-detoxifying enzymes, including catalase and glutathione reductase, decreases as the plant senesces. Free radicals play an important role in the aging process of plants, especially in membrane deterioration. They cause the peroxidation of

membrane lipids resulting in the loss of membrane integrity and membrane bound enzyme activities (Bowler and others 1998; Lacan and Baccou 1998).

Pulsed Ultraviolet Light

Pulsed ultraviolet light (PUV), also referred to as high intensity (100-1100 nm), is a composite of ultraviolet light (UV; 100-400 nm), visible light (400-700 nm), and infrared (IR; 700-1100 nm) at 54, 26, and 20% respectively (Oms-Oliu and others 2010). In food processing, the pulses of light used typically emit 1-20 pulses/s at an energy density in the range of about 0.01 to 50 J cm⁻². As described by Oms-Oliu and others (2010), the equipment consists of one or more lamps filled with inert gases, such as xenon, a power unit, and a high voltage connection that allows the transfer of a high current electrical pulse. PUV light is generated as electric current passes through the gas chamber of a lamp unit. During this process, the energy is exponentially increased as a result of being stored in a capacitor, and then the stored energy is released in short pulses (Demirci and Panico 2008).

Cao and others (2011) reviewed that the levels of bioactive compounds and antioxidant capacity of strawberries were enhanced by postharvest treatments, such as ultraviolet radiation, altered gas composition, and application of signaling molecules.

The PUV light has three mechanisms of action for killing microorganisms: Photochemical, photothermal, and photophysical mechanisms. In the photochemical mechanism, nucleic acids of the microorganisms are the main target of PUV treatment because DNA molecules are the primary targets of these UV wavelengths. The photothermal mechanism has a lethal effect on microorganisms due to the temporary overheating by UV light absorption of microorganisms. The photophysical mechanism is

effective on membrane and cell composition, influences proteins, membranes, and other cell materials, thought to be related to the destruction of nucleic acids.

The PUV treatment is a light-based technology and a promising alternative disinfection method to traditional applications, such as heat treatment for inactivating spoilage and pathogenic microorganisms in foods (Hsu and Moraru 2011). However, while product safety is an essential criterion for public health and safety, the eating quality and shelf life stability of the food should not be compromised. Two important quality parameters for consumer acceptability and fresh-market value are texture and color. These parameters are especially pertinent to the quality of fresh fruits. Additionally, physiochemical properties, such as soluble solids, pH, and titratable acidity, may also contribute to the quality of fruit, as they are primarily responsible for taste.

Pulsed UV Light Effects on Antioxidant Capacity

In many studies, it has been suggested that UV light has an increasing effect on antioxidant capacity of fresh fruits. Erkan and others (2008) analyzed the UV light effect on fresh strawberries and they obtained increased antioxidant capacity values of UV light treated strawberries. A similar study by Perkins-Veazie and others (2008) revealed that UV irradiation increased antioxidant capacity of blueberries. Similar results were obtained by Wang and others (2009) in another study about UV-C effects on flavonoid content and antioxidant capacity of blueberries. They observed significant increases in antioxidant capacity and phytochemical contents (phenolics and anthocyanins) of treated blueberries as compared to the control. These studies show that UV light may have an enhancing effect on antioxidant capacity in fresh fruits with slight differences.

Since the majority of the light (~54%) is UV light in a PUV system, PUV treatment may also increase significantly antioxidant capacity of fresh fruits.

Oms-Oliu and others (2010) studied on the PUV light effects on antioxidant capacity and quality properties of fresh cut mushrooms. They reported that PUV treatment enhanced the phenolic compounds, vitamin C content, and antioxidant capacity of fresh cut mushrooms while providing high quality. However, high fluencies reduced these attributes affecting texture and quality properties.

Based on the results obtained in these studies on the effect of UV light on antioxidant capacity of fresh fruits and vegetables, it is suggested that PUV treatment may have a similar enhancing effect on phytochemicals and antioxidant capacity while maintaining a high quality in products. However, the right conditions of treatment time, lamp intensity, and distance might be adjusted for each type of fruits and vegetables. The process conditions are as effective as the light absorption efficiency of food for a successful application. Many researchers try to find an easy method to obtain right conditions for each food sample, but the most effective way is still experimental measurements.

Pulsed UV Light Effects on Bioactive Compounds

Many environmental factors are effective on the regulation of plant development and the expression of plant genes. Light is one of the most important environmental factors. The maximization of a plant's photosynthetic productivity depends on its abilities, including capacity of sensing, evaluating, and responding to light quality, quantity, and direction. Ultra violet-A comes from the sun light, reaches Earth's surface, and causes serious effects on all living organisms (Guo and others 2008; Guo and Wang 2010). The UV- absorbing pigments are alerted to accumulate antioxidant

defenses to use against ROS compounds even at a low intensity of UV-B rays (Guo and others 2008).

Guo and others (2008) reviewed that the anthocyanin accumulation caused by UV-A exposure might have a role in the protection of the plant tissue from the potential damage by UV absorption. Low fluences of UV-B cause changes in the expression of genes as a response to stress, and an increase in the UV-absorbing pigments. UV-A can increase oxidative stress by inducing ROS generator compounds in plants. Flavonoids and anthocyanins of higher plants are examples of UV-absorbing pigments generated by organisms whereas melanin exposes the same traits in animals and humans (Guo and Wang 2010). The UV treatment may reduce fruit softening and enhance anthocyanin levels in strawberries since light is one of the important environmental factors in the generation of anthocyanin biosynthesis in plants (Wang and others 2011).

Pulsed UV Light Effects on Disinfection

Pulsed UV light is a rich source of UV light and PUV treatment exposes short, high power pulses of white light emitted from a light source, such as a Xenon lamp. The high proportion of UV light is mainly responsible for cell death in microorganisms. It is believed that the effectiveness of PUV light treatment on decontamination depends on the dose of the light energy, which is emitted by the target microorganism (Woodling and Moraru 2007). Pulsed UV light is used to inactivate not only spoilage microorganisms found in natural flora, but also pathogenic microorganisms that may pose a health risk to humans.

In a recent study by Levy and others (2011), the relevant factors on surface decontamination using pulsed light were studied. They observed different log reductions

with different treatment times. However, even in the same treatment conditions (treatment time and lamp distance), various results were observed. This variety was an evidence of other related factors, such as variety of microorganisms, the lamp input voltage, and substrate, which influenced the effectiveness of PUV treatment.

A study by Bialka and Demirci (2007), contrasting the efficacy of gaseous ozone, aqueous ozone, and pulsed UV-light (PUV) on decontamination of artificially contaminated blueberries (with *E. coli* and *Salmonella*). In the PUV experiment there were two independent conditions, treatment time and sample distance from the lamp. The amount of inactivated microorganisms increased with the increasing treatment time and decreasing lamp distance to the sample. Even though these conditions influenced the effectiveness of the PUV light, interaction between treatment time and lamp distance could not be explained in this study. Decreasing lamp distance and increasing treatment time produces loss of quality characteristics of the blueberries. Bialka and others (2007) indicated that PUV was a promising disinfection method on blueberries.

From another study of Bialka and others (2008) about PUV disinfection on artificially inoculated (with *E. coli* and *Salmonella*) small fruits (raspberries and strawberries) at varying UV doses and times, significant microorganism reductions of raspberries and strawberry surfaces were reported. Thus, there was no observable damage to the fruits at the applied UV doses. Both studies showed that PUV light was effective on surface decontamination of small fresh fruits.

Low pH values of fruit juices inhibit the growth of many microorganisms in juices. Even though fruit juices have an innate partial protection against spoilage and pathogenic microorganisms, they still need to be disinfected since some

microorganisms can tolerate the acidic environment and adopt for growth (Mazzotta 2001). Also, freshly squeezed fruit juices are in increasing demand despite the fact that these products are susceptible to spoilage and their shelf lives are limited (Jordan and others 2001).

In a recent study by Pataro and others (2011), the decontamination efficiency of PUV treatment on artificially inoculated (with *E. coli* and *Listeria innocua*) commercial apple and orange juices were studied. The lethal effect of PUV light on decontamination was affected by different energy doses of the light. The high inactivation levels were obtained when high energy amounts of PUV light were delivered to the juice sample. Also, it was found that *E. coli* cells were more susceptible to PUV treatment than *L. innocua* cells in both types of juices.

These studies show that PUV treatment may have a destructing effect on spoilage and pathogen microorganisms due to the UV light energy. It was observed in the literature that PUV light could reduce microorganisms (spoilage or pathogenic) by several logs depending on the variety. Pulsed UV light treatment application may be a promising method for decontamination of fresh fruits without any loss in quality or sensory attributes.

Justification of the Study

According to the USDA studies, among other fresh fruits, blueberries are regarded as the number one fruit with the highest rating in antioxidant capacity. This is the main attributing factor warranting its potential health benefits and status as a functional food. It has been estimated that the US per capita fresh blueberry consumption would increase 65% from estimated 26.6 ounces in 2008 to 44.0 ounces by 2015 (Blueberry Highbush Council 2010). With this predicted increase in consumption, there is an equal

demand for high-quality blueberry products with minimal changes in quality and nutritional profile and enhanced shelf life, post processing. As previously mentioned, PUV light is an emerging non-thermal technology, which has shown to be effective in the surface decontamination of blueberries without compromising their sensory and nutritional profile. The hypothesis of this study is that PUV exposure may have an effect on increasing their antioxidant capacity. In addition, there is little published information regarding the effect of PUV on the antioxidant system in blueberry fruit, enzymes involved in the synthesis of anthocyanins in blueberries, elucidation of the underlying mechanisms involved, and quality and sensory improvements in blueberries.

Research Objectives

1. To determine the effects of PUV light on the antioxidant capacity of blueberries as measured by the Oxygen Radical Absorbance Capacity (ORAC) assay, Ferric Reducing Antioxidant Power (FRAP) assay, and DPPH Free-Radical-Scavenging activity assay and to examine the effect of PUV light on the antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx).
2. To determine the effects of PUV light on decontamination of blueberries by enumeration of total aerobic plate count, total yeast and mold counts.
3. To determine the effects of PUV light on the temperature, compositional structure and the sensory attributes of fresh blueberries, such as color and texture.
4. To quantify total phenolic, flavonoid and anthocyanin contents of the PUV treated fresh blueberries.

2006. Isolation and identification of flavonoids (Page 48, Figure 2.1). In: Grotewold E, editor. The science of flavonoids. Ohio: Springer. p 47-69.]

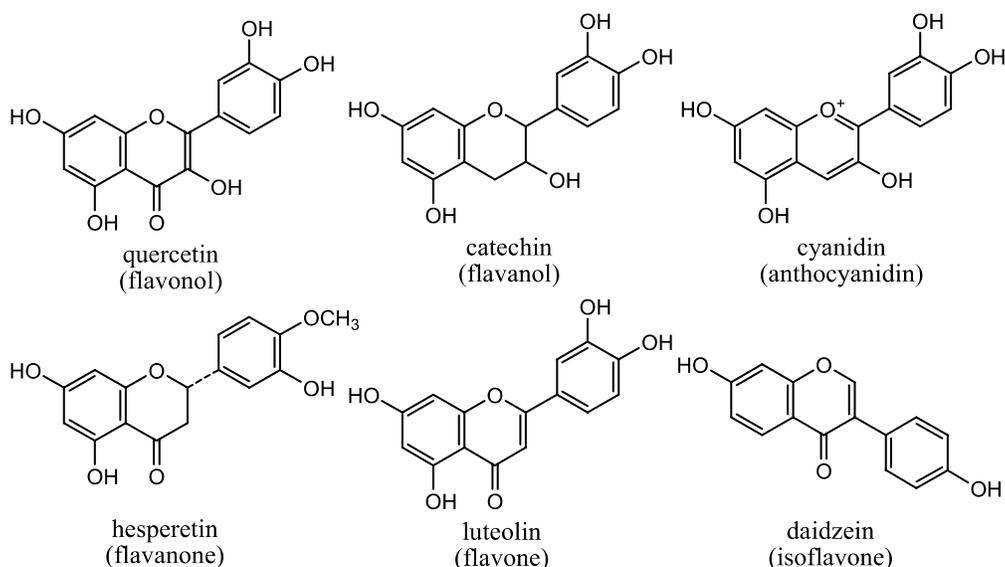


Figure 1-3. Representative aglycone structures of the common flavonoid subclasses. [Reprinted by permission from He J, Giusti MM. 2010. Anthocyanins: Natural colorants with health-promoting properties (Page 165, Figure 1). Annu Rev Food Sci Technol 1:163-87.]

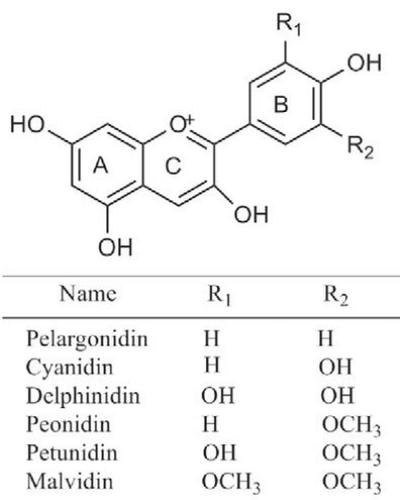


Figure 1-4. Chemical structures of the most common anthocyanidins in the nature. [Reprinted by permission from Wang BC, He R, Li ZM. 2010. The stability and antioxidant activity of anthocyanins from blueberries (Page 43, Figure 1). Food Technol Biotechnol 48(1):42-9.]

CHAPTER 2 ANTIOXIDANT CAPACITY OF FRESH BLUEBERRIES AS AFFECTED BY PULSED UV LIGHT

Antioxidant compounds inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Two basic categories of antioxidants are named as synthetic and natural. Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids, as well as ascorbic acid. On the other hand, synthetic antioxidants are generally compounds with phenolic structures of various degrees of alkyl substitution (Velioglu and others 1998).

Blueberries are well known for being a good source of natural antioxidants. Antioxidants are the defense mechanism of organisms against the harmful effects of oxidative stress caused by decay organisms and are efficient free radical scavengers in suppressing ROS in plant tissues (Wang and others 2010).

The major antioxidant capacity assays can be divided into two groups due to the basis of the chemical reactions involved: (1) hydrogen atom transfer (HAT) reaction based assays and (2) single electron transfer (ET) reaction based assays. ORAC is considered in the first group whereas FRAP and DPPH are considered in the second group (Huang and others 2005). ORAC, FRAP, and DPPH assays were used to evaluate the antioxidant capacities to scavenge free radicals and measure this activity spectrophotometrically.

The antioxidant enzyme system is a part of the defense mechanism of fruits and vegetables against oxidative stress. Plant cells have enzymatic and non-enzymatic defense mechanisms against oxygen toxicity. These mechanisms can delay the harmful effects of free radicals. Free radicals are controlled by an array of enzymes, such as

superoxide dismutase (SOD), catalase, ascorbate peroxidase, and glutathione reductase. Superoxide dismutases (SODs) are metalloenzymes and are a part of antioxidant defense. They can catalyze the dismutation of O_2^- into H_2O_2 while catalases and/or peroxidases remove H_2O_2 (Lacan and Baccou 1998; Ballester and others 2006). Hydroperoxides, including hydrogen peroxide, are reduced by glutathione peroxidase (GPx) using reduced glutathione to catalyze the reaction.

The UV light, used as a postharvest treatment, is proved to be beneficial to delay senescence in postharvest fruit and to control decay in many fruits and vegetables, which may be related to the antioxidant capacity (Erkan and others 2008). Erkan and others (2008) found that the UV-C exposure increased antioxidant capacity and enzyme activity of fresh strawberries. However, there is little available information about PUV light effects on antioxidant enzymes.

The objectives of this chapter were to determine the antioxidant capacities of PUV treated blueberries as measured by ORAC, DPPH, and FRAP assays and to determine the antioxidant enzyme activities of PUV treated blueberries.

Materials and Methods

Chemicals and Materials

Fresh early season (May - June) highbush blueberries (from cultivars, Star, Emerald, Jewel, Windsor, and Primadona) were handpicked from a local farm (Gainesville, FL) at commercial maturity stage from trees within the same grove. They were rapidly frozen for further analysis. Frozen blueberries were kept at room temperature for appropriate times before analysis.

AAPH (2,2' azobis (2-amidinopropane) dihydrochloride) was a product of Wako Chemicals Inc. (Richmond, VA). Trolox was a product of Acros Organics (Morris Plains,

NJ). Ferric chloride ($\text{FeCl}_3 \cdot \text{H}_2\text{O}$) and ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were purchased from Fisher Scientific Co. (Fair Lawn, NJ). TPTZ (2,4,6-tri (2-pyridryl)-s-triazine) was a product of MP Biomedicals, LLC (Solon, OH). DPPH (1,1-diphenyl-2-picryl-hydrazyl) was a product of CalbioChem (La Jolla, CA).

Superoxide Dismutase Assay Kit and Glutathione Peroxidase Assay Kit were purchased from Cayman Chemical Company (Ann Arbor, MI). Ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA) was a product of Sigma-Aldrich Co. (St. Louis, MO). HEPES (2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid), sucrose, Tris (hydroxymethyl) aminomethane, and EDTA (ethylenediamine tetraacetate acid disodium salt) were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Mannitol and dithiothreitol were purchased from Acros Organics (Morris Plains, NJ).

Pulsed UV Light Treatment

Blueberries were weighed, 20 g for antioxidant capacity assays and 3 g for antioxidant enzymes activity assays, using a balance accurate to three decimal places and placed in 70 mL aluminum dishes as a single layer (Fisher Scientific, Pittsburgh, PA). Samples were transferred to the Xenon PUV light system model RC: 847 (Xenon Corporation, Wilmington, MA) and treated at 3 pulses/s for 30, 60, 90, and 120 s at a distance of 13 cm from the PUV lamp in stationary mode.

Extract Preparation for ORAC, DPPH, and FRAP Analysis

Blueberry samples (control or PUV treated), each 20 g, were homogenized using a hand held homogenizer and extracted with a mixture of 20 mL of an acetone/water/acetic acid (70:29.9:0.1, v/v) solvent. The extraction tubes were sonicated for 30 min, and kept at room temperature for 2 h. The tubes were centrifuged

at 10,000 x g, 8000 rpm for 50 min at 4 °C and the supernatant were collected for ORAC, FRAP, and DPPH analysis.

Determination of Oxygen Radical Absorbance Capacity (ORAC)

The ORAC assay is a common antioxidant capacity assay and is based on the fluorescence decay of fluorescein, which is an indicator of damage from the reaction of fluorescein with peroxy radical (Huang and others 2005).

The ORAC of PUV treated and untreated blueberries were determined using a modified method of Huang and others (2002). In this method, the appropriately diluted (1:800) samples were analyzed using a Spectra Max Gemini XPS microplate reader (Molecular Devices, Sunnyvale, CA). Fluorescence was monitored using 485 nm excitation and 530 nm emissions at 1 min intervals for 40 min. The inhibition of decay of fluorescein in the presence of the peroxy radical generator 2, 2' azobis (2-amidinopropane) dihydrochloride (AAPH) was monitored. Briefly, 50 µL ORAC Phosphate Buffer (75 mM, ORAC-PB, pH 7.4) and diluted samples were added to the designed wells of a 96-well black plate (Fisher Scientific). This was followed by addition of 100 µL of the 20 nM fluorescein working Solution to all filled wells. The mixture was incubated at 37 °C for 10 min before the addition of 0.228 g of AAPH. After adding AAPH, the readings were recorded. The rate of fluorescence decay was monitored over time by calculating the area under the fluorescent decay curve and quantified using a standard curve of Trolox. The antioxidant capacities of the extracts were expressed µmole of Trolox Equivalent (TE)/g of extracted samples.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is one of the common assays used for the measurement of antioxidant capacities of blueberries. The reduction of the intense blue ferric tripyri-

dyltriazine complex to its ferrous form enables this colorimetric assay to measure the antioxidant capacity of the sample (Molan and others 2010).

The FRAP assay was conducted according to a method of Benzie and Strain (1996) with slight modifications. Briefly, 10 μL of appropriately diluted sample was combined with 290 μL of FRAP reagent into a 96 clear well plate (Fisher Scientific, Fairlawn, NJ). The FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6) with 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride ($\text{FeCl}_3 \cdot \text{H}_2\text{O}$) in the respective ratios, 10:1:1. The mixture was subsequently incubated for 10 min for 37 $^\circ\text{C}$. After incubation, the samples were analyzed at an absorbance using a Spectra Max Gemini XPS microplate reader (Molecular Devices, Sunnyvale, CA). The change in absorbance was compared to ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) standards (0.1 mM-1.0 mM) at 1 min intervals for 4 min. The concentration of Fe^{2+} was expressed as μmole of Fe^{2+}/g .

Determination of DPPH Free Radical Scavenging Activity (DPPH)

DPPH assay is a spectrophotometric method and is based on the reduction of DPPH free radical (DPPH^{\cdot}) by an antioxidant or by a radical species, resulting in a loss of absorbance in 515 nm (Fukumoto and Mazza 2000).



The free radical scavenging activity of blueberries was measured as described by Brand-Williams and others (1995) with some modifications. Briefly, 80 μL of each appropriately diluted extracts was added to 220 μL of 0.1 M DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical solution. The DPPH radical will be dissolved in 80% methanol, agitated, and incubated at ambient temperature for 30 min under restricted light.

Analysis was conducted using a Spectra Max Gemini XPS microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 517 nm. Free radical scavenging activity was calculated as the amount of extract required to decrease the initial absorbance of DPPH radical concentration by 50% (IC₅₀) as compared to the control according to the equation below.

$$\%DPPH = A_{\text{control}} - A_{\text{sample}} / A_{\text{control}} \times 100 \quad (2-3)$$

Superoxide Dismutase (SOD) Activity

The Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan) was used for the determination of SOD enzyme activity of blueberries. The fresh blueberry samples (3 g) were homogenized in 5-10 mL of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose per gram blueberry sample. The homogenate was centrifuged at 1,500 x g for 5 min at 4 °C. The supernatant was collected for assay and stored on ice. The samples were kept frozen for 1 month at -80 °C for further analysis. Briefly, 10 µL of the sample and 200 µL of the diluted tetrazolium salt solution (radical detector) were added to the wells of a 96-well plate. The reaction was initiated by adding 20 µL of diluted Xanthine Oxidase to all used wells. The 96-well plate was shaken for a few seconds to be mixed and incubated for 20 min at room temperature. The absorbance was read at 460 nm using a Spectramax 340³⁸⁴ spectrometry microplate reader (Molecular Devices Inc., Sunnyvale, CA). The SOD of blueberries were calculated with the equation below:

$$\text{SOD (U/mL)} = \left\{ \frac{\text{sample LR} - \text{y-intercept}}{\text{slope}} \right\} \times 0.23 \text{ mL} / 0.01 \text{ mL} \times \text{sample dilution} \quad (2-4)$$

Glutathione Peroxidase (GPx) Activity

The Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan) was used for the determination of the GPx activity of blueberries. The blueberry samples (3 g) were homogenized in 5-10 mL of cold buffer containing 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT per gram blueberry sample. The homogenate was centrifuged at 10,000 x g for 15 min at 4 °C. The supernatant was removed for the assay and stored on ice. The sample was stored for at least 1 month at -80 °C. Briefly, 20 µL of the sample, 100 µL of the Assay Buffer (50 mM Tris-HCl, pH 7.6, containing 5 mM EDTA), and 50 µL of the Co-Substrate Mixture (containing a lyophilized powder of NADPH, glutathione, and glutathione reductase) were added to the wells of a 96-well plate. The reaction was initiated by adding 20 µL of cumene hydroperoxide to all the used wells. The 96-well plate was shaken for a few seconds to be mixed. The absorbance was read once every minute at 340 nm using a Spectramax 340³⁸⁴ spectrophotometry micro-plate reader to obtain at least 5 time points. The change in absorbance (ΔA_{340}) per min was determined by the selection of two points on the linear portion of the curve and using the following equation:

$$\Delta A_{340} = [A_{340} (\text{Time 2}) - A_{340} (\text{Time 1})] / [\text{Time 2}(\text{min}) - \text{Time 1}(\text{min})] \quad (2-5)$$

And GPx activity was determined with the equation below:

$$\begin{aligned} \text{GPx activity} &= (\Delta A_{340}/\text{min})/0.00373 \mu\text{M}^{-1} \times 0.19 \text{ mL}/0.02 \text{ mL} \times \text{sample} & (2-6) \\ & \text{dilution} \\ &= (\text{nmole}/\text{min}/\text{mL}) \end{aligned}$$

Statistical Analysis

The data obtained were analyzed using the Statistical Analysis System (SAS[®]) version 9.1. Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The

data were tabulated as an average of triplicates \pm standard deviation, and the level of significance was determined at $P \leq 0.05$.

Results

Oxygen Radical Absorbance Capacity (ORAC)

The ORAC results from this study of PUV treated frozen blueberries and control group are shown in Table 2-1. The ORAC values observed among blueberries ranged from a low of 97.9 ± 4.1 to a high of 119.2 ± 4.8 $\mu\text{mole TE/g fruit}$ (Table 2-1). No significant differences ($P \leq 0.05$) were observed in ORAC values between control and PUV treatments of 60 and 90 s, but significant difference ($P \leq 0.05$) existed between control 30 and 120 s (Table 2-1). The difference amounted to nearly a 75% increase in ORAC values (30 s) relative to control.

Ferric Reducing Antioxidant Power (FRAP)

The effects of PUV light on antioxidant capacity in fresh blueberries (expressed as FRAP values) are shown in Table 2-2. The FRAP values observed among PUV treated and control blueberries ranged from a low of 22.5 ± 3.4 to a high of 27.5 ± 13.8 $\text{mmole of Fe}^{2+}/\text{g fresh weight}$. The FRAP values of PUV treated blueberries showed no significant difference ($P \leq 0.05$) as compared to untreated control blueberries (Table 2-2). A slight non-significant decrease when compared to control was observed when blueberries were treated for 30 s. A non-significant increase was observed from 30 s exposure to 90 s exposure. However, the FRAP value of PUV treated blueberries showed significant decrease ($P \leq 0.05$) after 90 s exposure (in 120 s exposure).

DPPH Free Radical Scavenging Activity (DPPH)

The scavenged DPPH radical values (expressed as percentage) of PUV treated and control fresh blueberries are shown in Table 2-3. The DPPH values obtained

among blueberries ranged from a low of 83.15 ± 4.48 to a high 84.99 ± 0.76 % DPPH (Table 2-3). No significant differences ($P \leq 0.05$) were observed between control and PUV treatments. Hence, there was no significant difference observed within the PUV treatment groups.

Superoxide Dismutase (SOD) Activity

Table 2-4 shows the SOD activity of control and PUV treated blueberries as U/g fresh weight. The SOD activity results observed were ranged from a low of 17.8 ± 5.5 to 28.7 ± 14.9 U/g fresh weight (Table 2-4). The SOD activities of blueberries PUV treated for 60 s, 90 s, and 120 s were not significantly difference ($P \leq 0.05$) from control (untreated) samples. However, a significant different was observed in blueberries PUV treated for 30 s as compared to control blueberries.

Glutathione Peroxidase (GPx) Activity

Table 2-5 shows the GPx activities of control and PUV treated blueberries as nmole/min/g fresh weight. The GPx activity results obtained from PUV treated blueberries ranged from a low of 40.1 ± 9.0 to a high of 116.0 ± 29.4 nmole/min/g fresh weight (Table 2-5). PUV treated blueberries for 30 s and 90 s showed no significant difference ($P \leq 0.05$) as compared to untreated blueberries. On the other hand, PUV treated blueberries for 60 s and 120 s showed significant difference ($P \leq 0.05$) as compared to untreated and PUV treated each group blueberries.

Discussion

Oxygen Radical Absorbance Capacity (ORAC)

Similarly, Wang and others (2009) evaluated the antioxidant capacity and individual flavonoid compounds in blueberries exposed to UV-C illumination for 1, 5, 10, and 15 min. They found an increase in ORAC values compared to the control. However,

their values ranged from a low of 40.4 ± 3.2 (control) to a high of 59.6 ± 2.0 $\mu\text{mole TE/g}$ (UV-C illumination at 6.45 kJ m^{-2}), which were lower than measured values of this study. Results indicated that PUV was also capable of increasing the antioxidant content of blueberries, which was achieved at much shorter exposure times than those of UV-C illumination. Reyes-Carmona and others (2005) suggested a mechanism for increased antioxidant capacity of the fruits, i.e., the upregulation or synthesis of antioxidant enzymes and polyphenolic compounds due to external stressor, such as PUV, as a defense mechanism during UV illumination.

Ferric Reducing Antioxidant Power (FRAP)

The data obtained in this chapter using FRAP assay showed no increasing effect on antioxidant capacity when blueberries were PUV treated for 30 s and 120 s as compared to control. However, the slight increase was observed when the exposure time was increased to 60 s and 90 s, it was not significantly different ($P \leq 0.05$). The mechanism underlying this increase may be originated from the UV light, the major component of PUV system. It was claimed before by several researches that UV light has an effect on the synthesis of secondary metabolites in fruits and vegetables. It accelerates the synthesis of secondary metabolites and therefore antioxidant capacity (Lamikanra and others 2005; Perkins-Veazie and others 2008; Alothman and others 2009). A study of Perkins-Veazie and others (2008) about UV-C light application enhancing the effect on the antioxidant capacity, measured by FRAP assay, showed that FRAP values increased with the application of UV-C light compared to fresh blueberries for 'Collin' and 'Bluecrop'. Dosage of UV-C light was the focal point of this research and different dosages were applied to blueberries. The FRAP values reduced in 'Collins' as dosage was changed from 0 or 1 kJ/m^2 while they increased in 'Bluecrop'

as dosage 4 kJ/m² was compared to 0 kJ/m². It was claimed that the activation of antioxidants by UV-C radiation might be dependent on exposure dosage, cultivar, and the individual flavonoid changes in blueberries.

DPPH Free Radical Scavenging Activity (DPPH)

A recent research of Huang and others (2012) revealed that blueberries scavenged almost all DPPH radicals (96.96%). The results of this research (both PUV treated for different times and untreated control group) were found to be a little bit lower than their results. The diversity of cultivar, variety, maturity, environmental conditions, and/or method steps may have caused this difference. In another recent research by Corrales and others (2012), the researchers observed that the radical scavenging activity of the UV-C radiated horchata (fresh tiger nuts' milk beverage) samples against DPPH radical decreased to a value of 71% as the dosage increased (an exposure of 4 J cm⁻²). They claimed that antioxidant groups decreased as dose dependent, since they interacted with free radicals, which occurred by oxidative processes induced by UV radiation. Antioxidant capacity of milk does not induce with UV-B or UV-C radiation as it happens in peppers or berries due to the absence of secondary metabolites in milk.

The data obtained using DPPH assay showed no significant difference between antioxidant capacity values of PUV treated and control blueberries. However, Fukumoto and Mazza (2000) claimed that using DPPH assay gives similar results to those of an oxidation method. On the other hand, the comparisons are not quantitative, since the compound's structural conformation of phenolics influences the reaction with DPPH radical. The antioxidant capacity values might have showed similar results because of this structural conformation.

Superoxide Dismutase (SOD) Activity

The SOD activity was significantly reduced when the blueberries were PUV treated for 30 s due to obtained data. However, there was no significant difference in SOD activity of the other PUV treatments (60, 90, and 120 s) when compared to control. The initial decrease may be a response to the oxidative stress of UV light on enzymes. However, as the time increased, SOD was activated, which might occur since PUV light induced oxidative stress and triggered antioxidant enzyme system. In a study of Erkan (2008), the UV treatment effect on antioxidant enzyme activity in strawberries was tested. They used three different UV illumination times and dosages, 1, 5, and 10 min, (0.43, 2.15, and 4.30 kJ m⁻²). They observed that the extracts of UV treated strawberries had higher SOD activities than those from control fruit.

Glutathione Peroxidase (GPx) Activity

Although the GPx activity of PUV light treated and untreated groups showed different responses to different exposure times, the longest PUV light treated blueberries showed the highest GPx activity. This high response to long PUV light treatment may have occurred in the demand of additional antioxidant enzymes when a limitation occurred other antioxidant defense systems, such as polyphenols, against oxidative stress caused by PUV light treatment. In a research of Erkan (2008) about the UV light effects on antioxidant enzymes, they used three different UV illumination times and dosages, 1, 5, and 10 min, (0.43, 2.15, and 4.30 kJ m⁻²). The obtained data showed that the highest GPx activity was observed in the longest UV treatment time (10 min).

Table 2-1. Antioxidant capacity of pulsed UV light treated blueberries expressed as the oxygen radical absorbance capacity (ORAC)

Treatment	$\mu\text{mole Trolox eq/g fresh weight}$
Control	119.2 \pm 4.8 b
PUV 30 s	131.7 \pm 14.7 a
PUV 60 s	121.4 \pm 14.7 b
PUV 90 s	117.4 \pm 5.1 b
PUV 120 s	97.9 \pm 4.1 c

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

Table 2-2. Antioxidant capacity of pulsed UV light treated blueberries expressed as the Ferric Reducing Antioxidant Power (FRAP)

Treatment	mmole of Fe ²⁺ /g fresh weight
Control	23.4±3.5 a,b
PUV 30 s	22.8±3.1 a,b
PUV 60 s	26.7±3.8 a,b
PUV 90 s	27.5±13.9 a
PUV 120 s	22.5±3.4 b

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

Table 2-3. Antioxidant capacity of pulsed UV light treated blueberries expressed as the DPPH Free Radical Scavenging Activity

Treatment	% DPPH
Control	84.6±1.8 a
PUV 30 s	85.0±0.8 a
PUV 60 s	83.2±4.5 a
PUV 90 s	84.1±2.4 a
PUV 120 s	83.3±2.8 a

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

Table 2-4. Superoxide dismutase activity of pulsed UV light treated and control fresh blueberries

Treatment	U*/g fresh weight
Control	25.9±7.7 a
PUV 30 s	17.8±5.5 b
PUV 60 s	19.6±5.6 a,b
PUV 90 s	20.5±7.4 a,b
PUV 120 s	28.6±14.9 a

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

*One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Table 2-5. Glutathione peroxidase activity of pulsed UV light treated and control fresh blueberries

Treatment	nmole/min/g fresh weight
Control	51.4±7.8 c
PUV 30 s	40.7±14.3 c
PUV 60 s	79.8±10.3 b
PUV 90 s	40.1± 9.0 c
PUV 120 s	116.0±29.4 a

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).



Figure 2-1. Experimental design image of blueberry configuration for PUV treatment.

CHAPTER 3 PULSED UV LIGHT DISINFECTION OF FRESH BLUEBERRIES

Spoilage microorganisms are a part of natural flora of fruits and vegetables and influence the shelf life and consumer acceptance of fresh products. High levels of sugars and other nutrients of fruits, such as berries, grapes, and citrus, and the low pH with an ideal water activity make fruits susceptible to fungal spoilage (Tournas and Katsoudas 2005). As the fruits and vegetables mature and senescence, the appropriate mediums (e.g. higher water activity, materials released from cells with cell rupture) allow more microbial growth. On the other hand, pathogenic bacteria can grow on the surface of fresh fruits. Fresh fruits and vegetables should be prepared with proper processing and sanitation procedures in order to have ready-to-eat status based on food regulations (Williams and others 2012).

Total aerobic plate (TAPC) and yeast and mold counts are indicative of the level of microorganisms in a specific product, which is not usually associated with food safety concerns. However, presence of these microorganisms plays an integral role in determining the quality, shelf life, and post-treatment processing contamination.

Pulsed UV light has broad spectra of UV, infrared, and visible light, composing a photo-physical process. Effective lethal dosage of intense light is produced to inactivate bacteria, fungi, virus, and oocysts. The killing effect of PUV radiation occurs by altering the normal structure of DNA and causing single strand breakage within its double helix. Other components of the yeast cell, such as protein and other cellular organelles, are also altered by PUV radiation. Pulsed UV light radiation is considered as an alternative surface decontamination method, which is used without the aid of chemicals, and has

advantages such as low cost, easy maintenance, and no film residue on the surface of the product (Williams and others 2012).

In this chapter, the objective was to determine the PUV light disinfection effects on the natural microflora of fresh blueberries.

Materials and Methods

Chemicals and Materials

Blueberries were handled and prepared for sampling as described in Chapter 2. Petrifilms for total aerobic plate count and yeast and mold count were purchased from 3M™ Microbiology (St. Paul, MN). Bacto™ Peptone was a product of BD Biosciences (Sparks, MD).

Pulsed UV Light Treatment

Blueberries were weighed (10 g) using a balance accurate to 1 g and placed in 70 mL aluminum dishes as a single layer (Fisher Scientific, Pittsburgh, PA). Samples were transferred to the Xenon PUV light system model RC: 847 (Xenon Corporation, Wilmington, MA) and treated at 3 pulses/s for 30, 60, 90, and 120 s at a distance of 13 cm from the PUV lamp in stationary mode.

Total Aerobic Plate Count and Yeast and Mold Count

In this experiment, microbial assays of fresh blueberries, as total aerobic plate and yeast and mold count, were performed using a modified method described by Lamikanra and others (2005). For these analyses, appropriate dilutions of the samples were prepared by using sterile 0.1% peptone water (prepared with deionized distilled water; ddH₂O) and subsequently homogenized using a stomacher. The homogenate was administered onto Total Aerobic Plate Count and Yeast and Mold Petrifilm plates (3M™ Microbiology, St. Paul, MN) with a pipette perpendicular to the Petrifilm plate,

placing 1 mL of sample onto the center of the bottom film. Using a spreader, the inoculum was evenly distributed over a circular area of the film. Total aerobic, yeast and mold counts were enumerated after incubation at 35 °C for 48 h and 120 h respectively, using a standard colony counter. Counting and data expression of both methods were validated and approved as the official methods as prescribed by the AOAC (McMohan and others 2003).

Statistical Analysis

The data obtained were analyzed using the Statistical Analysis System (SAS[®]) version 9.1. Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The data were tabulated as an average of triplicates \pm standard deviation, and the level of significance was determined at $P \leq 0.05$.

Results

Total Aerobic Plate Count

Table 3-1 shows the TAPC population of control and PUV treated blueberries as log CFU. The correlation between PUV light exposure time and final TAPC count is shown in Figure 3-1. The TAPC population of PUV treated blueberries ranged from a low of 6.96 ± 0.42 to a high of 8.92 ± 0.77 as log CFU (Table 3-1). Non-significant log reductions ($P \leq 0.05$) were observed when blueberries were PUV treated for 30 s and 60 s. Significant log reductions ($P \leq 0.05$) were observed in PUV treated blueberries for 90s and 120 s, 1.97 and 1.72 log reductions respectively.

Yeast and Mold Count

Table 3-2 shows the total yeast and mold population of control and PUV treated blueberries as log CFU. The correlation between PUV light exposure time and total

yeast and mold count is shown in Figure 3-2. The total yeast and mold population of PUV treated blueberries ranged from a low of 6.52 ± 0.20 to a high of 7.81 ± 0.61 as log CFU (Table 3-2). Significant log reductions ($P \leq 0.05$) were observed when blueberries were PUV treated for 90 s and 120 s as compared to untreated blueberries, 1.29 and 1.17 respectively. Thus, significant differences ($P \leq 0.05$) were observed between PUV treatments for 30 and 60 s and PUV treatments for 90 and 120 s. Non-significant reductions ($P \leq 0.05$) were observed when blueberries were PUV treated for 30 s and 60 s.

Discussion

Total Aerobic Plate Count

According to the obtained data, PUV treatment was effective in higher exposure time periods, 90 s and 120 s, more than lower exposure time periods, 30 s and 60 s, on total aerobic count of fresh blueberries. The Figure 3-1 showed a linear correlation ($R^2=0.85$) between PUV treatment time and log CFU of TAPC, which indicated that PUV exposure time was the major factor of inactivation. The killing effect of PUV light was a result of PUV damage on DNA structure of microorganisms. Manzocco and others (2011) used UV-C light for the impact on safety of fresh cut melon since UV-C light has an antimicrobial effect due to its ability to damage microbial DNA, causing cross-linking between neighboring thymine and cytosine in the same DNA strand. The DNA transcription and replication are blocked and cellular functions are limited, leading to cell death. They observed log reductions ranging from 0.40 to 2.14, 0 J/m^2 and $12,000 \text{ J/m}^2$ respectively as a result of cutting melon under UV-C light and exposure to UV-C light for different dosages and times (1, 5, and 10 min). The log reductions of 1.97 and 1.72, PUV treatments for 90 s and 120 s respectively, were observed in our study, which may

indicate that even short periods of PUV exposure are more effective than UV-C exposure.

In another study by Williams and others (2012), the PUV light surface disinfection effect was investigated on *Salmonella* inoculated tomatoes. Pulsed UV light treatment was combined with different sanitizer and observations were made at different storage periods. The researchers observed 1.35 log CFU reduction in control group after 60 s PUV exposure at 6.35 cm from the strobe. They claimed that PUV alone was not significant enough in reducing *Salmonella* population on tomato surface. The result might be affected by high inoculum level (10^6 CFU/mL) or the PUV radiation of 60 s might not be long enough to cause significant log reductions. In our study, the PUV radiations of 30 and 60 s were found to be not effective on significant reduction of TAPC as well.

Total Yeast and Mold Count

The Figure 3-2 showed that the inactivation effect on total yeast and mold increased when PUV treatment time increased, and showed linear correlation ($R^2=0.88$), as well as obtained in inactivation of TAPC. PUV treatments for 90 s and 120 s were more effective on reducing yeast and mold population in fresh blueberries than short time periods, 30 s and 60 s, as well in total aerobic plate count (Table 3-2). Pulsed UV light is well known for its rapid inactivation of pathogenic and spoilage microorganisms in foods. The major benefits of PUV light are obtaining significant microbial reductions in short treatment times (in 120 s at most in this study), limited energy cost, absence of residual compounds, and great flexibility (Oms-Oliu and others 2010).

In a recent research of Oms-Oliu and others (2010), the PUV disinfection effects on microbial growth in fresh-cut mushrooms were studied with different storage periods. The yeast and molds count ranged between 2.28 and 3.00 log CFU/g, PUV treated of 28 J cm⁻² and untreated blueberries respectively, without storage effect. In this study, yeast and mold count showed higher results, which might have occurred due to initial yeast and mold count and different PUV treatment conditions (time and dosage). However, higher log reductions of 1.29 and 1.17, respectively, were observed when PUV radiations of 90 and 120 s were used than the highest log reduction (0.72 log CFU/g) obtained by Oms-Oliu and others (2010).

Table 3-1. Total Aerobic Plate Count population of blueberries after pulsed UV light treatment

Treatment	Log CFU*/g fresh weight
Control	8.92±0.77 a
PUV 30 s	8.10±0.29 a,b
PUV 60 s	8.03±0.17 a,b
PUV 90 s	6.96±0.42 b
PUV 120 s	7.21±0.50 b

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

*CFU: Colony forming unit

Table 3-2. Total Yeast and Mold population of blueberries after pulsed UV treatment

Treatment	Log CFU*/g fresh weight
Control	7.81±0.61 a
PUV 30 s	7.45±0.19 a
PUV 60 s	7.33±0.17 a
PUV 90 s	6.52±0.20 b
PUV 120 s	6.64±0.18 b

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

*CFU: Colony forming unit

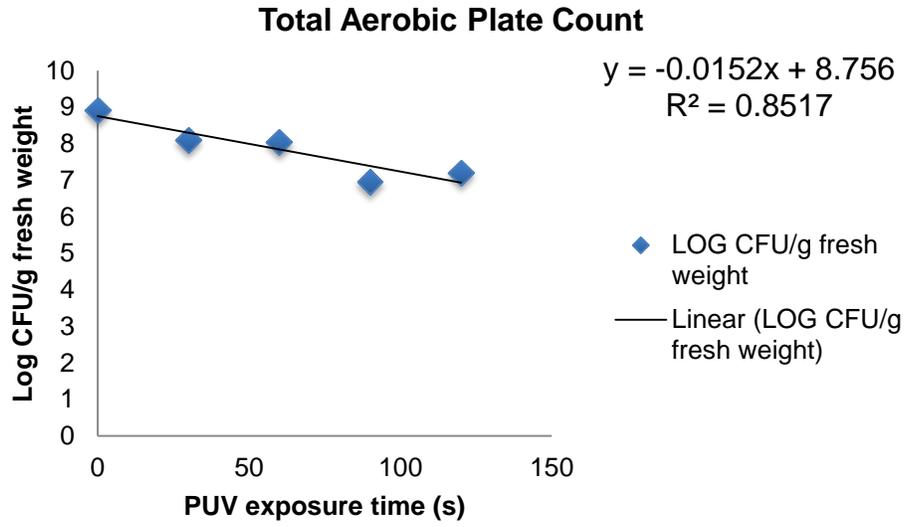


Figure 3-1. Total aerobic plate count correlation with Pulsed UV exposure time

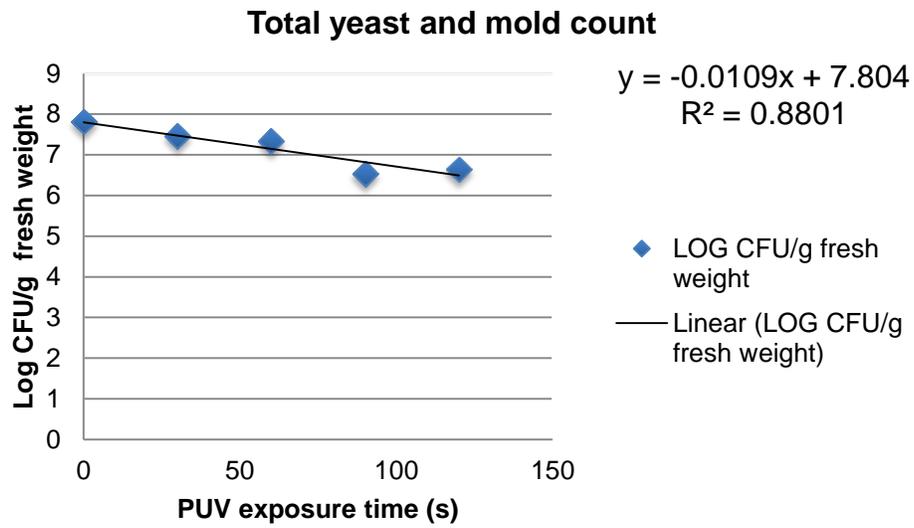


Figure 3-2. Total yeast and mold count correlation with Pulsed UV light exposure time

CHAPTER 4

EFFECT OF PULSED UV LIGHT ON SENSORY AND QUALITY TRAITS OF FRESH BLUEBERRIES

General appearance and taste of fruits and vegetables are the major factors for market, which directly affect consumer acceptance. Color is an important quality factor, determining fresh market value of blueberries and the suitability of the blueberries for processing or market. This highly complex trait is influenced by some factors, such as the total anthocyanin content, the quantity and structure of surface wax, the pH, the distribution of individual anthocyanins, and the formation of metal complexes of anthocyanins (Sapers and others 1984). As an important rheological parameter for textural quality, firmness has been reported in literature as a standard trait to reflect the quality of fresh fruits and vegetables. Duan and others (2011) stated that the loss of firmness was usually associated with fruit softening and related to the degradation of pectin in the cell walls as well as the metabolism of sugars. Additionally, vacuoles in the cell wall of blueberries as well as other fruits and vegetables release their water in the case of cell death or injury, leading to the disintegration of cell walls (Goulao and Oliveira 2008).

A stable pH is very important for blueberries, as well as all fresh fruits and vegetables, during processing and post-processing handling, since its hue and color are highly dependent on the pH change. This is because anthocyanin pigments may undergo reversible transformations with change of pH (Tomas-Barberan and Espin 2001). Appropriate pH values allow spoilage microbial growth, which has non-desired consequences. Blueberry organic acids, such as malic, chlorogenic, and phosphoric acid, increase, and blueberry citric acid decreases during postharvest storage (Zhao 2007).

Novel technologies, such as PUV light, have been used for disinfection as an alternative to thermal processing of foods (Oms-Oliu and others 2010). It is well known that foods treated with high temperatures are susceptible to thermal degradation, which may result in undesirable changes to their organoleptic and physiochemical properties. For PUV light, when the exposure time is short (e.g., seconds), there is a low increase in temperature because the photo-thermal effect of PUV light is minimal. However, prolonged exposure times (e.g., minutes) would result in temperature increase of the product as the photo-thermal effect is intensified and attributed to the infrared portion of the PUV spectra (Shriver and others 2011; Yang and others 2011).

The objective of this chapter was to determine the effects of PUV light on the temperature rise, the compositional structure of fresh blueberries, as soluble solids, pH, and titratable acidity, and the sensory attributes of fresh blueberries, as color and texture.

Materials and Methods

Chemicals and Materials

Blueberries were handled and prepared for sampling as described in Chapter 2. Sodium hydroxide (NaOH) was purchased from Fisher Scientific Co. (Fair Lawn, NJ).

Pulsed UV Light Treatment

Blueberries were weighed (10 g for color and texture analyses and 10 g for compositional analysis) using a balance accurate to 1 g and placed in 70 mL aluminum dishes as a single layer (Fisher Scientific, Pittsburgh, PA). Samples were transferred to the Xenon PUV light system model RC: 847 (Xenon Corporation, Wilmington, MA) and treated at 3 pulses/s for 30, 60, 90, and 120 s at a distance of 13 cm from the PUV lamp in stationary mode.

Color Analysis

The color of PUV treated and untreated (control group) blueberries was measured using a color machine vision system (CMSV), which consisted a Nikon D200 digital color camera (Nikon Corp, Japan), housed in a light box [42.5 cm (W) x 61.0 cm (L) x 78.1 cm (H)] (Wallat 2002). The camera (focal light, 35 mm; polarization, 18.44 mm) was connected to a computer and was used to capture the images prior to color analysis. The LensEye software (Engineering and Cybersolutions Inc., Gainesville, FL) was used to analyze the color of PUV treated blueberries based on the values of the L (lightness), a* (redness), and b* (yellowness) system. The CMVS was calibrated with a standard blue tile (L: 58.24; a*: -4.74; b*: -42.44) (Labsphere, North Sutton, NH). In this study, color was reported as the L*a*b* values from an average of each sample. All samples were aligned in the center of the light box before acquiring images.

Texture Analysis

A texture analyzer (TA.XT Plus, Texture Technologies Corporation, Scarsdale, NY) was utilized to evaluate the texture of the PUV treated and untreated blueberries. In this research, the “compression test” was utilized. The texture analyzer was set to speed of 2.0 mm/s. The texture was measured by the surface of the horizontally aligned blueberry to a total distance of 5 mm in a given time(s) using a TA-212 5/16” Dia (radius of curvature 33.02/162.56 cm) subjecting 50 kg of force.

Compositional Analysis

Soluble Solids (SS) were determined using a digital refractometer (Leica Mark II Abbe Refractometer, Buffalo, NY). Control and PUV treated blueberries were macerated and filtered before analysis. Results were expressed as Brix^o. Degrees Brix is the sugar content of an aqueous solution. A refractometer measures the angle of

refraction, which depends on the composition of the solution, concentration of dissolved substances.

The pH of the pureed blueberries was measured using a pH meter (Fisher Scientific Accumet® Basic AB15/157, Pittsburg, PA). A pH meter is used to measure the pH (acidity or alkalinity) of a liquid with a special measuring probe (a glass electrode) connected to an electronic meter.

As described by Almenar and others (2008), titratable acidity (TA) of 10 g the macerated blueberry samples was added to 50 mL of dionized distilled water (ddH₂O) and titrated with sodium hydroxide (NaOH, 0.1 N) until a pH of 8.2 is obtained. The TA values were calculated and results were reported as % equivalent weight of the predominant acid (Malic acid/g fresh weight).

$$\% \text{ Malic Acid} = N \times V \times \text{Eqn wt} / W \times 10 \quad (4-1)$$

Temperature Rise

Temperatures of PUV light treated blueberry samples were measured using a non-contact infrared thermometer (OS423-LS, Omega Engineering Inc., Stamford, Ct). Infrared thermometers infer temperature using a portion of the thermal radiation emitted by the object of measurement.

Statistical Analysis

The data obtained were analyzed using the Statistical Analysis System (SAS®) version 9.1. Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The data were tabulated as an average of triplicates ± standard deviation, and the level of significance was determined at $P \leq 0.05$.

Results

Color Analysis

Significant differences ($P \leq 0.05$) were observed among all treatment groups in the degree of lightness (L^*), greenness (a^*), and blueness (b^*) values compared to the control (Table 4-1). The L^* values indicated that the blueberries darkened in color after prolonged PUV exposures. No significant differences ($P \leq 0.05$) were observed between those treated for 30 s and 60 s relative to control. However, those treated for 90 and 120 s ($L^*=17.23 \pm 1.94$, 15.50 ± 1.75 respectively), were darkest in color as compared to control ($L^*=20.51 \pm 1.93$) and other treatment groups. Overall, the results showed that PUV light decreased b^* values (i.e., more dark blue) of the samples as compared to the control.

Texture Analysis

As shown in Table 4-1, the firmness values of PUV treated vs. untreated (control) blueberries showed no significant difference ($P \leq 0.05$). The obtained values ranged from a low 8.82 ± 0.05 Newtons (N) in blueberries PUV treated for 120 s to a high of 10.29 ± 0.04 N in untreated blueberries (control).

Compositional Analysis

Soluble solids: Although the mean SS in blueberries measured in this study showed a high of $11.67 \text{ }^\circ\text{Brix} \pm 0.04$ at with PUV 30 s and a low $10.86 \text{ }^\circ\text{Brix} \pm 0.04$ at PUV 60 s (as shown in Table 4-1), there was no significant difference ($P \leq 0.05$) among all PUV treatment groups compared to control.

pH: Table 4-1 shows the average pH values after the blueberries were PUV treated compared to control. The pH value ranged from a low of 3.07 ± 0.02 at PUV 90 s to a high of 3.21 ± 0.04 at PUV 120 s, but overall, there was no significant difference ($P \leq$

0.05) in pH value among the PUV treated blueberries and no difference either compared to control. This indicates that PUV exposure up to 120 s did not have an effect on the pH of fresh blueberries.

Titrateable acidity: In this experiment, titrateable acidity (TA) was reported as % malic acid on the basis of fresh fruit weight. The TA values in Table 4-1 ranged between $0.40\% \pm 0.13$ at PUV 120 s and $0.47\% \pm 0.15$ in the control.

Temperature Rise

According to Table 4-3, the surface temperature ranged from a low of $22.5\text{ }^{\circ}\text{C} \pm 0.7$ in untreated (control) blueberries to a high of $35.5\text{ }^{\circ}\text{C} \pm 1.2$ in PUV treated blueberries for 120 s. It is noted that there was a few seconds delay before the temperature reading, when the sample was removed from the treatment chamber upon completion of the PUV treatment, so the instantaneous surface temperature could possibly be a bit higher. It was observed that the surface temperature was not significantly different ($P \leq 0.05$) among control, PUV 30 and 60, but significantly different after 90 s (Table 4-3).

Discussion

Color Analysis

A similar result was observed by Moreno and others (2007), who investigated the effects of ionizing radiation (0, 1.1, 1.6 and 3.2kGy) on blueberry color. With a higher dosage of irradiation, there was a decrease in the b^* values resulting in darker fruit. They hypothesized that the darkening of the fruit color in the treated blueberries may be attributed to co-pigmentation where anthocyanins form complexes with flavonoid compounds, causing an increase in color intensity. Other associative mechanisms during treatment could be the activation of enzymes, which were associated with the synthesis of anthocyanins that could act as UV screens to reduce the damaging effects

of UV on the genetic materials in plant tissues (Tomas-Barberan and Espin 2001). In a separate study (Silva and others 2005), similar L* values compared to our control were reported for blueberry cultivars including Premier, Bluecrop, and Highbush, with L* being 20.0, 20.9, and 20.4, respectively. These results suggest that exposure to PUV light might have resulted in the darkening of the fruits.

Texture Analysis

The experimental results suggest that PUV treatment in the time range tested in this study did not have an effect on the firmness of the blueberries. In contrast, in a study conducted by Silva and others (2005), they studied variations in the physiochemical (pectin, pH, TA, fiber, skin toughness) differences that led to difference in the firmness in several cultivars of blueberries. They found that the texture ranged from a high of 7.28 N to a low of 3.58 N in the Climax and Jersey cultivars, respectively. Their values were lower than those reported in this study, which may be attributed to varietal differences.

Compositional Analysis

Soluble solids: Pulsed UV light did not have an effect on SS metabolism, which may vary according to blueberry cultivar. Perkins-Veazie and others (2008) also reported that exposure to UV-C light did not affect SS content, but slight variations may be attributed cultivar and storage conditions.

pH: A similar trend was observed by Perkins-Veazie and others (2008). They reported that after the UV-C exposure (1-15 min, 8 cm from UV lamp), there was a slight increase in blueberry pH values, but there was no significant effect ($P \leq 0.05$). The pH values they reported were 3.40-3.60 and 3.20-3.40 in the Colins and Bluecrop varieties, respectively, which were slightly higher than the values we obtained.

Titrateable acidity: Titrateable acidity results of this study were comparable with those of Perkins-Veazie and others (2008), who reported a TA of 0.44 and 0.54% malic acid equivalents in the Collins and Bluecrop variety, respectively, after UV-C treatments. These results showed that there was no significant effect ($P \leq 0.05$) of PUV light on TA even after exposure for 120 s. This may imply that PUV light (within 120 s) had no degradative effect on TA.

Temperature Rise

Temperature rise to between 45 °C and 54 °C has been reported to improve sensory and nutritional quality of some horticulture products like tomato (Lurie and others 2006; Rajchl and others 2009) or low-temperature storage quality (Vlachonasios and others 2001) without damage to the product. In contrast, the recorded surface temperature of blueberries after 120 s PUV light exposure generally did not exceed 54°C, so there should not be any negative impact of the temperature rise on the blueberry quality in this study.

Table 4-1. L*, a*, and b* color values of PUV treated and control fresh blueberries

Treatment	Lightness (L-)	Blueness (-b)	Greenness (-a)
Control	20.51±1.93 a	-7.29±0.93 c	-5.25±0.30 b
PUV 30 s	20.40±1.56 a	-6.76±0.39 c	-5.46±0.29 b
PUV 60 s	19.38±2.54 a	-6.12±1.12 b,c	-5.36±0.16 b
PUV 90 s	17.23±1.94 b	-5.04±1.02 a,b	-5.30±0.17 b
PUV 120 s	15.50±1.75 b	-4.35±0.51 a	-4.52±0.69 a

Means (in columns) with same letter not significantly different according to the Tukey's Studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

Table 4-2. Physiochemical properties of pulsed UV light treated blueberries

Treatment	pH	Titrateable acidity (% Malic acid)	Soluble solids (°Brix)	Firmness (Newtons)
Control	3.16±0.06 a	0.48±0.15 a	11.6±0.5 a	10.30±0.03a
PUV 30 s	3.21±0.04 a	0.41±0.02 a	11.7±0.5 a	8.92±0.04 a
PUV 60 s	3.13±0.04 a	0.41±0.11 a	10.9±0.2 a	9.32±0.05 a
PUV 90 s	3.07±0.02 a	0.42±0.10 a	10.9±0.1 a	9.51±0.03 a
PUV 120 s	3.21±0.03 a	0.40±0.13 a	11.4±0.4 a	8.83±0.05 a

Means (in columns) with same letter not significantly different according to the Tukey's Studentized Range Test $P \leq 0.05$. Data is expressed as mean \pm standard deviation (SD).

Table 4-3. Surface temperature values of PUV treated and control fresh blueberries.

Treatment	Temperature (°C)
Control	22.5±0.7 b
PUV 30 s	23.0±0.4 b
PUV 60 s	25.1±0.3 b
PUV 90 s	32.7±0.7 a
PUV 120 s	35.5±1.2 a

Means (in columns) with same letter not significantly different according to the Tukey's Studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).



Figure 4-1. Standard blue tile used in color analysis.



Figure 4-2. Color image of control fresh blueberries.

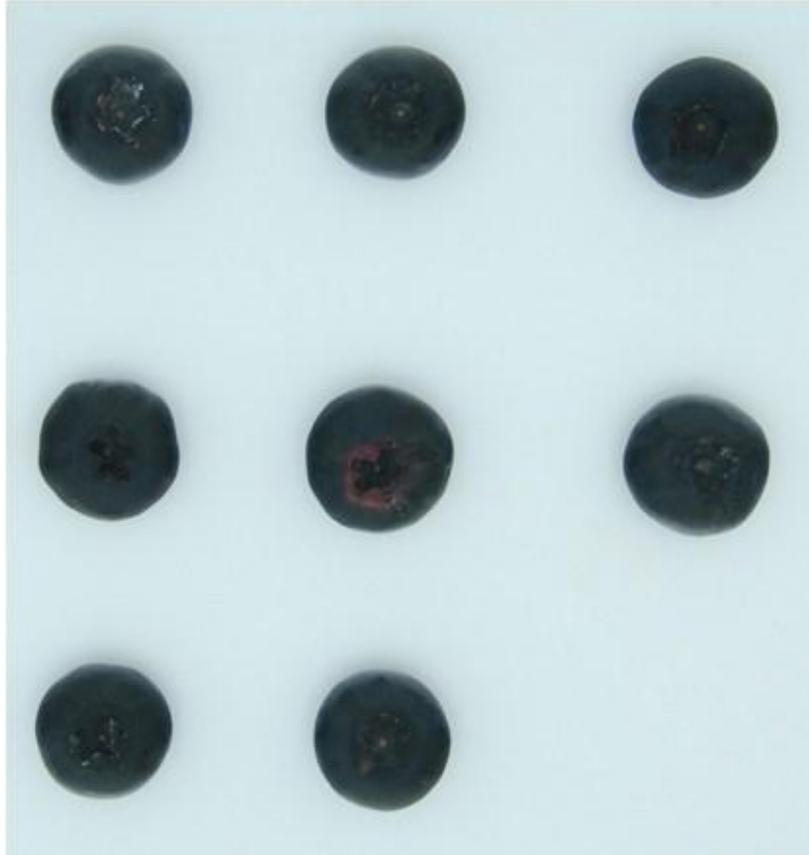


Figure 4-3. Color image of fresh blueberries after PUV treatment for 120 s.

CHAPTER 5 TOTAL PHENOLICS, TOTAL FLAVONOIDS, AND ANTHOCYANINS OF FRESH BLUEBERRIES AS AFFECTED BY PULSED UV LIGHT

Fruits and vegetables have several health promoting compounds, such as fiber and high concentrations of phenolic acids, flavonoids, vitamins, and minerals and are a rich source of dietary antioxidants. Phytochemicals may contribute to the protective effects of a fruit- and vegetable-rich diet even though they are not essential to survive (Borges and others 2010). Phenolic compounds are secondary metabolites and are a major class of antioxidants found in all plants, often at high concentrations in fruits and vegetables. Anthocyanins are the most important group of water-soluble pigments and are responsible for the bright colors, such as orange, red, and blue depending on environmental pH values (You and others 2011). High phytochemical content is one of the desired traits due to studied health effects of fruits and vegetables.

The quality of light reaching plants affects the growth and development of plants. The pigment and colorless phytochemical contents of some crop plants are affected by the changes in the level of UV light in growing systems (Ordidge and others 2010). As reviewed by Ordidge and others (2010), tomatoes cultivated under UV-transparent films had 20% higher total phenolic and phenolic acid contents than tomatoes cultivated under UV blocking plastic films.

The objective of this chapter is to determine the effects of PUV light on total phenolic, total flavonoid, and anthocyanin contents of fresh blueberries.

Materials and Methods

Chemicals and Materials

Blueberries were handled and prepared for sampling as described in Chapter 2. Folin-Ciocalteu's phenol reagent and gallic acid were products of Sigma-Aldrich (St.

Louis, MO). Sodium carbonate (NaCO_3) and aluminum chloride (AlCl_3) were purchased from Acros Organics (Morris Plains, NJ). (+)-Catechin (hydrate) was a product of Cayman Chemical Company (Ann Arbor, MI). Sodium nitrate (NaNO_2) and sodium hydroxide (NaOH) were purchased from Fisher Scientific Co. (Fair Lawn, NJ).

Pulsed UV Light Treatment

Blueberries were weighed (20 g) using a balance accurate to 1 g and placed as a single layer in 70 mL aluminum dishes as a single layer (Fisher Scientific, Pittsburgh, PA). Samples were transferred to the Xenon PUV light system model RC: 847 (Xenon Corporation, Wilmington, MA) and treated at 3 pulses/s for 30, 60, 90, and 120 s at a distance of 13 cm from the PUV lamp in stationary mode.

Extract Preparation for Total Phenolics, Flavonoids, and Anthocyanin Analysis

The blueberries were macerated using a Bio Homogenizer M 133/1281-0 (Biospec Products Inc., Bartlesville, OK) and the extraction of phenolic compounds was performed as described by Kim and others (2003). A mixture consisting of 20 g of the sample and 200 mL of 80% methanol were sonicated for 30 min and filtered using Whatman no.2 filter paper. Solvent was removed using a rotary evaporator (Büchi Rotavapor 2025, Gardner Denver Thomas Inc. and Niles, IL) at 40 °C. The extracts were stored at -20 °C until further analysis. Extracts were used for the determination of total phenolics, total flavonoids, and anthocyanins.

Determination of Total Phenolic Content

Total phenolics were determined using a modification of Folin-Ciocalteu's method as described Kim and others (2003). Briefly, 12.5 μL of appropriately diluted sample was added to 50 μL of distilled water (ddH_2O) in a 96-well plate (Fisher Scientific). Following that step, 12.5 μL of 2 N Folin-Ciocalteu's phenol reagent was added to the

mixture. After 5 min, 7% sodium carbonate (NaCO_3) solution (125 μL) was added to the mixture. Prior to spectrometric analysis, the samples were incubated for 90 min at 25 $^\circ\text{C}$. The absorbance of the sample was measured at 750 nm versus a reagent blank using the microplate reader, Spectramax 340³⁸⁴ spectrophotometer (Molecular Devices Inc., Sunnyvale, CA). A standard curve for total phenolics was developed using gallic acid solution. Total phenolic concentration was expressed in mg of gallic acid equivalents (GAE)/g of fruit.

Determination of Total Flavonoid Content

A standard colorimetric assay (Kim and others 2003) with slight modifications was used to quantify total flavonoid content. To a 96-well plate, 25 μL of the diluted sample was added to 125 μL of ddH₂O. Subsequently, 7.5 μL of 5% sodium nitrate (NaNO_2) was added to the mixture. The mixture was allowed to stand for 5 min and then 15 μL of 10% aluminum chloride (AlCl_3) was added. The mixture was incubated at ambient temperature (25 $^\circ\text{C}$) for an additional 5 min. Following that step, 50 μL of 1 M sodium hydroxide (NaOH) was added to the mixture. The mixture was immediately diluted by the addition of 27.5 μL of ddH₂O and the absorbance of the mixture was measured using a Spectramax 340³⁸⁰ spectrophotometer (Molecular Devices Inc., Sunnyvale, CA) at 510 nm against a reagent blank prepared with ddH₂O. For the purpose of quantifying total flavonoids in the blueberries, changes in the absorbance of the analyte were compared to a catechin standard. The total flavonoids were calculated as mg catechin equivalent (CE)/g of fruit.

Determination of Anthocyanin Content

Total anthocyanin amount was determined by means of the pH differential method (Giusti and Wrolstad 2001). Two buffer systems were used: potassium chloride (KCl)

buffer, (pH 1.0; 0.025 M) and sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2$) buffer, (pH 4.5, 0.4 M). An aliquot of the blueberry extract was adjusted to pH 1.0 and another aliquot to pH 4.5. The difference in absorbance was accordant to the anthocyanin content. An aliquot of the blueberry extract (1.0 mL) was transferred to a 20 mL volumetric flask, diluted to volume with pH 1.0 buffer, and mixed. A second aliquot of the blueberry extract (1.0 mL) was placed in a 20 mL volumetric flask, diluted to volume with pH 4.5 buffer, and mixed. The solutions were incubated at ambient temperature for 20 min. Absorption was measured using a Beckman Coulter DU[®]730 Life Science UV/VIS Spectrophotometer (Brea, CA) at 510 and 700 nm. Results were calculated according to the equation below and expressed as mg of cyanidin-3-glucoside per g of fruit.

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5} \quad (5-1)$$

Statistical Analysis

The data obtained were analyzed using the Statistical Analysis System (SAS[®]) version 9.1. Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The data were tabulated as an average of triplicates \pm standard deviation, and the level of significance was determined at $P \leq 0.05$.

Results

Total Phenolic Content Analysis

Table 5-1 shows the total phenolic content of control and PUV treated blueberries as mg GAE/g. Total phenolics in blueberries ranged from a low of 2.08 ± 0.84 (control) to a high of 3.07 ± 0.47 mg GAE/g fruit (PUV 90 s) (Table 5-1). There was no significant difference ($P \leq 0.05$) in total phenolics between PUV 30 s and control, but a significant increase ($P \leq 0.05$) was found for PUV 60 and 90 s compared to control. The highest

percentage increase relative to control was 48% at PUV 90 s, indicating that PUV had a significant ($P \leq 0.05$) enhancing effect on total phenolics within 90 s exposure.

Total Flavonoid Content Analysis

Table 5-1 shows the total flavonoid content of control and PUV treated blueberries as mg CE/g fruit. Total flavonoid content ranged from a low of 0.15 ± 0.01 (control) to a high of 0.20 ± 0.02 mg CE/g fruit (PUV 30 s). It increased significantly ($P \leq 0.05$) at all PUV exposure times compared to control (Table 5-1), but the increase at PUV 30 s was significantly higher ($P \leq 0.05$) than the other 3 PUV times.

Anthocyanin Content Analysis

Table 5-1 shows the anthocyanin content of control and PUV treated blueberries as mg/g fruit. It was observed that PUV stimulated an increase in blueberry anthocyanins, which ranged from a low of $0.738 \text{ mg/g} \pm 0.27$ (control) to a high of 0.962 ± 0.24 mg/g fruit (PUV 120 s) (Table 5-1). Significant differences ($P \leq 0.05$) were observed in all PUV treated samples relative to control, however, no significant differences were observed among the samples PUV treated for 30, 60, and 90 s.

Discussion

Total Phenolic Content Analysis

A decrease in total phenolics was observed at PUV 120 s, which had a significantly ($P \leq 0.05$) lower value than PUV 90 s, but comparable with control. Phenolic decrease in our experiments may be attributed to increased polyphenoloxidase (PPO) activity, which may have resulted in oxidation of these compounds as suggested by Moreno and others (2007). According to Agarwal (2007), enzymes such as PPO function as antioxidants against oxidative stress, induced by the UV-B spectrum of PUV, which may have unregulated their activity resulting in the

oxidation of phenols to quinones. However, the fact that the total phenolics at 120 s was not significantly different ($P \leq 0.05$) from control, suggested that PUV did not have a degradative effect on the total phenolic content.

Similar trends were observed by Wang and others (2009). They found an increased level of total phenolics proportionate to the time of UV-C exposure. They reported a low of 3.12 ± 0.06 (control) to a high of 4.97 ± 0.09 mg GAE/g at the energy strength of 2.15 kJ m^{-2} . Similar to the trend of our study, higher doses of UV radiation were associated with a decrease in total phenolics.

Total Flavonoid Content Analysis

Increase in phenolic compounds such as flavonoids could be attributed to the upregulation of enzymes such as flavonol synthases (FLS), which is implicated in the biosynthesis of flavonoids (Winkel-Shirley 2002). However, no significant difference ($P \leq 0.05$) was observed in total flavonoids among PUV 60, 90, and 120 s, indicating that the total flavonoid content in fresh blueberries could be easily elicited at a short PUV exposure (30 s), but a longer exposure (60-120 s) did not significantly increase flavonoid content compared to 30 s. The main underlying mechanism for this phenomenon was that high doses of UV light might result in too much stress on the blueberry, consequently inhibiting further flavonoid synthesis (Wang and others, 2009).

Anthocyanin Content Analysis

Wang and others (2009) also found that higher UV-C doses increased anthocyanin content of blueberries, but only up to a dose of 4.30 kJ m^{-2} . In their experiments, UV-C doses were 0, 0.43, 2.15, 4.30, and 6.45 kJ m^{-2} , corresponding to anthocyanin contents of 2.02, 2.38, 2.87, 3.11, and 2.42 mg/g fruit, respectively. One apparent difference observed in our experiments as compared to literature was that our

anthocyanin content was lower in value than that reported by Wang and others (2009). Their reported values ranged from a low of 2.02 mg/g fruit (0 kJ m⁻²) to a high of 3.11 mg/g fruit (4.30 kJ m⁻²) after UV-C illumination. This may be due to varietal difference and environmental conditions relevant to geographical location of harvest and climate (Reyes-Carmona and others 2005).

Table 5-1. Total phenolic, flavonoid, and anthocyanin contents of PUV treated and control fresh blueberries

Treatment	Phenolics (mg GAE/g Fruit)	Flavonoids (mg CE/g Fruit)	Anthocyanins (mg/g Fruit)
Control	2.08±0.84 b	0.147±0.01 c	0.738±0.28 c
PUV 30 s	2.46±1.42 b	0.196±0.02 a	0.916±0.16 b
PUV 60 s	3.05±0.22 a	0.180±0.01 b	0.882±0.11 b
PUV 90 s	3.07±0.47 a	0.183±0.02 b	0.851±0.13 b
PUV 120 s	2.24±0.44 b	0.190±0.02 b	0.962±0.24 a

Means (in columns) with the same letter are not significantly different according to the Tukey's studentized Range Test $P \leq 0.05$. Data are expressed as mean \pm standard deviation (SD).

CHAPTER 6 CONCLUSIONS

In conclusion, PUV light was found to have enhancing effects on antioxidant capacity as well phytochemical content (total phenolics, flavonoids, and anthocyanins) and antioxidant enzymes while maintaining other quality characteristics (sensory, compositional, and temperature) in this study. The well-known disinfection effect of PUV light was witnessed by the observed decrease in total aerobic plate and yeast and mold count, which are microflora of fresh blueberries. Other fruits, rich in secondary metabolites, can be treated with various times and/or distances from PUV lamp to observe enhancing effect on antioxidant capacity and/or phytochemical contents in future research. The PUV light effects on enzymes responsible for synthesis of secondary metabolites can be examined further since there is not enough scientific information about these effects.

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BIOGRAPHICAL SKETCH

Senem Guner was originally from Ankara, Turkey. She received her bachelor's degree in food engineering from Ankara University in 2007. After a one-long experience in the bakery business, she received a scholarship from the Ministry of Education of Turkey in 2008, to continue her education abroad in the United States of America. She attended to University of California UC Davis Intensive English Course before she entered the food science Master of Science program at the University of Florida under the supervision of Dr. Wade Yang. In her time as a Master of Science student Senem had one publication in the Sustainable Agriculture Research journal. Senem plans to continue further her education and pursuit a PhD degree upon her completion of her master's degree.