AN ABSTRACT OF THE THESIS OF

Ruyi Wu for the degree of Master of Science in Food Science and Technology
presented on December 12, 2008.

Title: Effects of Refrigeration Storage and Processing Technologies on the Bioactive
Compounds and Antioxidant Capacities of Blackberries (‘Marion’ and ‘Evergreen’).

Abstract approved:

Yanyun Zhao

The objective of this project was to investigate the effects of refrigeration
storage and processing technologies on the quality and nutraceutical benefit of
blackberries. ‘Marion’ and ‘Evergreen’, the two major blackberry varieties in Oregon,
were evaluated in this study. For refrigeration storage, fresh fruit were packed in clam-shell containers right after harvest and stored at 2.0 ± 0.2 °C and 95 ± 2% relative
humidity for 9 or 7 days for ‘Marion’ and ‘Evergreen’, respectively. For evaluating
processing effect, fresh fruit were individually quick frozen at -23 °C and then subjected
to different processing treatments including freeze drying, hot-air drying, canning-in-water, canning-in-20° Brix sucrose syrup, and making into jam. Processed berry
products were stored at room temperature at 25 ± 2 °C and 30 ± 2% relative humidity
for up to 6 months. Physicochemical properties including pH, titratable acidity (TA),
total soluble solids (TSS) and moisture content (MC), bioactive compounds including
total phenolics (TPC) and total monomeric anthocyanins (ACY), and antioxidant
capacities evaluated as radical scavenging activity (RSA), oxygen radical absorbance
capacity (ORAC) and ferric reducing ability of plasma (FRAP) were monitored in fresh
blackberries and processed blackberry products right after processing and during
storage. Frozen fruit were used as a control for evaluating processing effects. TA
reduced by 36.8% and 46.2% in ‘Marion’ and ‘Evergreen’, respectively (P<0.05) at the
end of refrigeration storage compared to that at day 0. TPC and ACY decreased
significantly in both varieties. ORAC declined by 20% and FRAP increased by 18.75%,
respectively in ‘Evergreen’ (P<0.05) while remained stable in ‘Marion’ during
refrigeration storage. Right after processing, TA in all the processed ‘Marion’ products
lost significantly, while TSS increased in dried blackberries and blackberry jams of both
varieties. Freeze drying increased TPC by 27% and 21% in ‘Marion’ and ‘Evergreen’,
respectively (P<0.05), whereas hot-air drying reduced ACY by 56% in ‘Marion’ and
84% in ‘Evergreen’ (P<0.05). The content of measured bioactive compounds lost 31%
to 70% (P<0.05) in canned products except TPC in ‘Marion’ canned-in-water. ORAC
and FRAP lost 21%-61% in canned ‘Marion’ (P<0.05). RSA increased in canning-in-
water fruit while decreased in canning-in-sucrose syrup for both varieties. Making
blackberries to jams resulted in extensive losses of bioactive compounds and
antioxidant capacity in both varieties. Over 6–month storage, TA deceased 9% and 12%
in ‘Marion’ and ‘Evergreen’ jams, respectively (P<0.05). The significant losses of ACY
in hot-air dried, canned blackberries and blackberry jams, and RSA in frozen, dried and
canned blackberries, ORAC in hot-air dried and canned-in-water blackberries of both
varieties were also observed. Two varieties of blackberries varied in physicochemical
properties, content of bioactive compounds and antioxidant capacities, with higher
values of TA, MC, TPC, ACY, RSA and FRAP observed in ‘Marion’ than those in ‘Evergreen’.
Effects of Refrigeration Storage and Processing Technologies on the Bioactive Compounds and Antioxidant Capacities of Blackberries (‘Marion’ and ‘Evergreen’)

by

Ruyi Wu

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APPROVED:

______________________________________________________________
Major Professor, representing Food Science and Technology

______________________________________________________________
Head of the Department of Food Science and Technology

______________________________________________________________
Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My Signature below authorizes release of my thesis to any reader upon request.

______________________________________________________________
Ruyi Wu, Author
This comprehensive work can not be completed successfully by me alone. Here is my sincere acknowledgement to all the individuals generously contributing to my research and giving support in my everyday life in Corvallis.

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CHAPTER 1

Introduction

Berry fruit are pronounced in vitamins, minerals, folic acid, dietary fiber and phytochemicals, particularly phenolic compounds including flavonoids, tannins and phenolic acids. Epidemiological evidences have promoted the consumption of berry fruit in regular diet against chronic diseases via bioavailable phytochemicals participating in chemical reactions and biological metabolism in human body (1-5).

As one of the most popular berry crops, blackberry (*Rubus spp.*) is high in anthocyanins, ellagic acid, and antioxidant capacity with variation of phytochemical concentrations by genotype, climate, harvest season, location and cultivation conditions (6-9). Thorny ‘Marion’ (*Rubus ursinus*) and thornless ‘Evergreen’ (*Rubus laciniatus*) are the two widespread cultivars of trailing blackberries grown in Oregon and dominantly utilized for processing (10, 11). Recently, more interests have arisen in analyzing the composition of polyphenolics, anthocyanins and ellagintannins in fresh blackberries by High Performance Liquid Chromatography (HPLC) (12-14).

Fresh blackberries are easily bruised by rough handling after harvest because of their soft tissue. In addition, blackberries are extremely perishable with only 2-3 days of
shelf life at -0.6-0°C and 90-95% relative humidity (15). Shrinkage, bleeding, changes of physicochemical and nutritional properties, and mold growth commonly occur during post-harvest refrigeration storage as a result of moisture loss, mechanical injury, fruit respiration, and microbiological contamination (16-20).

Accordingly, multiple technologies have been employed to process fresh blackberries into various value-added products for long-term preservation. For instance, freeze processing aims to prolong the shelf-life of blackberries by inactivating bacteria and enzyme activity and suppressing respiration of fruit at low temperature (21). Canning and jam processing thermally destroy pathogenic and spoilage microorganisms, inactivate enzymes, and eventually eliminate oxygen in the final products (22). Drying removes water from fresh fruit and minimizes water activity essential for microorganism growth (23). Besides extension of the shelf-life, applications of post-harvest processing technologies also diversify the types of blackberry products. For example, juices blended with blackberries and other fruit offer delicious and nutritional liquid products to the market (24). Moreover, blackberry incorporated candies, cereals, yogurt, ice creams, smoothies, and pies have become more popular.

Therefore, it is worthwhile investigating the impacts of post-harvest storage and processing on the significant bioactive compounds and antioxidant capacities of blackberries, thus establishing the scientific references for consumers when making purchase decision and for manufacturers to advance the technologies satisfying both safety and nutrition requirements. Accordingly, the objective of this study was to
evaluate the effects of post-harvest refrigeration storage, processing technologies including freezing, hot-air drying, freeze drying, canning-in-syrup and canning-in-water, and jam manufacturing, as well as post-processing storage in room conditions on the basic physicochemical properties, important bioactive compounds, and antioxidant capacities of ‘Marion’ and ‘Evergreen’ blackberries.

**Literature cited**


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CHAPTER 2

Literature Review

2.1 Introduction

With benefits from climatic and soil conditions suitable for the growth of blackberry plants, Pacific Northwest especially Oregon in the United States is one of the largest production regions and leading suppliers of blackberry fruit in the world (1). Blackberries are well-known as an excellent nutritional resource for a healthy diet in humans and have been nourishing people as a type of small fruit for centuries (2, 3).

This chapter will introduce the history, botanical classification, cultivars, production and physiological changes during ripening of blackberries, and summarize the chemical composition, health benefits and quality characteristics of fresh blackberries. Pre-harvest practices related to the quality of blackberries at harvest and post-harvest processing technologies applied for preserving fresh blackberries are covered as well.
2.2 Overview of blackberries

2.2.1 History and botanical classification of blackberries

Blackberries are in Plantae Kingdom, Magnoliophyta Division, Magnoliopsida Class, Rosaceae Family, and Rubus Genus. In the United States, species \( R. allegheniensis \), \( R. argutus \), \( R. cuneifolius \), and \( R. Canadensis \) grow in the north while \( R. trivialis \) is a southeastern species. The Pacific Northwest region of the US has native species \( R. ursinus \) and non-native species \( R. lacinatus \) introduced from Europe in 1860 (4).

Blackberry plants with trailing, erect or semi-erect canes are generally cultivated in either sandy or clay soil with pH from 5 to 7. They can be propagated by cutting root, suckering, layering tip and cutting leaf stem. Pinky white flowers of blackberries are generated in the second year of planting. Blackberry (\( Rubus spp. \)) fruit are formed on a delicate cluster of fragile drupelets with attractive color (5).

2.2.2 Cultivars of blackberries

Trailing, erect, and semi-erect are recognized as the three major types of blackberries grown in the United States classified by cane architecture. Erect blackberries with firm texture but weak flavor were mainly bred in Arkansas for the fresh market. The cultivars of erect blackberries harvested from July to August include ‘Cherokee’, ‘Arapaho’, ‘Natchez’, ‘Shawnee’, ‘Kiowa’, ‘Chickasaw’, ‘Quachita’, ‘Apache’, and ‘Navaho’ (Table 2.1). Semi-erect blackberries with high yield were mainly bred in Maryland and Illinois for the fresh market as well. Their cultivars

‘Marion’ blackberries with dark red color, medium size, medium seeds and excellent flavor grown in very vigorous and thorny plant and ‘Evergreen’ blackberries with dark black color, small size, large seeds and good flavor grown in vigorous and thornless plant stand for the native *Rubus ursinus* species and the imported *Rubus laciniatus* species of trailing blackberries, respectively. Both ‘Marion’ and ‘Evergreen’ are in high yielding and favorable to be commercially processed into different blackberry products for year-round availability (6).
Table 2.1 Types and cultivars of blackberries

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Erect blackberries</th>
<th>Semi-erect blackberries</th>
<th>Trailing blackberries</th>
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<tr>
<td></td>
<td>Cherokee</td>
<td>Loch Tay</td>
<td>Marion</td>
</tr>
<tr>
<td></td>
<td>Arapaho</td>
<td>Loch Ness</td>
<td>Evergreen</td>
</tr>
<tr>
<td></td>
<td>Natchez</td>
<td>Hull Thornless</td>
<td>Black diamond</td>
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<tr>
<td></td>
<td>Shawnee</td>
<td>Triple Crown</td>
<td>Waldo</td>
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<tr>
<td></td>
<td>Kiowa</td>
<td>Doyle’s</td>
<td>Black Pearl</td>
</tr>
<tr>
<td></td>
<td>Chickasaw</td>
<td>Black Satin</td>
<td>Obsidian</td>
</tr>
<tr>
<td></td>
<td>Quachita</td>
<td>Smoothstem</td>
<td>Metolius</td>
</tr>
<tr>
<td></td>
<td>Apache</td>
<td>Dirksen Thornless</td>
<td>Siskiyou</td>
</tr>
<tr>
<td></td>
<td>Navaho</td>
<td>Thornfree</td>
<td>Silvan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chester Thornless</td>
<td>Nightfall</td>
</tr>
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Source: Adapted from Finn et al. (6).

2.2.3 Production of blackberries

The planted area of blackberries increased 45% in ten years from 1995, reaching 49,508 acres with 154,643 tons of worldwide production in 2005. Mainly through alternative year production system and machine harvest system, Oregon supplied 25,185 tons of blackberries in 7,754 acres in 2005, which topped in cultivation area and total production of blackberries in the United States and accounted for approximate 16% of worldwide production of blackberries. ‘Marion’ and Thornless ‘Evergreen’ were reported as two important cultivars composing of 61% and 11% of blackberries in Oregon, respectively (7).

2.2.4 Physiological ripening of blackberries

Fruit ripening refers to the process of fruit developing to edible food. There are a series of physical and chemical changes involved in fruit ripening. Non-climacteric pattern of ripening is recognized in blackberries (8).
First of all, softening occurs in blackberries as the maturity increases, which has been attributed to the breakdown of cellular substances such as pectin, cellulose, hemicellulose and other polysaccharides through hydration (9).

Secondly, glucose, fructose and sucrose are three main carbohydrates partly from the hydrolysis of starch within fruit. These sugars accumulate during fruit ripening, resulting in increased soluble solids content or brix (10). In contrast, organic acids are consumed as one of the substrates in respiration course of fruit and decomposed under light and ambient temperature.

Thirdly, chlorophyll degrades concurrently with the synthesis of anthocyanins, representing the color changes of blackberries from green, red, to black as maturity progresses (11).

Fourthly, polymerization leads to a reduction in phenolics, flavor compounds formed and antioxidant capacity enhanced as detected in blackberries from previous studies (12, 13). Finally, the production of ethylene, a traditional physiological index in fruit ripening, is not consistent in all the blackberry cultivars (14).

2.2.5 Chemical composition of blackberries

Nutrition fact of fresh blackberries (*Rubus spp.*) used as reference is updated and accessible in national nutrient database released by USDA (15). But chemical compositions of blackberries are subjected to variability in cultivars, harvest seasons, locations, maturity stage and other pre-harvest factors. The principal chemical compositions in fresh blackberries are elaborated as follows.
2.2.5.1 Carbohydrates

Following water, carbohydrates are the second most abundant components in fresh blackberries. They are the essential element which provides consumers with the fundamental sensory attribute of sweetness in most fruit. Fruit ripening actually is the process of accumulation of sugars and reduction of acids. The contents of subgroup of carbohydrates in 100g of fresh blackberries are listed in Table 2.2 (15), indicating glucose and fructose are the two major sugars in ripe blackberries in general.

Table 2.2 Carbohydrate composition of fresh blackberry fruit (100g)

<table>
<thead>
<tr>
<th>Carbohydrate (g)</th>
<th>Sugar (g)</th>
<th>Sucrose (g)</th>
<th>Glucose (g)</th>
<th>Fructose (g)</th>
<th>Maltose (g)</th>
<th>Galactose (g)</th>
</tr>
</thead>
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<tr>
<td>9.61±0.00</td>
<td>4.88±0.69</td>
<td>0.07±0.00</td>
<td>2.31±0.30</td>
<td>2.40±0.37</td>
<td>0.07±0.00</td>
<td>0.03±0.03</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (n=2-5).
Source: Adapted from USDA national nutrient database for standard reference (15).

2.2.5.2 Organic acids

Similar to carbohydrates, organic acids are another indicator to evaluate the basic taste of sourness in most fruit. Moreover, it is a factor affecting the stabilization of pigments in blackberries. According to the study by Kafkas et al. in investigating five cultivars of blackberries, ‘Navaho’, ‘Chester Thornless’, ‘Jumbo’, ‘Bursa 2’, and ‘Loch Ness’ grown in Turkey, malic acid accounts for 0.6% to 1.1% on weight, thus statistically a major organic acid detected in fresh blackberries by HPLC compared to ascorbic acid and citric acid (16).
2.2.5.3 Phenolic compounds of blackberries

As product from plant secondary metabolism, phenolics can be classified into phenolic acids, flavonoids, hydrolyzable tannins and other phytochemicals such as lignans, sterols, and stilbenes (17). Phenolics in fruit are biologically synthesized as plants grow and fruit ripen generally through hydroxylation, methylation, esterification and glycosylations involving Phenylalanine Ammonialyse (PAL), Cinnamate 4-Hydroxylase (C4H) and Hydroxycinnamate CoA Ligase (CoAL), which are affected by environmental conditions such as light, temperature and nutrients in the plants and pre-harvest practices as well (17). From the standpoint of sensory properties, phenolics provide consumers with the perception of bitterness, astringency and the color of fruit (17).

According to Macheix et al., hydroxybenzoic acids (HBA) and hydroxycinnamic acids (HCA) are the two dominant types of phenolic acids in blackberries (17). Zadernowski et al. reported that free, ester bonded and glycoside bonded phenolic acids constitute 3.3%, 53.1% and 43.6% of total phenolic acids respectively in *Rubus plicatus*. There are significantly more HCA existing in blackberries than HBA. Specifically, salicylic is in the highest fraction (524.1 mg/Kg of dry matter of fresh berries) of hydroxybenzoic acids (HBA) (18). Hydroxycaffeic (627.6 mg/Kg of dry matter of fresh berries), m-coumaric (596.6 mg/ Kg of dry matter of fresh berries) and 3, 4-dimethoxycinnamic (501.9 mg/Kg of dry matter of fresh berries) topped as hydroxycinnamic acids (HCA) in phenolic acids profile (18).
As the water-soluble pigments belong to the category of flavonoids in fruit and vegetables, anthocyanins are responsible for the appealing color of blackberries. They are in glycosidic form of anthocyanidins (17). Depending on the positions and numbers of hydrogen, hydroxyl, and methoxyl groups in the basic chemical structure, six anthocyanidins including Pelargonidin (Pg), Cyanidin (Cy), Delphinidin (Dp), Peonidin (Pn), Petunidin (Pt), and Malvidin (Mv) are naturally occurring and widely recognized in berry fruit (19). Cho et al. reported that cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, cyanidin 3-malonylglucoside, and cyanidin 3-dioxalylglucoside are the five common anthocyanins present in blackberries. Among them, cyanidin 3-glucoside was identified as primary composition (20). Wu et al. indicated that nonacylated anthocyanins contributed 94% and 97% to the total level of anthocyanins in the overall of four blackberry varieties studied and ‘Marion’ blackberry, respectively, and 90% and 76% of anthocyanins in overall blackberries and ‘Marion’ blackberry, respectively are monoglycosides (21).

Total anthocyanin contents in five genotypes of blackberries harvested in Oregon in 2000 were reported as 164 mg/100g fresh weight (2). Significantly higher contents of anthocyanins (245 mg/100g fresh weight) were identified in blackberries without specific information about harvest time and location in another study (21). Results in these two studies were both roughly in the range of 114.39 to 241.54 mg/100g of fresh blackberries with six cultivars, ‘Apache’, ‘APF-12’, ‘Arapaho’, ‘Chickasaw’, ‘Kiowa’, and ‘Navaho’ (20). Significantly lower contents of anthocyanins (88.7 mg/100g fresh weight) were in ‘Black Diamond’, ‘Smoothstem’, ‘Thornless Boy Sembes’, ‘Darrow’,
‘Chester’, ‘Hull Thornless’, and ‘Black Satin’ blackberries (22). These numbers also fell in the range of anthocyanin content from 70.3 to 201 mg/100g fresh weight of 18 varieties of blackberries harvested in 51 locations (23).

Flavonol is considered to be another largest group of flavonoids mainly in the flesh of blackberries. There were 102 to 160 mg of flavonol composed of quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 3-xyloside, quercetin 3-xylosylglucuronide, and quercetin 3-glucosylxyloside in every Kg of fresh weight of ‘Apache’, ‘APF-12’, ‘Arapaho’, ‘Chickasaw’, ‘Kiowa’, and ‘Navaho’ blackberries (20). ‘Evergreen’ blackberries showed a significantly higher content of flavonol than that of ‘Marion’ blackberries harvested in Oregon in 1999 (24).

Tannins are polyphenolics polymerized with proteins, carbohydrates and other molecules, providing astringency in fruit and vegetables (25). They are traditionally divided into hydrolysable tannins and condensed tannins based on structural variations. Hydrolysable tannins are created by esterification of hydroxyl groups in carbohydrates usually glucoside and carboxyl groups in phenolic acids such as gallic acid, hexahydroxydiphenic acid, and ellagic acid (17).

Like other berries, blackberries are abundant in hydrolysable tannins (19). More studies have focused on the analysis of ellagitannins in blackberries. It has been confirmed that ellagitannins are mostly distributed in seeds other than flesh (24, 26). Six ellagitannins including pedunculagin, casuarictin/potentillin, castalagin/vescalagin, lambertianin A/sanguin H-6, lambertianin C, and labertianin D have been identified by virtue of HPLC-ESI-MS (High Performance Liquid Chromatography-ElectroSpray
Ionization-Mass Spectrometry) instrument (27). Besides ellagitannins, free, acylated and glycosylated ellagic acids are also available in blackberry fruit. Ellagic acid derivatives were significantly higher in the whole ‘Evergreen’ blackberries (3.62 mg/100g FW) than in ‘Marion’ blackberries (1.64 mg/100g FW) (24).

Condensed tannins or proanthocyanidins are another division of tannins rich in the kingdom of plants. Structurally, they are complex of flavonoids linked through carbon-carbon bonds which are not hydrolysable (28). Contents of proanthocyanidins composed of catechin and epicatechin were only detected in blackberry seeds ranging from 48.8 to 58.5 and 9.26 to 11.3 mg/100g FW, respectively (24).

In addition, blackberries contain secoisolariciresinol, which is a kind of lignans resulted from the interactions between two cinnamic acids and approved to have anticancer effect (29).

### 2.2.5.4 Enzymes in blackberries

Enzymes play an important role in boosting chemical reactions which consequently result in quality deterioration of berry fruit in color, texture, flavor, and nutritional value during ripening, post-harvest handling, and storage. Peroxidase and polyphenol oxidase actively participate in enzymatic browning by catalyzing the oxidation of phenols to quinones which further interact with proteins to form complex with large molecular weight (30, 31), resulting in the consumption of phenolics and the brown color on fruit. Tissue softening partly caused by the decomposition of cell wall materials involves enzymes as well, such as cellulase and hemicellulase. Polygalacturonase and pectate
lyase break down pectin, while glucanase, xyloglucan, transglycosidase, xylanase, and β-xylosidase decompose hemicellulose (32).

2.2.5.5 Vitamins and minerals

Blackberries are excellent source of numerous essential vitamins including vitamin C, vitamin B groups, vitamin A, vitamin K, and folate, and minerals including potassium, magnesium, calcium, phosphorus, and iron. The distribution of nutrients in 100g of fresh blackberries released by USDA is summarized in Table 2.3 and 2.4.

Table 2.3 Vitamin composition of fresh blackberry fruit (100g)

<table>
<thead>
<tr>
<th>Vitamin A (IU)</th>
<th>Vitamin C (mg)</th>
<th>Vitamin E (mg)</th>
<th>Vitamin K (mcg)</th>
<th>Folate (mcg)</th>
<th>Choline (mg)</th>
<th>β Carotene (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>214±39.42</td>
<td>21±0.00</td>
<td>1.17±0.05</td>
<td>19.8±2.08</td>
<td>25±2.01</td>
<td>8.5±0.00</td>
<td>128±23.65</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (n=4-5).
Source: Adapted from USDA national nutrient database for standard reference (15).

Table 2.4 Mineral composition of fresh blackberry fruit (100g)

<table>
<thead>
<tr>
<th>Ca (mg)</th>
<th>Fe (mg)</th>
<th>Mg (mg)</th>
<th>P (mg)</th>
<th>K (mg)</th>
<th>Na (mg)</th>
<th>Zn (mg)</th>
<th>Cu (mg)</th>
<th>Se (mcg)</th>
<th>Mn (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29±6.74</td>
<td>0.62±0.08</td>
<td>20±1.26</td>
<td>22±0.79</td>
<td>162±0.00</td>
<td>1.0±0.00</td>
<td>0.53±0.04</td>
<td>0.165±0.03</td>
<td>0.4±0.00</td>
<td>0.646±0.23</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (n=2-5).
Source: Adapted from USDA national nutrient database for standard reference (15).

2.2.6 Health benefit of blackberries

Several potential health benefits associated with the intake of phytochemicals in blackberries have been confirmed by many scientific evidences. For example, polyphenolics demonstrated an anti-cancer effect through quenching inflammatory and
inhibition of carcinogenic proliferation (33). It was also found that phytochemicals reduced the risk of coronary heart disease in many pathways, such as intervening the LDL oxidation and biosynthesis of cholesterol (34). Furthermore, Barbara et al. declared that polyphenolics relieved the stress in nervous system against brain aging (35). Finally, two recent biological studies claimed that anthocyanins were effective in the prevention of obesity (36, 37).

2.2.7 Quality of fresh blackberries

The objective of cultivation practice, pre-harvest, and post-harvest handling is to provide high quality of blackberries in fresh market and for subsequent processing. The quality of blackberries is generally evaluated by the following quality characteristics.

2.2.7.1 Fruit size

Fruit size has direct effect on the marketability and acceptance of blackberries in both fresh market and processing plant. Traditionally, large fruits are preferred by consumers (38). Moyer et al. reported that a 100 g of blackberries contained 27 to 108 fruits based on an investigation on five genotypes (2). Eyduran et al. recorded that fresh blackberries weighed 2.01 g (cv. ‘Black Satin’) to 9.25 g (cv. ‘Black butte’) per fruit (Table 2.5) (39).
Table 2.5 Average fruit weight of fresh blackberries

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Cherokee</th>
<th>3.03</th>
<th>Arapaho</th>
<th>3.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g/berry)</td>
<td>2.30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Black Satin</th>
<th>Ness</th>
<th>Chester Thornless</th>
<th>5.19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g/berry)</td>
<td>2.01</td>
<td>2.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Marion</th>
<th>Nightfall</th>
<th>Black Butte</th>
<th>9.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g/berry)</td>
<td>5.10</td>
<td>6.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Adapted from Eyduran et al. (39).

2.2.7.2 Color

The shiny dark red or black color of blackberries is very attractive to consumers. Fading of the color and degradation of the pigments are associated with quality deterioration of blackberry fruit from the consumer standpoint. Colorimeters are widely used to measure the surface color of fruit by using the Hunter L*a*b* system (40, 12, 41). Color parameters such as L*, a*, and b* stand for lightness, red to green, and yellow to blue, respectively. L* values of four dull-black thorny erect blackberries were 20.4 to 25.3 (40). L*, a*, and b* values of nine wild Turkey blackberries harvest in 2005 were 17.35, 10.41 and 3.62, respectively (12). The complete colorimetric profile of nine thornless blackberries (‘Bursa-1’, ‘Bursa-2’, ‘Bursa-3’, ‘C. Thornless’, ‘Bartin’, ‘Loch Ness’, ‘Navaho’, ‘Jumbo’, and ‘D. Thornless’) is summarized in Table 2.6 (41).

Table 2.6 Range of colorimetric parameters of thornless blackberries

<table>
<thead>
<tr>
<th>Colorimetric measurements</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Range</td>
<td>10.5-14.5</td>
<td>3.7-7.4</td>
<td>1.3-3.4</td>
<td>4.0-8.1</td>
<td>16.7-24.8</td>
</tr>
</tbody>
</table>

Source: Adapted from Turemis et al. (41).
2.2.7.3 Firmness

Fruit firmness is considered to be one of the critical texture properties because blackberries are very susceptible and fruit softening occurs as a result of degradation of pectin existed in the cell wall of fruit (42). Firmness of blackberries could be measured by shear, extrusion, compression, and penetration force using a Texture Analyzer (43, 11). The means of shear, extrusion, compression and penetration force applied on frozen and thawed ‘Thornfree’ blackberries harvested in Spain at the commercial maturity stage were reported as 16.02N, 20.11N, 20.63N and 8.85N, respectively (43). For fresh ‘Navaho’ harvested in the US in 1996, the compression and penetration force decreased during ripening approximately from 42 N to 2 N and from 0.9 N to 0.2 N, respectively (11).

2.2.7.4 Fruit decay

Spoilage microorganisms mostly fungi grow and propagate rapidly by sourcing nutrients from fruit tissues under warm weather and refrigeration storage condition with high humidity, which consequently causes fruit decay (44). The physical damages of fruit skin during and after harvest facilitate the overspread of microbial infection, thus accelerating the decay of blackberries (44). Specifically, blackberries are prone to grow grey molds (Botrytis cinerea) in the high moisture environment (45, 46). The occurrence of fruit decay is detrimental for marketing fresh blackberries. Hence decay rate is a necessary criterion to assess the quality of the fruit. The percentage of decay depends on the cultivar and maturity stage, ranging from 0 to 40.6% after 7-d
refrigeration storage in ‘Choctaw’, ‘Cheyenne’, ‘Navaho’ and ‘Shawnee’ blackberries (47).

2.2.7.5 Weight loss

Weight loss is a basic parameter to evaluate quality of blackberries during post-harvest storage (48, 49). Loss of moisture is the major reason for weight loss of berries as a result of post-harvest respiration and transmission (50). Besides, evaporation of aroma compounds also partly contributes to weight loss in the fruit. A 0.8% to 3.3% of weight loss was found in four blackberry cultivars (‘Choctaw’, ‘Cheyenne’, ‘Navaho’ and ‘Shawnee’) with three ripen stages (mottled, shiny black and dull black) during 7-d refrigeration storage (47).

2.2.7.6 Total soluble solids, Brix, and acidity

Total soluble solids (TSS) reflect seeds and sugar content in the blackberries. The seediness affects the taste and chewability of the fruit and the processing of fresh blackberries (38).

Brix degree is defined as the percentage (w/w) of sugar (sucrose) present in food samples. Fructose, glucose and sucrose are the three primary types of sugar and malic acid is the dominant organic acid in blackberries (51).

Sugar content gradually elevates but the acidity drops as fruit ripen. The ratio of sugar and acidity varies in different varieties of blackberries at harvest and is a good indicator of balance between sweetness and sourness of fruit. Moreover, the sugar
content of fruit determines the amount of sugar addition to make shelf-stable blackberry products, such as jams, jellies, and juices (38). The stability of some bioactive compounds is heavily influenced by pH and acidity of the fruit.

Reyes-Carmona et al. reported a range of 2.33 (wild) to 4.28 (‘Evergreen’) for pH, 7.5% (wild) to 16.1% (‘Evergreen’) for TSS, and 1.02% (‘Evergreen’) to 4.22% (wild) citric acid equivalent for titratable acidity (TA) in 11 cultivars of blackberries harvested in different locations (52). Perkins-Veazie et al. also found TSS as low as 4.7% in mottled ‘Cheyenne’ and TA 0.8% in dull black ‘Navaho’ and TA 0.84% in ‘Chester’ at harvest (47).

2.2.7.7 Nutritional values

Blackberries are excellent sources of dietary fibers, vitamins and minerals (Table 2.3 and 2.4). Blackberries are well-known for their high content of antioxidants, total phenolics, anthocyanins, ellagic acids, and other phytochemicals (13, 24). The exact composition of nutraceuticals in blackberries is highly dependent on numerous pre-harvest and post-harvest factors (1, 48).

2.2.8 Pre-harvest factors affecting quality of fresh blackberries

2.2.8.1 Genotype and cultivars

Perkins-Veazie et al. found that erect blackberry cultivars of ‘Arapaho’ and ‘Navaho’ had higher ratio of TSS and acidity, firmer texture at 5 °C or 10 °C, and longer shelf-life (7-10d) at 2 °C than ‘Choctaw’ and ‘Shawnee’ blackberries (49).

Siriwoharn et al. found that physicochemical parameters including TSS, TA and Brix/TA, total phenolics, total anthocyanins, quantitative compositional distribution of anthocyanins and polyphenolics, and antioxidant capacity evaluated by ORAC and FRAP were considerably varied among blackberry cultivars (‘Marion’, ‘Waldo’, ‘Evergreen’, ‘Chester’, ‘Silvan’, ‘NZ’, and ‘ORUS’). ‘Evergreen’ demonstrated the highest antioxidant capacity in both ORAC (75.5 µmol TE/g FW) and FRAP values (91.5 µmol TE/g FW) (13).

Reyes-Carmona et al. indicated that genotypes, other than climate and region, was a major factor affecting acidity, soluble solid content, polyphenols, and antioxidant capacity in ripen ‘Brazos’, ‘Tupi’, ‘Comanche’, and wild blackberries harvested in Mexico, and ‘Marion’, ‘Evergreen’, and ‘Siskiyou’ harvested in Oregon (52).

### 2.2.8.2 Maturity

Some studies have addressed the effect of maturity stage on the quality of blackberry fruit. Perkins-Veazie et al. found that mottled (50% black) erect blackberry cultivars, ‘Cheyenne’, ‘Navaho’, ‘Choctaw’, and ‘Shawnee’, were firmer, more acidic,
and less sweet than shiny black and dull black fruit of same blackberry cultivars. More anthocyanins were accumulated in dull black blackberry fruit but deteriorated more rapidly \( (47) \). Woods et al. studied thornless blackberries including ‘Apache’, ‘Arapaho’, ‘Chester’, ‘Loch Ness’, ‘Navaho’, and ‘Triple Crown’, and reported that pH increased, TA declined, TSS/TA ratio increased, and antioxidant capacity increased along with increased fruit maturity at harvest from red, mottled, shiny black to dull black \( (55) \).

Siriwoharn et al. also observed a significant increase of total anthocyanins and TSS and loss of acids along with the ripening in ‘Marion’ and ‘Evergreen’ blackberries harvested in Oregon in 2002 \( (13) \).

Wang and Lin investigated three cultivars of thornless blackberries, ‘Chester’, ‘Hull’, and ‘Triple Crown’ in Maryland and found that the anthocyanins were synthesized while the level of total phenolics declined with the development of blackberry fruit. Interestingly, ORAC values consistently decreased for fruit from green to pink stage then increased from pink to ripe stage \( (56) \).

Tosun et al. confirmed the decrease in total phenolics and increase in anthocyanins along with maturity in a study on blackberries harvested in Turkey in 2005 \( (12) \).

2.2.8.3 Climate, location, season and year

The qualities of berry crops and berry fruit are strongly dependent on macroclimate such as temperature, light, carbon dioxide, and water, as well as microclimate such as spacing between plants, pruning, and training during pre-harvest practice \( (48) \).
Connor et al. reported that antioxidant and total phenolic content of blackberries harvested from both New Zealand and Oregon turned out significantly different in 12 shiny-black blackberries across the years which may result from the slightly changed climate (53). Qian and Wang found that volatile acids, alcohols, aldehydes, ketones, terpenes, terpenoids, and esters varied in ‘Marion’ and ‘Evergreen’ blackberries grown in 1999, 2001, and 2002 in Oregon (57).

2.2.8.4 Cultivation technique

The optimal amount of water and nutrients including N, P, K, Ca, and Mg influences crop growth, leaf production, and fruit ripening. The lack of water may result in soft and less sweet fruit, but the excess of water may promote fruit bruise and decay. Application of sufficient and balanced nutrients is critical for the quality of berry fruit at harvest (48).

In addition, the organic and sustainable cultivation practices produced significantly higher fresh-weight based total phenolics in frozen, freeze-dried, and air-dried ‘Marion’ blackberries harvested in Oregon than conventional ones (58).

2.2.8.5 Pests and diseases

Occurrence of pests and diseases, such as yellow vein disease (YVD), phragmidium violaceum, and arthropod pests jeopardized the production of blackberry crops and the safety, quality, and processing properties of blackberry fruit (59-61). On the other side,
the residual of herbicides, pesticides, and any other chemicals used against disease and pest infection of berry crop could cause some safety concerns as well (62).

2.3 Post-harvest handling technologies

2.3.1 Introduction

Post-harvest handling practices basically involve four steps: harvesting, pre-cooling, packaging, transportation and storage (63). Fresh blackberry fruit are extremely perishable with a very short shelf-life. Hence, retaining quality and extending shelf-life of fresh fruit throughout post-harvest handling are essential. A diverse array of storage conditions, package technologies, and post-harvest handling technologies has been investigated and introduced to the blackberry industry.

2.3.2 Refrigeration storage

The principle of refrigeration storage is to slow down the metabolism of fruit by reducing temperature (64). Several previous studies have addressed the effect of refrigeration storage on the quality of blackberries (65, 66). Cultivars mostly affect the preservation of quality of fresh blackberries during refrigeration storage. Perkins-Veazie et al. observed that shiny black erect blackberry cultivars, ‘Navaho’, ‘Cheyenne’, and ‘Shawnee’ were successfully stored in plexiglass boxes under 2 °C and 95% relative humidity for 7 days without significant changes in firmness, pH, soluble solids, and titratable acidity, but up to 3.4% of initial weight was lost (65). Antunes et al. found that
‘Brazos’ and ‘Comanche’ blackberries had 9 days of shelf-life at 2 °C. Fruit lost mass, titratable acid and soluble solids but gained pH along with the storage time (66).

Harvest maturity is also important to the performance of fresh blackberries after harvest. Perkins-Veazie et al. reported that ‘Navaho’ blackberries harvested at 60% ripeness was marketable after 21 days while ‘Shawnee’ blackberries deteriorated after 14 days of storage at 2 °C and 95% relative humidity in polyethylene clamshell boxes with paper absorbents. However, decay rate surged in both varieties of blackberries during refrigeration storage (49).

Again, Perkins-Veazie et al. found that erect blackberries ‘Navaho’ and ‘Shawnee’ deteriorated more rapidly and lost more weight at 5 °C than at 2 °C storage (49, 67). As for antioxidant capacity, Perkins-Veazie et al. reported no significant changes in ORAC values of erect blackberries, ‘Navaho’, ‘Kiowa’, ‘Shawnee’, ‘Arapaho’, and ‘Choctaw’ harvested in Oklahoma in 1998 after 7 days of storage at 2 °C and 95% relative humidity (68).

2.3.3 Controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP)

CAS is to modify the composition of gas in the environment where fruit are stored for prolonging the shelf-life of fresh fruit (44). The quality of fruit can be better preserved in atmosphere composed of a high concentration of carbon dioxide and low concentration of oxygen, leading to delayed respiration and transpiration of fruit, controlled growth of aerobic microorganism and insects, and lowered enzymatic
activity. However, the high percentage of carbon dioxide may potentially retard the production of fruit aroma compounds and result in off-flavor (44). CAS generally enriches carbon dioxide up to 10-20% and reduces oxygen to 5-10% in blackberry (44). Perkins-Veazie and Collins found that CAS effectively reduce the incidence of decay and retain titratable acid in both ‘Navaho’ and ‘Arapaho’ blackberries during the storage at 2 °C (69). However, Agar et al. reported CAS not effective in retaining ascorbic acid in thornfree blackberries (70).

On the same basis of CAS principles, modified atmosphere packaging (MAP) aims to change the rate of respiration within the food and transpiration between food and air outside the package by adjusting the regular composition of the atmosphere inside the food package. Gas composition, storage temperature, and package material with different permeabilities need to be carefully selected according to the characteristics of food, the cultivar or type of raw food materials, maturity of fresh produce, and some mechanical injuries for the well-signed MAP (71, 72). Few studies investigated the effectiveness of MAP technology to extend the shelf-life of blackberries, but the grape berries (73), apples (74), blueberries (75) and cherries (76).

2.3.4 Edible coatings

As a novel “invisible packaging” technology, edible coatings have been developed with the purpose of extending the shelf-life of fresh fruits and vegetables. Edible coatings work as gas and water barriers to minimize the respiration rate and moisture
loss. Antimicrobial agents may be incorporated into edible coatings to control microorganism infection in fresh produce (77-79).

Polysaccharides, proteins and lipids may be used for forming coatings applied on berry fruit. Several studies showed chitosan (1, 4-linked 2-amin -2-deoxy–β–D-glucan) coated fresh strawberries with improved qualities including reduced weight loss and decay and elevated firmness (78-82). Meneghel et al. reported that sodium alginate coating on ‘Comanche’ blackberries provided ready-to-eat fruit with comparable physical, chemical and sensory properties to fresh ones (83).

Protein-based coatings, such as caseinate and whey protein not only controlled gas transfer during post-harvest ripening, but also provided additional proteins (84, 85). The effectiveness of protein based coatings could be advanced by addition of lipids and combination with other physical technologies such as irradiation (86). Lipids with moisture resistant properties are traditionally used to retain fruit quality by holding sufficient water in fruit tissues (85).

Previous studies have shown that kiwifruits, pineapple, and cherries have extended shelf-life via coating of Semperfresh™ which is a sucrose-fatty acid ester (87-89). However, to our best knowledge, no published study has evaluated the application of protein and lipid based coating on fresh blackberries.

### 2.3.5 Other possible technologies

Exposure to ultraviolet (UV) light gave rise to an increase in antioxidant capacity and a reduction in strawberry, blueberry and mango decay (90-92), and helped maintain
physicochemical properties, antioxidant capacity, and firmness in boysenberry fruit (93). Gamma ray specifically inhibited mold growth under refrigerated storage (94, 95). However, the carcinogenic radioactivity perception of consumers and the possible high price both limited the wide application of irradiated food (96). Refrigeration storage at 2°C with 0.3 ppm ozone treatment destroyed fungi and preserved the color and anthocyanins in thornless blackberries for 12 days (97).

Treatments of fruit using methyl jasmonate (MJ), melaleuca alternifolia (tea-tree oil or TTO) and ethanol (EtOH) successfully prevented the decay, improved antioxidant capacity and radical scavenging activity of ‘Triple Crown’ blackberries (98). Besides, recent study further reported that methyl jasmonate (MJ) treatment on thornless ‘Chester’, ‘Hull’, and ‘Triple Crown’ blackberries remarkably increased flavonoid content, antioxidant and anti-cancer capacities (99). Thus volatile application may be a promising technology to stimulate the increase of nutritional contents and health benefit of blackberries (99).

2.4 Processing of blackberries

2.4.1 Introduction

Due to their short production season and limited shelf-life, fresh blackberries are commonly processed into frozen and dried products which are then applied to confectionary, baking, cereal, dairy, and formulated food industry, or made into canned fruit, juices, jams and jellies for providing diverse blackberry products in the market.
The quality of raw fruit and the specific processing technology directly impact the quality of the final processed products.

The principles and research achievements of the major processing technologies applied to fresh blackberries are summarized in the following sections. The principles of these processing technologies are to destroy microorganisms, reduce water content, and inhibit enzyme activity, thus resulting in safe, high quality, and longer shelf-life products. On the other hand, these processing technologies may decrease the nutritional contents, advance the structural transformation, and diminish biological activities of some nutrients (100).

2.4.2 Freezing

During the freezing process, temperature and water activity of fresh food drop down to decelerate chemical, microbiological, and biochemical reactions. The quality of frozen food is challenged by moisture migration, drip loss, large crystals, flavor sublimation, and solute precipitation which in turn is determined by freezing rate and subsequent storage conditions (101).

Freezing rate is influenced by the characteristics of food samples such as size, surface area, heat conductivity, processing parameters such as temperature and type of freezing methods, and package material (101). The typical freezing methods used in berry processing include air-blast freezing, spiral belt freezing, fluidized bed freezing, and cryogenic freezing (102).
Liquid nitrogen application with high freezing rate had least impact on the structure of frozen wild blackberries compared with static and plate freezing methods (103). González et al. reported that Spanish wild blackberries harvested at commercial ripening stage in 1996 showed significant drop in total anthocyanins, total phenolics, and ellagic acid from 306.26 to 258.84 mg cyanin-3-glu/100 g FW, 977 to 903.61 mg GAE/100 g FW, 25.93 to 19.34 mg/100 g FW, respectively after cryogenic freezing by liquid nitrogen at -80°C, while total anthocyanins and phenolics stayed stable against ellagic acid conversely rallied during the storage of frozen blackberries packed in polyethylene bags for up to 6 months at -24°C (104). Schmidt et al. also pointed out that individual quick freezing (IQF) had good retention of antioxidant activity and total phenolics in North America wild blueberries and cultivated blueberries grown in New Jersey and Michigan (105). In contrast, Ngo et al. found that air-blast freezing removed the glossiness on the surface of six genotypes of strawberries, ‘Ovation’, ‘Totem’, ‘Puget Reliance’, ‘2273-1’, ‘1843-1’, and ‘1723-1’ obtained in Oregon in 2005 (106).

Vacuum impregnation of corn syrup, pectin and minerals has shown to be effective pretreatments prior to freezing for maintaining the firmness and preventing drip loss of frozen blackberries (107).

2.4.3 Canning

Canning is a thermal process for preserving food by destroying pathogenic and spoilage microorganisms during processing and preventing their growth during post-processing storage (108). To perform a successful canning process, numerous factors
have to be considered such as pH of the food, heat resistance of microorganisms, types of container, and storage conditions (108). Canned blackberries are produced by going through several procedures including filling fruit into metal cans, hot-syrup infusion, exhausting, sealing, and applying high heat (109).

A very few studies have addressed the quality of canned blackberries although canned fruit are the traditional shelf-stable products on the market. Hager et al. reported that 22% and 27% of ORAC and 10.5% and 17.8% of total monomeric anthocyanins accompanied with darkening of polymeric color were lost in 40° Brix Sweetose syrup and water canned 2005 shiny black ‘Apache’ blackberries harvested in Arkansas, respectively. Afterwards, a 6 months post-processing storage at 25 °C led to 60.6% and 65.8% losses in total monomeric anthocyanins, respectively, but no significant changes in ORAC were observed (110). Consistent results were found in a similar study on fully ripen ‘Bluecrop’ blueberries harvested in Arkansas in 2005. Brownmiller et al. found that 46% and 42% of ORAC and 28% and 34% of total monomeric anthocyanins accompanied with darkening of polymeric color were lost in blueberries canned in 40° Brix Sweetose syrup and water, respectively right after canning process. The following room temperature storage didn’t change ORAC of the canned blueberries, but huge decline in total monomeric anthocyanins (71% and 62% for canned-in-syrup and canned-in-water blueberries, respectively) was observed (111). Ngo et al. also showed that 68.8% of total anthocaynins and 23.5% of total phenolics in the whole canned strawberries in 20° Brix sucrose syrup were lost after canning and 60 days of room temperature storage (106).
However, Chaovanalikit and Wrolstad indicated that in canned Bing cherries with 19° Brix sucrose syrup, no apparent loss of anthocyanins until storage for 5 months was observed, while total phenolics remarkably increased after processing and during 5 month of storage at both 2°C and 22 °C (112).

2.4.4 Dehydration

Dehydration serves as a food preservation method by releasing bounded water and removing water from tissues of food. The efficiency of dehydration varies with processing conditions such as temperature, air-flow velocity, time, dehydration method, and physical properties of food samples such as shape, dimension, size, surface area, density, and original moisture content (113). Apparent changes include shrinkage, bleeding and solute diffusion, and nutritional loss, which are related to the specific dehydration process. Water and gas resistance, as well as light protection of the package play important roles in the quality of dehydrated food during storage. Commonly used dehydration methods are sun drying, hot-air drying, spray drying, vacuum drying, and freeze drying (114).

Asami et al. reported that freeze drying was comparable to individually quick freezing under air-blast tunnel at -32°C in respect to holding total phenolic in ‘Marion’ blackberry cultivated by conventional, organic, and sustainable practices and grown in Oregon, whereas air-drying significantly lowered total phenolic content from 15.6% to 21.1% compared to frozen controls (58). Yurdugul claimed that freeze-dry was also an
excellent technology for preserving physical, physicochemical, and nutritional qualities of dried ‘alpine’ strawberries (115).

Combinations of multiple dehydration technologies such as microwave-vacuum drying, microwave drying with osmotic dehydration pretreatment, and innovation of new technologies such as sequential infrared radiation and freeze-drying (SIPFD) have become the trend in the research field to improve drying efficiency, reduce drying cost, and assure quality of dried berries (116-118).

### 2.4.5 Jam and jelly processing

Jam and jelly processes use combination of heat, sugar, and acid to kill microorganisms, inactivate enzymes, and lower water activity with the gel formation. Uniform dissolution of ingredients and building up polysaccharides cross-linked network holding water molecules by interaction between pectin and sugars under acidic condition are critical for successful manufacture of jams and jellies. A few operation units such as homogenizing, boiling, filling, and gel-setting are involved in jam and jelly processing (119).

Amakura et al. showed stable total phenolic content (76.23-76.91 mg/100 g fresh fruit) and DPPH radical scavenging activity during blackberry jam processing (120). Häkkinen et al. also found that making strawberry jams exerted a minor effect on quercetin and kaempferol which are two specific flavonoids (121). Nevertheless, a study by Ngo et al. concluded that long-time heat treatment during jam manufacture
was detrimental to the bioactive compounds, leading to an approximate 70% and 20% reduction of total anthocyanin and total phenolic content in ‘Totem’ strawberries (106).

2.4.6 Juice and concentrate

Berry juices supply the market as a liquid form of product. The traditional fruit juice production is charted in Figure2.1 (122). Addition of appropriate enzymes catalyzes cellular collapse and juice release in berry juice production (123). But endogenous polyphenol oxidase is responsible for the loss of polyphenolics throughout all the steps of juice processing (124).

Hager et al. reported that an approximate 67% loss of total monomeric anthocyanins, 55% loss of ORAC value as well as a small increase in polymeric color occurred in juice processing of shiny black ‘Apache’ blackberries (110). And over the 6 months of room temperature storage, ORAC value of blackberry juice showed no significant changes while total monomeric anthocyanins fell by 69-75% (110).

Brownmiller et al. reported consistent results were demonstrated in a similar study on ‘Bluecrop’ blueberries, in which more total monomeric anthocyanins were removed and less polymeric color were formed in blueberry juice clarified by centrifuge compared to non-clarified juice (111).

Skrede et al. revealed that most anthocyanins in frozen ‘Bluecrop’ blueberries were not completely extracted into juice but were enriched in its press-cake. Because of the heat treatment involved in production and enzymatic degradation in juice portion, the leftover anthocyanins were limited in the final juice product (124).
Figure 2.1 Diagrammatic scheme of juice processing (122).
2.4.7 Berry wine

Blackberries have been widely used for fruit wine manufacturing. In the production of blackberry wine, juice is extracted from washed fresh or frozen fruit by milling, crushing, maceration, or pressing with the aid of enzyme and heating treatments, followed by the fermentation of juice with yeast, sugar and acid at 20-25 °C, maturation at 7-15 °C, pasteurization or hot-filling (125). Addition of urea and SO₂ during fermentation is conducted for elevating the fermentation rate and inhibiting the growth of wild yeast. (125). Blending with other sweeteners, flavor compounds, and stabilizers are optional prior to pasteurization and filling for improving the organoleptic characters of blackberry wine (125). Unfortunately, the loss of anthocyanins during blackberry wine production was detected (126).

2.4.8 Other applications of blackberries

On the demand of functional food in the worldwide market and thanks to the advancement of food technology, blackberries have been extensively incorporated into conventional foods based on its recognized health benefits, appealing color, and fantastic flavor, consequently enhanced the nutritional aspects of conventional foods and expanded the diversity of food for consumers.

Dairy products such as yogurts, ice-creams, smoothies, bakery products such as pies, energy bars, muffins, cakes, and cereals are the major food matrix to carry blackberries. Furthermore, blackberries are significant contributors to blended fruit juice popular in the market. Nonetheless, the bioavailability of food products fortified
with blackberries other than the individual blackberry fruit is unknown. A synergistic effect with nutrients has been proposed in the fundamental research (34), but the interactions among different bioactive compounds and between basic food matrix and nutraceticals, as well as their impact on health benefit and bioavailability in blackberry products are likely trends for future research.

2.5 Conclusion

Post-harvest handling and processing technologies are critical for delivering blackberry fruit with high quality and safety from field through processing plant to dining table at home. Although abundant blackberry products are available for satisfying consumers’ demands for tasty and nutritious products, limited information is available about the impacts of post-harvest handling and processing technologies on the quality and nutritional value of blackberries.

Therefore, it is necessary to explore how the basic quality, nutritional contents, and bioactive compounds of blackberries change over refrigeration storage and during common processing procedures and subsequent storage in order to provide consumers with various forms of blackberry products at a maximum nutritional level.
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CHAPTER 3

Effects of Refrigeration Storage and Processing Technologies on the Bioactive Compounds and Antioxidant Capacities of Blackberries (‘Marion’ and ‘Evergreen’)

Ruyi Wu¹, Balz Frei², James A. Kennedy¹, and Yanyun Zhao¹,³

¹Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331, USA.
²Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA.
³Corresponding author: E-mail: yanyun.zhao@oregonstate.edu

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ABSTRACT

Blackberry fruits have attractive color, unique flavor, and contain bioactive compounds associated with a number of health benefits that have been confirmed by scientific evidences. However, their perishable nature and short harvest season make it challenging to preserve their quality. Multiple post-harvest processing technologies have been applied to extend the shelf-life of fresh blackberries. This study investigated the effects of refrigeration storage and different processing technologies including freezing, freeze drying, hot-air drying, canning and jam processing, as well as 6-month subsequent storage at room temperature on the physicochemical properties (pH, titratable acidity (TA), total soluble solids (TSS) and moisture content (MC)), bioactive compounds (total phenolics (TPC) and total monomeric anthocyanins (ACY)), and antioxidant capacities evaluated as radical scavenging activity against DPPH (RSA), oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) of two blackberry varieties, ‘Marion’ and ‘Evergreen’.

Refrigeration storage at 2.0 ± 0.2 °C and 95 ± 2% relative humidity (RH) decreased TA by 36.8% in ‘Marion’ at 9 days and 46.2% in ‘Evergreen’ at 7 days. TPC and ACY fluctuated in ‘Marion’ while continuously declined in ‘Evergreen’ along with refrigeration storage time. Similar to TPC and ACY, RSA fluctuated in ‘Marion’, but was stable in ‘Evergreen’. ORAC decreased by 20% and FRAP increased by 18.75% in ‘Evergreen’ at the end of refrigeration storage, while no significant changes in ‘Marion’ were observed.
TA declined in all processed ‘Marion’. The increases in TSS of dried fruit and jams were observed in both blackberry varieties. Freeze drying increased TPC (21%), ACY (5.5%), and RSA (14%) in ‘Evergreen’ and TPC in ‘Marion’ (27%) compared to frozen control. Hot-air drying resulted in significant (P<0.05) loss of ACY (56%), ORAC (37%), and FRAP (27%) in ‘Marion’, and TPC (37%), ACY (84%), and RSA (13%) in ‘Evergreen’. Canning significantly reduced the content of bioactive compounds by 31% to 70% in both varieties except TPC in canned-in-water ‘Marion’. ORAC and FRAP reduced in canned ‘Marion’ by 21% to 61%. Canning-in-water increased RSA (22% in ‘Marion’ and 8% in ‘Evergreen’) while canning-in-20° Brix sucrose syrup decreased RSA by 31% in both varieties. Jam processing resulted in 67% to 84% loss of TPC and ACY, 78% to 80% loss of RSA, and 65% to 77% loss of ORAC and FRAP in both blackberry varieties.

The 6-month post-process storage at 25 ± 2 °C and 30 ± 2% RH decreased TA of jams by 9% in ‘Marion’ and 12% in ‘Evergreen’, but no significant (P>0.05) changes in TA in canned and dried samples. ACY declined in hot-air dried and canned blackberries from 21% to 57%, and jams from 56% to 66% in both varieties, RSA consistently fell in all frozen, dried, and canned samples from 24% to 86%, ORAC reduced in hot-air dried (13% for ‘Marion’ and 45% for ‘Evergreen’) and canned-in-water samples (68% for ‘Marion’ and 38% for ‘Evergreen’) during room storage.

‘Marion’ had significantly (P<0.05) higher TA, MC, TPC, ACY, RSA and FRAP values than those of ‘Evergreen’, but lower pH, TSS, and ORAC values. TA, TPC,
RSA, and FRAP were positively correlated to each other in both fresh and processed blackberry products.
INTRODUCTION

Berry fruits have been widely recognized as an excellent source of bioactive phenolic compounds including flavonoids, phenolic acid, and tannins (1), providing positive impacts both individually and synergistically against cardiovascular disease, cancer, inflammatory, aging, obesity, diabetes, and other chronic diseases (2-7).

Blackberries have attracted substantial attention nowadays due to their high anthocyanins and ellagitannin content, high antioxidant activities, and favorable flavor demonstrated in numerous studies (8-10). The production of blackberries in the United States was 35,099 tons in 2005, which accounted for the highest portion of the worldwide production of blackberries in 2005 (11). Unfortunately, the fragile surface and high post-harvest respiration rate of blackberries contributes significantly to their quality, nutritional, and microbiological deterioration, resulting in limited shelf-life and diminished health benefits for fresh market (12). Refrigeration storage is the most common practice used for fresh blackberry preservation (13-15).

Due to the short production season and perishable characteristics of blackberries, most fresh fruit is processed into frozen, dried, and canned forms, or processed into jams, jellies, and juices for longer storage to satisfy various markets and consumer demands (16). Freeze processing is generally considered as the least destructive preservation technology for phenolic compounds in berries and is recommended as a pretreatment for manufacturing other berry products, although the physical and nutritional quality of frozen berries can be affected by freezing methods, package materials, storage conditions, and blackberry varieties and maturity stage (17).
Drying is a traditional method used to preserve fresh berries by reducing their water activity. Dried fruit have many different applications in snacks, breakfast, and formulated foods (18). The quality of dried berries is determined by the type of drying method applied, water activity of the final product, and package and storage conditions. For ‘Marion’ blackberry, freeze drying has been shown to retain higher phenolics than that of hot-air drying (19). Freeze drying has also been shown to help preserve anthocyanins and antioxidant capacity of Saskatoon berries (20).

Canning process uses high temperature to destroy microorganisms and creates hermetical sealing (21). Our previous study reported that the canning of Oregon strawberries in syrup increased their total phenolics and anthocyanins substantially (22). A recent study also concluded that canning increased polymeric color, but decreased anthocyanin content and antioxidant capacity of blackberries, while storage at 25 °C did not change antioxidant capacity significantly, but led to the continuous gain of polymeric color and loss of anthocyanins (23).

Berry jams are one of the most important dietary forms of berry fruit consumption (24). Previous studies have indicated that a small portion of flavonols was lost when making strawberry jams via cooking berries with sugar for 30 min (25) while total phenolics were well retained during blackberry jam processing (26).

The extent to which nutraceuticals preservation in blackberry products heavily depends on the specific processing technology, the variety of the blackberries, production location, maturity and time when harvested, and storage conditions (16). Therefore, more comprehensive studies are necessary to understand the impact of
different processing technologies on the nutraceutical benefits of various forms of blackberry products. Such information is essential for processors to better design the processing procedures and for consumers to understand the health benefit of product when purchasing.

The objective of this study was to investigate the changes of total phenolics, total monomeric anthocyanins, and antioxidant capacities in two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage of fresh fruit and when the fruit were subjected to different processing treatments including freezing, drying, canning, jam-making, and subsequent 6-month storage in room condition. Basic physicochemical properties of fresh and processed berries were also investigated.

MATERIALS AND METHODS

Fruit. Two blackberry varieties, ‘Marion’ and ‘Evergreen’, were obtained from Scenic Fruit Co. (Gresham, OR, USA) and RainSweet, Inc. (Salem, OR, USA) in early July and early September, 2007, respectively. Fresh fruit were shipped under refrigeration to the Department of Food Science & Technology (FST) at Oregon State University the day after harvest. For refrigeration storage, fresh fruit were packed in 10 oz (300 g) clam-shell containers and stored at 2.0 ± 0.5 ºC and 95 ± 2% RH at FST pilot plant under dark. For preparing frozen samples, fresh fruit were washed with 20 ppm chlorinated water for 80 sec prior to freezing process. ‘Marion’ fruit were individually frozen on stainless steel trays in an air blast freezer at -23 ºC in the FST pilot plant, while fresh ‘Evergreen’ fruit were individually frozen at the RainSweet facility under
similar conditions. Frozen fruit were packed into 2 L wide-mouth glass jars (Ball Corp., Muncie, IN, USA) with lids and stored at -23 ± 2 °C for up to 6 months (Figure 3.1).

**Figure 3.1** Flow diagram of experimental design. Con: Control (frozen); FD: Freeze drying; HD: Hot-air drying; Can: Canning; Jam: Jam processing.

**Dry processing.** Both freeze and hot-air dryings were applied in this study. For freeze drying, whole fruits were laid on aluminum trays in a freeze drier (Hull Corp., Hatboro, PA, USA) and frozen under a condenser temperature of -45 °C for about 1 h. After the chamber temperature of the drier reached to -45 °C, vacuum was applied for achieving a maximum vacuum pressure of 30 Pa. It took approximately 72 h for the fruit to be fully dried at the shelf temperature (the temperature on the shelf of the
chamber when drying) of 35 °C and 50% RH. For hot-air drying, whole fruit were dried in the MP-2000 Enviro-Pak conventional drier (Division of Tech-Mark, Inc., Clackamas, OR, USA) under a shelf temperature of 50 °C and 50% RH with 50% air speed for 48 h. Dried blackberries were packaged in moisture-resistant plastic Ziploc bags (Johnson & Son, Inc., Racine, WI, USA) and stored in desiccators (Space Saver Vacuum desiccators, Scienceware, Pequannock, NJ, USA) with drierite (anhydrous calcium sulfate, W.A. Hammond drierite company Ltd., Xenia, OH, USA) under dark at 25 ± 2 °C and 30 ± 2% RH for up to 6 months.

**Jam processing.** To make blackberry jams composed of 45% fruit and 55% sugar, frozen blackberries were thawed overnight at room temperature and then crushed in a stainless steel kettle (DN-30 Groen MFG. Co., Chicago, IL, USA). Crushed fruit were weighed and the Brix of the fruit was measured by using a refractometer (RFM81 Multi Scale Automatic, Bellingham+Stanley Inc., Atlanta, Georgia). The pH of ‘Evergreen’ blackberries was titrated (Brinkmann Digital Buret, BRAND, W-Germany) to 3.2 by 1% W/V citric acid (Integra Chemical Company, Renton, Washington, USA). The fruit puree was transferred into a jacketed steam kettle (D-20 Groen MFG. Co., Chicago, IL, USA) heated up to ~49 °C, subsequently mixed with pre-measured amounts of cane sugar (G&H, distributed by Domino Foods Inc., Yonkers, NY, USA) and 150 grade rapid-set pectin (Pacific pectin Inc., Oakhurst, CA, USA) by stirring to avoid clumping. Fruit mixtures were heated to boiling, and the Brix was checked until reaching approximately 68 °Brix. The finished product was hot-filled into pasteurized 400 mL Mason jars with caps (Ball Corp., Muncie, IN, USA). The filled jars were inverted to
pasteurize the lids for 3 min and then allowed to cool and set up in the upright position at room temperature. Blackberry jams were stored in the dark cardboard boxes at 25 ± 2 °C and 30 ± 2% RH for up to 6 months.

**Canning processing.** Approximate 210 g of frozen blackberries were filled into No. 300 plain can (300 X 407, Ball Corp., Muncie, IN, USA) with C-enameled ends. A 180 g of boiling 20 °Brix sucrose syrup or distilled (DI) water were added into the can, leaving ½ inch headspace. The cans with the lids on went through a steam tunnel (Dixie Canner, Athens, Georgia, USA) at around 95 °C and heated up. After coming out of the steam tunnel, the hot cans were immediately sealed by using the Automatic Master-Sealer (Canning devices, Inc., Manitowoc, WI, USA). The sealed cans were placed in boiling water for 15 min and then cooled in cold running water. The temperature of the first can coming out of the steam tunnel and the vacuum of the finished cans were checked by a thermometer (Weston, Model 2261, CSI International Inc., USA) and vacuum gauge (Marshalltown, Model 89011, USA), respectively during the processing. After cooling down, the cans were stored in the dark cardboard boxes at 25 ± 2 °C and 30 ± 2% RH for up to 6 months.

**Measurement of pH, total soluble solids and titratable acidity.** A 20 g of fresh, frozen and canned blackberries and blackberry jams, or 5 g of freeze and hot-air dried blackberries were crushed and homogenized with 180 mL of DI water separately for 1 min by using a 12-speed blender (Osterizer, Jarden Corp., Mexico). Total soluble solids (TSS) of the homogenized solution were measured by a refractometer (RA-250, kem, Kyoto Electronics Manufacturing Co. Ltd, Japan) right after blending. The solution was
then filtered through a filter paper (Qualitative 15 cm Whatman International Ltd. Maidstone, England) and 30 mL of filtered solution was collected for testing pH using a pH meter (IQ240, IQ Scientific Instruments, Inc., San Diego, CA, USA) and titratable acidity (TA) by titrating (Brinkmann Digital Buret, BRAND, W-Germany) with 0.1 N aqueous NaOH (J.T.Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) until a final pH of 8.2 was reached. TA results were expressed as mass percentage of malic acid based on fresh or dried weight of blackberry samples.

Measurement of moisture content. Approximate 10 g of blackberry samples (M_i) (TL-204 Analytical Balance, Denver Instrument Company, city, State, USA) were placed into a pre-weighed glass dish (M_d). Samples were dried in a convection oven (STM 40, Precision Scientific Inc., Chicago, IL, USA) at 70 °C for 6 h and then removed to a vacuum oven (Forma Scientific Inc., Marietta, OH, USA) set at 30 Pa vacuum and 70 °C until fully dry. The final weight (M_f) of dried sample with the dish was measured. Moisture content (MC) on a fresh weight basis was calculated as:

\[
MC = \frac{(M_i + M_d) - M_f}{M_i} \times 100\%
\]

Sample extraction. The extraction of blackberry samples for analyzing bioactive compounds and antioxidant capacity was conducted according to a modified method by Rodriguez -Saona and Wrolstad (27). A 10 g of fresh, frozen, canned blackberries, or 2 g of hot-air and freeze dried sample was powdered under liquid nitrogen in a stainless steel Waring blender (Model 1001, Waring Products Co., Winsted, CT, USA). Fresh, frozen and canned blackberries were extracted by 100% aqueous acetone with 0.01% (v/v) HCl (EMD Chemicals Inc., Gibbstown, NJ, USA) once followed by 70% (v/v)
aqueous acetone with 0.01% (v/v) HCl twice with 0.5 min of ultrasound in an ultrasonic cleaner at 50/60 Hz, 117 V and 185 W (Branson B-220H 19, 6”W X 10”L X 4”D, SmithKline Co., Shelton, CT, USA). The mixtures were centrifuged at 4000g for 5 min (CL International Clinical Centrifuge, International Equipment Co., Boston, MA, USA), the supernatants were gathered in plastic bottles, and 50 mL chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) was added into the bottles to partition the filtrate. After the solvent in bottles was centrifuged at 3000g for 30 min under refrigerated conditions, the chloroform was discarded through the separation funnels (Pyrex, Corning Inc., Lowell, MA, USA) and the aqueous portion was transferred to the boiling flask (Kontes, Kimble Chase Life Science and Research Products, Vineland, NJ, USA). After removing the remaining organic solvents in the extract by rotary evaporator (Brinkmann Instruments, Westbury, NY) under vacuum pressure of 30 Pa and with immersion of the flask with samples in water bath (CMS, Model No: 392-142, Lab-Line Instruments Inc., Melrose park, IL, USA) at 40°C for 1 min, the extract was transferred to a 50 mL volumetric flask (Kontes, Kimble Chase Life Science and Research Products, Vineland, NJ, USA) and then transferred into 2.0 mL microtubes (Axygen Scientific, Union city, CA, USA). Triplicate extractions were performed for each blackberry sample throughout all the experiments.

**Analysis of total phenolic content (TPC).** TPC was determined by the Folin-Ciocalteau colorimetric method (28). A 0.5 mL of diluted sample extract and 0.5 mL of 50, 100, 150, or 200 ppm gallic acid (Sigma Chemical Co. MO, USA) solutions were vortexed with 7.5 mL DI water and 0.5 mL Folin-Ciocalteau reagent (Sigma Chemical
in a series of test tubes, respectively, and a 0.5 mL DI water was used as control. After setting up at room temperature for 10 min, the solutions were mixed with 3 mL of 20% (w/v) Na$_2$CO$_3$ (Sigma Chemical Co. MO, USA) and placed in water bath at 40°C for 20 min. Samples taken out of the water bath were immediately cooled to room temperature in an ice bath for 3 min. The absorbance of the samples and standards were measured spectrophotometrically (UV160U Shimadzu spectrophotometer, Kyoto, Japan) at 765 nm. TPC was calculated as mg of gallic acid equivalents (GAE) per gram fresh weight (FW) or dried weight (DW) of sample.

**Analysis of total monomeric anthocyanins.** Total monomeric anthocyanins were quantified by the pH-differential method (29). The sample extract was diluted in both 0.025 M potassium chloride (EMD Chemicals Inc., Gibbstown, NJ, USA) buffer (pH=1.0) and 0.4 M sodium acetate (EMD Chemicals Inc., Gibbstown, NJ, USA) buffer (pH=4.5), respectively. After equilibration at room temperature for 15 min, the absorbance of two dilutions was analyzed by a UV160U spectrophotometer (Shimadzu Corp., Kyoto, Japan) at both 510 nm and 700 nm. Total monomeric anthocyanins (mg cyanidin-3-glu equivalent/g FW or DW) were calculated according to the equation:

$$\left[ (A_{510} - A_{700})_{pH=1.0} - (A_{510} - A_{700})_{pH=4.5} \right] \times 449.2 \times DF \times 1000 / 26900$$

**Analysis of radical scavenging activity.** The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was carried out to measure the radical scavenging activity of blackberry samples (30). A 0.5 mL of diluted sample extract and 0.004, 0.01, 0.02, 0.03, or 0.04 mg/mL ascorbic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) were mixed with 1 mL of DPPH solvent (Kasel Kogyo Co. Ltd, Tokyo, Japan) in a
series of 2.5 mL disposal cuvettes (PLASTIBRAND, Postfach, Germany). The solution was set at room temperature for 5 min and the absorbance was determined by a spectrophotometer at 517 nm. The absorbance of ascorbic acid was used as standard, and radical scavenging activity of samples was reported as mg of ascorbic acid equivalents (AAE) per gram FW or DW of sample.

**Analysis of antioxidant capacity.** Oxygen radical absorbance capacity (ORAC) assay (31) and ferric reducing ability of plasma (FRAP) assay (32) were conducted to evaluate antioxidant capacity of blackberry samples. To measure ORAC, 30 µl sample (diluted as necessary) and 0, 10, 20, 40 µM Trolox were placed in pre-warmed plates, and then 200 µl pre-warmed PE reagent (solution of β-Phycoerythrin) was added into each plate. After the filled plates were incubated for 1 h at 37 °C, 70 µl AAPH reagent (2,2’-azobis(2-amidino-propane) dihydrochloride, MW = 271.17) was added into each plate to trigger the chemical reaction. The changes in PE fluorescent value of samples were read kinetically and recorded every 2 min and lasted for 2 h. The area of positive changes in the curve was measured as antioxidant capacity. The ORAC values were finally expressed as micromoles of Trolox equivalent (TE) per gram of FW or DW sample. To measure FRAP, 40 µl sample (diluted as necessary) and 0, 62.5, 125, 250, 500 µM Trolox were placed in plates, and a 300 µl of pre-warmed FRAP reagent (30 mL of 300 mM acetate buffer with 3 mL of 10 mM 2,4,6-tri(2-pyridil)-s-triazine solution and 3 mL of 20 mM FeCl₃ solution ) was added into each plate. After the filled plates were incubated for 15 min at 37 °C, the absorbances of solutions were analyzed at
550 nm. The final FRAP values were also reported as micromoles of Trolox equivalent (TE) per gram of FW or DW sample.

**Statistical analysis.** The measurements of all the parameters were completed at every storage time for both blackberry varieties with three replicates. Data analyses were performed by analysis of variance (ANOVA) and general linear model (GLM) using SAS statistical software 9.01 (SAS institute, Cary, NC). Multiple comparisons among the treatments with significant differences tested in ANOVA were conducted by using LSD (least significant difference) at P<0.05.

**RESULTS AND DISCUSSION**

**Effect of refrigeration storage on physicochemical parameters, bioactive compounds and antioxidant capacities of blackberries.** Fresh ‘Marion’ and ‘Evergreen’ blackberries were able to be stored at 2.0 ± 0.2 °C and 96 ± 2% RH for 9 and 7 days, respectively before significant quality deterioration and decay occurred (<10% decay). According to the ANOVA results, refrigeration storage significantly (P<0.05) affected pH, TA, TPC, ACY, and RSA in ‘Marion’, and TSS, TA, TPC, ACY, ORAC, and FRAP in ‘Evergreen’ (Table 3.1).
Table 3.1 ANOVA results of two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage (2.0 ± 0.2 °C, 95 ± 2% RH)

<table>
<thead>
<tr>
<th>Factor</th>
<th>pH</th>
<th>TSS</th>
<th>TA</th>
<th>MC</th>
<th>TPC</th>
<th>ACY</th>
<th>RSA</th>
<th>ORAC</th>
<th>FRAP</th>
</tr>
</thead>
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* =p< 0.05; ** =p< 0.01; *** =p< 0.001; ns= no significance.

TSS: total soluble solids; TA: titratable acidity; MC: moisture content; TPC: total phenolics content; ACY: total monomeric anthocyanins; RSA: radical scavenging activity; ORAC: oxygen radical absorbance capacity; FRAP: ferric reducing ability of plasma.

df: degrees of freedom; SS: sum of squares; MS: mean square; F: calculated F-value; Pr: probability.
The pH value of ‘Marion’ increased from initial 3.20 to 3.33 while TA declined from initial 2.50% to 1.58% after 9 days of storage (Figure 3.2). Meanwhile, TA of ‘Evergreen’ decreased 46.15% during the 7 days of storage (P<0.05), from initial 0.52% to 0.28% (Figure 3.2). These results can be explained that organic acids were consumed due to fruit respiration and exposure to light during post-harvest storage. TSS of ‘Marion’ didn’t change (P>0.05) throughout the refrigeration storage, same as the ‘Evergreen’ after 3 days (Figure 3.2), indicating that the sugar gaining during fruit ripening may compensate for the lost sugar, used as a substrate for fruit respiration (33-35).
Figure 3.2 Changes of selected physicochemical properties of two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage (2.0 ± 0.2 °C, 95 ± 2% RH).
Distinctive trends on TPC were observed in two blackberry varieties during refrigeration storage (Figure 3.3). In ‘Marion’, TPC didn’t change at the 1st day of storage (57.57-55.27 mg GAE/g DW), rose significantly by the 3rd day (66.38 mg GAE/g DW), but decreased to 49.38 mg GAE/g DW at the 9th day. In ‘Evergreen’, TPC was reduced 27.72% at the 3rd day of storage (P<0.05), but no significant change during the rest of storage, was 22.49 mg GAE/g DW at day 7. These different patterns may attribute to the diverse constitutions or proportions of phenolic compounds in two varieties, as well as different polyphenoloxidases activities that are essential to the phenolics degradation in fruit (33, 35).

ACY showed similar changes as TPC during refrigeration storage (Figure 3.3). In ‘Marion’, ACY decreased from 11.02 to 9.39 mg cyanidin-3-glu equivalent/g DW at the 1st day, but increased at the 2nd day, and then fluctuated afterwards, reached the lowest number of 9.17 mg cyanidin-3-glu equivalent/g DW at day 9 (P<0.05). ACY in ‘Evergreen’ increased 13.73% after the 1st day of storage (P<0.05), but decreased continuously during the rest of storage, had a 25% reduction from day 1 to day 7 (P<0.05). According to Kalt et al. (36), ambient temperature at some extent promoted the formation of anthocyanins, and the stability of anthocyanins was impacted by pH, light, enzymes and even fruit braisins (37). The ACY in both blackberry varieties declined at the end of refrigeration storage probably because the elevated pH during storage accelerated the degradation of anthocyanins and the refrigeration temperature was too low to accumulate anthocyanins (36).
With respect to antioxidant capacity, RSA in ‘Marion’ showed a similar trend as ACY (Figure 3.4), suggesting that anthocyanins probably had great contribution to radical scavenging activity against DPPH radicals during refrigeration storage. No change in RSA for ‘Evergreen’ was observed. No changes in ORAC for ‘Evergreen’ during the first 5 days of storage (278.61-302.26 umol TE/g DW), but a 20.08% decrease during the last 2 days (day 5 to 7) (P<0.05), while the FRAP values of ‘Evergreen’ increased 13.72% during the 1st day of storage, and continuously increased during the rest of storage, reached to 241.86 umol TE/g DW at day 7, 18.75% higher than the value at day 0 (P<0.05) (Figure 3.4). For ‘Marion’, no significant changes (P>0.05) in ORAC and FRAP were observed during refrigeration storage with a mean value of 174.59 umol TE/g DW and 757.09 umol TE/g DW, respectively (Figure 3.4).

In previous studies, no significant change in ORAC was reported for blackberries stored at 2 °C (13), neither for blueberries stored above 10 °C for 8 days (36), but significant increases in ORAC and anthocyanins were found in strawberries and raspberries stored at room temperature (36). Therefore, increasing the post-harvest storage temperature may potentially promote the synthesis of anthocyanins and other bioactive compounds.
Figure 3.3 Changes of bioactive compounds in two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage ($2.0 \pm 0.2^\circ C$, $95 \pm 2\%$ RH).
Figure 3.4 Changes of antioxidant capacities in two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage (2.0 ± 0.2 °C, 95 ± 2% RH).
Effect of processing treatments on physicochemical parameters, bioactive compounds and antioxidant capacities of blackberries. Overall, processing technologies and post-processing storage at room temperature significantly \((P<0.05)\) affected the measured physicochemical parameters, bioactive compounds and antioxidant capacities of both blackberry varieties (Table 3.2).
Table 3.2 ANOVA results of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2 °C, 30 ± 2% RH)

<table>
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</tr>
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<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

| Storage Time           |                           |                            |
| df                    | 2                          | 5                          |
| SS                    | 0.002                      | 8.43                       |
| MS                    | 0.0008                     | 1.69                       |
| F                     | 0.26                       | 36.57                      |
| Pr>F                 | 0.77                       | <0.0001                    |

TSS: total soluble solids; TA: titratable acidity; MC: moisture content; TPC: total phenolics content; ACY: total monomeric anthocyanins; RSA: radical scavenging activity; ORAC: oxygen radical absorbance capacity; FRAP: ferric reducing ability of plasma. df: degrees of freedom; SS: sum of squares; MS: mean square; F: calculated F-value; Pr: probability.
Comparing with frozen samples, pH increased in hot-air dried (8%) and canned ‘Marion’ (4% and 9% in canned-in-water and canned-in-syrup, respectively), and TA decreased in processed ‘Marion’, with the greatest loss (75%) in jam, followed by canned-in-syrup (69%), canned-in-water (42%), hot-air dried (29%), and freeze dried (15%) (Figure 3.5). Whereas no significant changes in TA and pH of hot-air dried and canned ‘Evergreen’, a 53% increase in TA of freeze dried ‘Evergreen’ was observed. Besides, jam processing of ‘Evergreen’ resulted in a 32% decrease and 60% increase in pH and TA, respectively, probably due to the addition of citric acid during jam processing.

Blackberry jams had 460% and 400% higher TSS than frozen ‘Marion’ and ‘Evergreen’ respectively (Figure 3.5), right after processing due to the addition of sugar and water evaporation during cooking. Drying removed most of the water from fresh berries, resulting in 340% and 424% increase in TSS of freeze-dried ‘Marion’ and ‘Evergreen’, and 306% and 189% increase in hot-air dried ‘Marion’ and ‘Evergreen’, respectively (Figure 3.5). Canned-in-water ‘Marion’ and ‘Evergreen’ had 38% and 53% lower TSS than frozen samples, respectively, possibly due to the diffusion of water from canning solution to the fruit and leaching of the solutes from fruit to the solution, which was confirmed by the higher moisture content of canned-in-water blackberries compared to frozen samples (Figure 3.5). The combined effects of water diffusion and sugar addition in canned-in-syrup products led to a 39% increase in TSS for ‘Marion’, but no significant change in TSS for ‘Evergreen’ was observed (P>0.05).
Figure 3.5 Physicochemical properties of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2°C, 30 ± 2% RH) (FC: Frozen control; FD: freeze dried blackberries; HD: Hot-air dried blackberries; CW: Canned-in-water blackberries; CS: Canned-in-sucrose syrup blackberries; Jam: Blackberry jam).
Figure 3.5 Physicochemical properties of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2°C, 30 ± 2% RH) (FC: Frozen control; FD: freeze dried blackberries; HD: Hot-air dried blackberries; CW: Canned-in-water blackberries; CS: Canned-in-sucrose syrup blackberries; Jam: Blackberry jam) (Continued).
Processing technologies showed different effects on the bioactive compounds of the two blackberry varieties (Figure 3.6). Freeze drying, recognized as the best technology to make high-quality dried products (38), increased TPC of ‘Marion’ and ‘Evergreen’ 27% and 21% on a dry weight basis, respectively. Freeze drying also increased ACY 5.5% in ‘Evergreen’, but decreased ACY 25% in ‘Marion’. Although the enzymes in blackberries were inactivated by freezing at low temperature, they may recover during drying at 35 °C shelf temperature, thus accelerating the degradation of monomeric anythocyanins and the polymerization with tannins, polysaccharides and other flavonoids (39, 40), which may explain the observed losses of ACY in ‘Marion’. The destruction of anthocyanins in freeze dried ‘Marion’ was not as high as that in hot-air dried fruit because the low temperature and vacuum in freeze drying protected the fruit phenolics from oxidation. On the other hand, fruit cell destruction during freezing and ice sublimation contributed to the extraction of anthocyanins (19), which possibly led to the observed increase (5.5%) in anthocyanins in ‘Evergreen’ and explained the higher TPC in freeze dried blackberries than that in frozen controls. Moreover, different compositions of anthocyanins in the two blackberry varieties could be another plausible reason for the varying contents of anthocyanins observed on freeze dried samples (41).

In contrast, hot-air drying promoted the oxidation and condensation of phenolic compounds (19), resulting in 56% and 84% reduction in ACY of ‘Marion’ and ‘Evergreen’, respectively (Figure 3.6). The higher loss of TPC (37%) was found in hot-air dried ‘Evergreen’ than in ‘Marion’, probably due to the smaller size and larger surface area of ‘Evergreen’ than those of ‘Marion’.
Canned-in-water fruit had 47% and 35% reduction in ACY in ‘Marion’ and ‘Evergreen’, respectively, and 31% decrease in TPC in ‘Evergreen’ compared with frozen controls, while canned-in-syrup had 36% and 54% TPC reduction in ‘Marion’ and ‘Evergreen’, respectively, and 70% ACY reduction in both varieties (Figure 3.6). These results may be attributed to the thermal degradation of ACY and preferential dissolution of ACY into canning solution (23). Anthocyanins are the predominant phenolic compounds in blackberries, it is expected that TPC had a similar trend as anthocyanins. However, less deduction of TPC than that of ACY in both varieties was observed, probably due to the undergoing polymerization of monomeric anthocyanins which produced polymers detected as phenolics and consumed large amount of monomeric anthocyanins during canning process (23).

Blackberry jams showed the lowest TPC and ACY among all processed products, only 33% of TPC and 20% of ACY retained comparing with frozen controls (Figure 3.6). This may be explained as condensation and polymerization of phenolic compounds were accelerated after cellular crush and long-time cooking of the fruit under the exposure to oxygen and heat during jam processing (25).
Figure 3.6 Bioactive compounds of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2 °C, 30 ± 2% RH) (FC: Frozen control; FD: freeze dried blackberries; HD: Hot-air dried blackberries; CW: Canned-in-water blackberries; CS: Canned-in-sucrose syrup blackberries; Jam: Blackberry jam).
Different processing technologies showed different impacts on the antioxidant capacities of the blackberries (Figure 3.7). Freeze drying didn’t change RSA, ORAC and FRAP of the fruit except 14% increase in RSA of ‘Evergreen’. Hot-air dry resulted in 37% and 27% decrease in ORAC and FRAP of ‘Marion’, respectively, and 13% decrease in RSA of ‘Evergreen’. Antioxidant capacity was also remarkably reduced in canned-in-syrup fruit with 31% loss in RSA and 50-60% loss in ORAC of both varieties and 58% loss in FRAP of ‘Marion’. Canning-in-water increased RSA of ‘Marion’ 22%, decreased ORAC and FRAP of ‘Marion’ 34% and 21%, respectively, whereas increased RSA and FRAP of ‘Evergreen’ 8% and 110%, respectively. Although the degradation of phenolics theoretically diminishes antioxidant capacity of heat-treated blackberries, some polymers formed through Maillard reaction during thermal process may possibly compensate or even increase the ability against certain type of radicals (42). Similar to TPC and ACY, the greatest loss of antioxidant capacity was detected in jams with about 20% RSA, 20%-30% ORAC, and 30-35% FRAP remained in finished jams compared to frozen controls.
Figure 3.7 Antioxidant capacities of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2 °C, 30 ± 2% RH) (FC: Frozen control; FD: freeze dried blackberries; HD: Hot-air dried blackberries; CW: Canned-in-water blackberries; CS: Canned-in-sucrose syrup blackberries; Jam: Blackberry jam).
Figure 3.7 Antioxidant capacities of two blackberry varieties (‘Marion’ and ‘Evergreen’) after processing and during room temperature storage (25 ± 2°C, 30 ± 2% RH) (FC: Frozen control; FD: freeze dried blackberries; HD: Hot-air dried blackberries; CW: Canned-in-water blackberries; CS: Canned-in-sucrose syrup blackberries; Jam: Blackberry jam) (Continued).
Effect of post-processing storage on physicochemical parameters, bioactive compounds and antioxidant capacities of blackberries. During post-process room storage at 25 ± 2 °C and xx RH, significant changes in pH were only observed in jams and canned-in-syrup fruit in both blackberry varieties (Figure 3.5). The pH of the jams increased by 6% and 10% in ‘Marion’ and ‘Evergreen’, respectively, while the pH of canned-in-syrup blackberries rose by 9% in ‘Evergreen’, fell by 3.5% in ‘Marion’ at the end of 6 month storage. A 16% decrease in TA and 20% increase in TSS of ‘Marion’ over 6 months of frozen storage at -23 °C were observed (Figure 3.5), possibly caused by the temperature fluctuation in the freezer and oxidation of the fruit. After 6 months of room storage, TA of ‘Marion’ and ‘Evergreen’ jams lost 9% and 12%, respectively, TSS in canned-in-water and canned-in-sugar ‘Marion’ decreased by 5% and increased by 4%, respectively (Figure 3.5), as a result of water and sugar penetrated into the fruit, and TSS of hot-air dried ‘Marion’ was down 17%, while up 20% in freeze dried ‘Marion’. No significant changes in TA of canned and dried fruits (both varieties) were observed during room storage (Figure 3.5). Moisture content of canned blackberries slightly increased over 6-month of room storage, by 1.1% for canned-in-water samples for both varieties and 1.3% for canned-in-syrup ‘Marion’ (P<0.05) attributing from gradual water penetration to canned fruit immersed in the syrup. Moisture content in dried samples was continuously lost by 13% in hot-air dried ‘Marion’ and 35% in hot-air dried ‘Evergreen’, and 15% in freeze-dried ‘Evergreen’ at the end of 6-month storage (Figure 3.5), suggesting the water loss of samples through package.
Canned fruit (both varieties) showed increased TPC during room storage, where TPC of canned-in-water ‘Evergreen’ increased 40% and that of canned-in-syrup ‘Marion’ increased 22% at the end of 6-month storage (Figure 3.6). This is partly because of the destroyed fruit cellular structure after long-term immersing in canning solution, making the phenolics extraction easier (25, 43). At the end of 6-month room storage, ACY was significantly destroyed in all thermally processed ‘Marion’ and ‘Evergreen’, including jams (56%, 66%), canned-in-water (21%, 57%), canned-in-syrup (42%, 53%), and hot-air dried (48%, 24%) (Figure 3.6), as a result of ACY’s condensation and complexion with other chemical compounds accelerated by heat-stable peroxidase in shelf-stable products under room temperature (23).

RSA fell down significantly in all blackberry samples except jams, 39% and 82% reduction in frozen, 29% and 53% in freeze dried, 47% and 86% in hot-air dried, 62% and 24% in canned-in-water, and 49% and 32% in canned-in-syrup ‘Marion’ and ‘Evergreen’ after 6-month room storage, respectively (Figure 3.7). These results could be explained by the bioactive compounds against DPPH free radicals were possibly sensitive to oxygen and gradually leached into the canning solution during room storage. ORAC decreased 13% and 45% in hot-air dried, and 68% and 38% in canned-in-water ‘Marion’ and ‘Evergreen’, respectively (Figure 3.7). Finally, there was 18% decrease in FRAP of frozen ‘Marion’, 57% loss in ‘Marion’ jams, 34% decrease in canned-in-syrup ‘Evergreen’, whereas 28% increase in canned-in-water ‘Marion’ and 17% increase in freeze dried ‘Evergreen’ (Figure 3.7).
Comparison of our results about fresh blackberries with previously reported data. Table 3.3 reported the mean values of physicochemical parameters and bioactive compounds of two blackberry varieties prior to cold storage.
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</table>

Data are expressed as means ± standard deviation (n=3).

TSS: total soluble solids, %; TA: titratable acidity, g malic acid/100g FW; MC: moisture content, %; TPC: total phenolics content, mg GAE(Gallic acid equivalent/g DW); ACY: total monomeric anthocyanins, mg cyanidin-3-glu equivalent/g DW; RSA: radical scavenging activity, mg AAE(Ascorbic acid equivalent)/g DW; ORAC: oxygen radical absorbance capacity, umol TE (Trolox equivalent)/g DW; FRAP: ferric reducing ability of plasma, umol TE (Trolox equivalent)/g DW.
The pH and TSS of ‘Marion’ (3.16, 12.85 %) and ‘Evergreen’ (4.40, 17.85%) (Table 3.3) were in good agreement with those of ripe ‘Marion’ and ‘Evergreen’ reported by Reyes-Carmona et al. (44) and Siriwoharn et al. (8) (3.13 and 13.5%, and 4.28 and 15.7 %, respectively), while our TSS of ‘Evergreen’ was much higher than that in Greece (11.5 %) (45). TA of ‘Marion’ (2.45%) reported as percent of malic acid equivalent in this study (Table 3.3) was significantly higher than 1.28% for ripe ‘Marion’, similar to 2.32% for under-ripe ‘Marion’ unveiled as percent of citric acid equivalent reported by Siriwoharn et al. (8), and our TA of ‘Evergreen’ (0.52% malic acid equivalent) was close to 0.47% citric acid equivalent for overripe fruit reported by the same author (8).

TPC in ‘Marion’ at harvest, 10.35 mg GAE/g FW or 57.57 mg GAE/g DW (Table 3.3), was consistent with the values of 10.05 mg GAE/g FW for blackberries harvested in 2002 (8), but higher than 8.44 mg GAE/g FW for the same variety of fruit harvested in 1999 (9). However, the mean TPC of ‘Evergreen’, 7.97 mg GAE/g FW or 31.46 mg GAE/g DW was nearly 20% lower than that reported by Siriwoharn et al. (8), 9.60 mg GAE/g FW for ripe fruit, but 50% higher than that measured in Greece in ‘Evergreen’ (2007) at 20.61 mg GAE/g DW (45).

ACY of ‘Marion’ at the harvest from this study (1.98 mg cyd-3-glu equivalent/g FW or 11.02 mg cyd-3-glu equivalent/g DW) (Table 3.3) was slightly lower than that of ‘Marion’ reported by Siriwoharn et al. (8, 9), 2.21 and 2.25 mg cyd-3-glu equivalent/g FW, while ‘Evergreen’ (1.92 mg cyd-3-glu equivalent/g FW or 7.58 mg cyd-3-glu
equivalent/g DW) had higher value than that of the same variety reported by Siriwoharn et al. (8, 9), 1.31 and 1.54 mg cyd-3-glu equivalent/g FW.

ORAC in ‘Marion’ at the harvest, 41.67 umol TE/g FW or 231.57 umol TE/g DW (Table 3.3), was about two third of that reported by Siriwoharn et al. (8), 60.9 umol TE/g FW for ripe fruits, but still significantly higher than 20.3-24.6 umol TE/g FW of fruit juices from other varieties (‘Chester thornless’, ‘Hull thornless’, ‘Triple Crown’) (46). ORAC in ‘Evergreen’ at harvest, 75.43 umol TE/g FW or 297.87 umol TE/g DW (Table 3.3), was significantly higher than that in ‘Marion’ in this study, and coincided with the number reported by Siriwoharn for the same variety (8), 75.5 umol TE/g FW.

As opposite to ORAC values, FRAP of ‘Evergreen’ (51.57 umol TE/g FW or 203.66 umol TE/g DW) (Table 3.3) was significantly lower than that of ‘Marion’ (147.38 umol TE/g FW, 819.46 umol TE/g DW) (Table 3.3), and both of them ran out of the range of 63.5-97.3 umol TE/g FW in several selected varieties of blackberries reported by Siriwoharn et al. (8).

The physicochemical properties and bioactive compounds of fresh blackberries are influenced by the fruit variety, genotype, maturity stage, harvest location and time, environmental condition and practice technology of cultivation, extraction procedures and solvents, and even the testing assays based on distinctive mechanisms. For example, ORAC_{PE} is considered to underestimate antioxidant capacity compared to ORAC_{FL} because of the binding effect between B-PE and polyphenolics (47, 48). This may explain the ORAC_{PE} values of ‘Marion’ and ‘Evergreen’ from this study (231.57 umol TE/g DW and 297.87 umol TE/g DW, respectively) were significantly lower than
the ORAC\textsubscript{FL} values of the same varieties harvested in Oregon (423.0 umol TE/g DW and 366.8 umol TE/g DW) reported by Reyes-Carmona et al. (44).

**Comparison of our results about processed fruit with previously reported data.** Total monomeric ACY of frozen (2.48 mg/g FW), canned-in-syrup (2.21 mg/g FW) and canned-in-water ‘Apache’ blackberries (2.04 mg/g FW) right after processing reported by Hager et al. (23) were significantly higher than those of ‘Marion’ (1.42, 0.54, 0.52 mg/g FW) and ‘Evergreen’ blackberries (1.32, 0.47, 0.61 mg/g FW) from this study (Figure 3.6). Canning process resulted in about 60% loss of ACY in ‘Marion’ and ‘Evergreen’ while minor loss in ‘Apache’ blackberries. Throughout 6-month storage, total monomeric ACY was stable in both frozen ‘Marion’ (1.42-1.42 mg/g FW) and ‘Evergreen’ (1.30-1.33 mg/g FW) (Figure 3.6), whereas degraded 25%-44% in canned ‘Marion’ and 52%-62% in ‘Evergreen’ (Figure 3.6), which was consistent with data reported by Hager et al. (23), in which were 65.8% and 60.6% losses in canned-in-syrup and canned-in-water ‘Apache’ blackberries, respectively. Similar to ACY, ORAC of frozen (97.2 umol TE/g FW), canned-in-syrup (75.8 umol TE/g FW), and canned-in-water ‘Apache’ blackberries (71.1 umol TE/g FW) right after processing (23) were significantly higher than those of ‘Marion’ (70.17, 34.65, 32.42 umol TE/g FW) and ‘Evergreen’ blackberries (54.88, 31.95, 43.67 umol TE/g FW) from this study (Figure 3.7). Significant losses of ORAC were detected in canned-in-water ‘Marion’ (68%) and ‘Evergreen’ (38%) (Figure 3.7), but not in ‘Apache’ blackberries (23) at the end of room temperature storage.
Our results revealed that the initial TPC of frozen ‘Marion’ blackberries was about 5.00 mg GAE/g FW and showed comparable amount to hot-air dried samples (Figure 3.6). However, TPC of frozen ‘Marion’ (4.00 mg GAE/g FW) reported by Asami et al. (19) was lower than our results and the value decreased significantly after hot-air drying.

**Comparison between ‘Marion’ and ‘Evergreen’ blackberries.** Two blackberry varieties evaluated in this study showed statistical differences in all measured physicochemical and nutraceutical parameters (Table 3.4).
Table 3.4 Mean comparison of physicochemical parameters, bioactive compounds and antioxidant capacities of two blackberry varieties (‘Marion’ and ‘Evergreen’) throughout refrigeration storage (2.0 ± 0.2 °C, 95 ± 2% RH)

<table>
<thead>
<tr>
<th>Variety</th>
<th>pH</th>
<th>TSS</th>
<th>TA</th>
<th>MC</th>
<th>TPC</th>
<th>ACY</th>
<th>RSA</th>
<th>ORAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marion</td>
<td>3.19</td>
<td>±0.10 b</td>
<td>±0.33 a</td>
<td>±0.01 a</td>
<td>±6.51 a</td>
<td>±0.74 a</td>
<td>±7.96 a</td>
<td>±51.90 b</td>
<td>±140.61 a</td>
</tr>
<tr>
<td></td>
<td>±0.10 b</td>
<td>±0.33 a</td>
<td>±0.01 a</td>
<td>±6.51 a</td>
<td>±0.74 a</td>
<td>±7.96 a</td>
<td>±51.90 b</td>
<td>±140.61 a</td>
<td></td>
</tr>
<tr>
<td>Evergreen</td>
<td>4.42</td>
<td>±0.89 a</td>
<td>±0.10 b</td>
<td>±0.01 b</td>
<td>±4.80 b</td>
<td>±0.78 b</td>
<td>±1.42 b</td>
<td>±29.50 a</td>
<td>±14.43 b</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard deviation (n=3); Values with the same letters are not significant different (LSD, p< 0.05).

TSS: total soluble solids, %; TA: titratable acidity, g malic acid/100g FW; MC: moisture content, %; TPC: total phenolics content, mg GAE (Gallic acid equivalent/g DW); ACY: total monomeric anthocyanins, mg cyanidin-3-glu equivalent/g DW; RSA: radical scavenging activity, mg AAE (Ascorbic acid equivalent)/g DW; ORAC: oxygen radical absorbance capacity, umol TE (Trolox equivalent)/g DW; FRAP: ferric reducing ability of plasma, umol TE (Trolox equivalent)/g DW.
‘Marion’ had significantly (P<0.05) higher TA, MC, TPC, ACY, RSA and FRAP values than those of ‘Evergreen’, but lower pH, TSS, and ORAC values. The pH and TSS of ‘Evergreen’ was about 130% higher than those of ‘Marion’, while TA of ‘Marion’ was about 500% higher than that of ‘Evergreen’ (Table 3.4). These differences can lead to the different flavors of the two varieties: ‘Marion’ tasted sour, while ‘Evergreen’ was sweet. More seeds in ‘Evergreen’ would be another possible reason for its high TSS value (9). According to Mitcham (49), Mazza and Miniati (50), the lower pH that is favorable to form and stabilize ACY is probably responsible for the higher ACY content in ‘Marion’ than that in ‘Evergreen’. The higher ACY content of ‘Marion’ was corresponded to its higher TPC than those of ‘Evergreen’, which confirmed the results by Siriwoharn and Wrolstad (9). Scavenging activity against DPPH radicals and FRAP in plasma in ‘Marion’ were about 500% and 300% higher than those in ‘Evergreen’, respectively, possibly because ‘Marion’ contains more antioxidants, such as ascorbic acid that is capable of reducing DPPH radicals and ferric at low pH (51). Whereas, the smaller fruit size and larger surface area of ‘Evergreen’ probably resulted in 160% higher ORAC than those of ‘Marion’ (52). Last but not least, slightly higher moisture content was observed in ‘Marion’ (82%) than that in ‘Evergreen’ (76%), making ‘Marion’ juicier (Table 3.4).

The means of all parameters measured in all the processed blackberry products were compared statistically (Table 3.5).
Table 3.5 Mean comparison of physicochemical parameters, bioactive compounds, and antioxidant capacities of processed blackberry (‘Marion’ and ‘Evergreen’) during room temperature storage (25 ± 2 °C, 30 ± 2% RH)

<table>
<thead>
<tr>
<th>Variety</th>
<th>pH</th>
<th>TSS</th>
<th>TA</th>
<th>MC</th>
<th>TPC</th>
<th>ACY</th>
<th>RSA</th>
<th>ORAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marion</td>
<td>3.23</td>
<td>33.49</td>
<td>6.54</td>
<td>0.49</td>
<td>27.33</td>
<td>4.01</td>
<td>27.52</td>
<td>240.30</td>
<td>513.86</td>
</tr>
<tr>
<td></td>
<td>±0.10 b</td>
<td>±23.21 a</td>
<td>±2.89 a</td>
<td>±0.35 a</td>
<td>±9.99 a</td>
<td>±2.56 a</td>
<td>±12.08 a</td>
<td>±123.64 a</td>
<td>±217.44 a</td>
</tr>
<tr>
<td>Evergreen</td>
<td>4.12</td>
<td>31.97</td>
<td>2.00</td>
<td>0.48</td>
<td>23.23</td>
<td>3.52</td>
<td>18.20</td>
<td>209.51</td>
<td>301.91</td>
</tr>
<tr>
<td></td>
<td>±0.45 a</td>
<td>±23.74 a</td>
<td>±0.79 b</td>
<td>±0.36 a</td>
<td>±8.67 b</td>
<td>±3.00 a</td>
<td>±10.85 b</td>
<td>±101.59 a</td>
<td>±131.03 b</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard deviation (n=3); Values with the same letters are not significant different (LSD, p< 0.05).
TSS: total soluble solids, %; TA: titratable acidity, g malic acid/100g DW; MC: moisture content, %;
TPC: total phenolics content, mg GAE (Gallic acid equivalent/g DW); ACY: total monomeric anthocyanins, mg cyanidin-3-glu equivalent/g DW; RSA: radical scavenging activity, mg AAE (Ascorbic acid equivalent)/g DW; ORAC: oxygen radical absorbance capacity, umol TE (Trolox equivalent)/g DW; FRAP: ferric reducing ability of plasma, umol TE (Trolox equivalent)/g DW.
‘Marion’ had significantly lower pH and higher TA than ‘Evergreen’ after processing and during room temperature storage (P<0.05) (Table 3.5). TSS and MC of processed two blackberry variety products didn’t show differences as seen in fresh fruit during refrigeration storage (Table 3.4). Means of TPC, RSA, and FRAP in ‘Marion’ products over room storage were nearly 46%, 35%, and 68% of those in fresh ‘Marion’ over refrigeration storage, but still showed significantly (P<0.05) higher values than that of processed ‘Evergreen’ products (Table 3.5). There were no significant differences between two varieties in means of ACY and ORAC of processed blackberry products observed over room storage, but about 40% and 50% of ACY in fresh ‘Marion’ and ‘Evergreen’ were remained in processed ‘Marion’ and ‘Evergreen’ products, respectively over room storage (Table 3.5).

**Correlation among measured physicochemical parameters, bioactive compounds and antioxidant capacities.** Correlation coefficients among the measured physicochemical parameters, bioactive compounds, and antioxidant capacities in two blackberry varieties during refrigeration and room temperature storage are presented in Tables 3.6 and 3.7, respectively.
Table 3.6 Correlation coefficients for physicochemical parameters and bioactive compounds measured in two blackberry varieties (‘Marion’ and ‘Evergreen’) during refrigeration storage (2.0 ± 0.2 °C, 95 ± 2% RH)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TSS</th>
<th>TA</th>
<th>MC</th>
<th>TPC</th>
<th>ACY</th>
<th>RSA</th>
<th>ORAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td>0.85***</td>
<td>-0.97***</td>
<td>-0.94***</td>
<td>-0.95***</td>
<td>-0.87***</td>
<td>-0.96***</td>
<td>0.75***</td>
<td>-0.91***</td>
</tr>
<tr>
<td>TSS</td>
<td>-</td>
<td>1</td>
<td>-0.91***</td>
<td>-0.87***</td>
<td>-0.84***</td>
<td>-0.79***</td>
<td>-0.89***</td>
<td>0.71***</td>
<td>-0.9***</td>
</tr>
<tr>
<td>TA</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.95***</td>
<td>0.95***</td>
<td>0.89***</td>
<td>0.97***</td>
<td>-0.74***</td>
<td>0.94***</td>
</tr>
<tr>
<td>MC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.95***</td>
<td>0.91***</td>
<td>0.97***</td>
<td>-0.80***</td>
<td>0.9***</td>
</tr>
<tr>
<td>TPC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.91***</td>
<td>0.94***</td>
<td>-0.76***</td>
<td>0.9***</td>
</tr>
<tr>
<td>ACY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.9***</td>
<td>-0.63***</td>
<td>0.82***</td>
</tr>
<tr>
<td>RSA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-0.77***</td>
<td>0.92***</td>
</tr>
<tr>
<td>ORAC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-0.65***</td>
</tr>
<tr>
<td>FRAP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

* = p< 0.05; ** = p< 0.01; *** = p< 0.001; ns = no significance.

TSS: total soluble solids; TA: titratable acidity; MC: moisture content; TPC: total phenolics content; ACY: total monomeric anthocyanins; RSA: radical scavenging activity; ORAC: oxygen radical absorbance capacity; FRAP: ferric reducing ability of plasma.
### Table 3.7 Correlation coefficients for physicochemical parameters and bioactive compounds measured in processed blackberry (‘Marion’ and ‘Evergreen’) during room temperature storage (25 ± 2°C, 30 ± 2% RH)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TSS</th>
<th>TA</th>
<th>MC</th>
<th>TPC</th>
<th>ACY</th>
<th>RSA</th>
<th>ORAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.31**</td>
</tr>
<tr>
<td>TSS</td>
<td>-0.27**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.36***</td>
</tr>
<tr>
<td>TA</td>
<td>-0.71***</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>0.12</td>
<td>-0.86***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC</td>
<td>0.0003</td>
<td>-0.23*</td>
<td>0.53***</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACY</td>
<td>0.04</td>
<td>-0.12</td>
<td>0.48***</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSA</td>
<td>-0.19</td>
<td>-0.22*</td>
<td>0.58***</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAC</td>
<td>0.01</td>
<td>-0.07</td>
<td>0.55***</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>-0.31**</td>
<td>-0.07</td>
<td>0.77***</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = p< 0.05; ** = p< 0.01; *** = p< 0.001; ns = no significance.

TSS: total soluble solids; TA: titratable acidity; MC: moisture content; TPC: total phenolics content; ACY: total monomeric anthocyanins; RSA: radical scavenging activity; ORAC: oxygen radical absorbance capacity; FRAP: ferric reducing ability of plasma.
Results showed that TSS had positive correlation to pH (r=0.85), but negative to TA (r= -0.91) (Table 3.6), demonstrating the typical physicochemical changes with acid converting to sugar in fruit during post-harvest storage (33). TA was positively, while pH was negatively correlated to ACY, TPC, RSA and FRAP of fresh blackberries during refrigeration storage (Table 3.6), and TA also correlated to TPC (r=0.53), RSA (r=0.58), ORAC (0.55) and FRAP (0.77) of processed blackberry products during room temperature storage (Table 3.7), indicating the low pH and high acidic environment stabilizing ACY. Moisture content of processed blackberry products was negatively related to TSS during room temperature storage (Table 3.7), which could be explained by the loss of water corresponded with increase of soluble solids in the fruits.

FRAP, RSA and TPC were positively correlated in both fresh and processed blackberries because all these 3 assays were based on the electron transfer reaction to quantify the reduction capacity of samples against Ferric (Fe III), DPPH radical and F-C reagent, respectively. The strong correlation between TPC and RSA, TPC and FRAP of both fresh and processed blackberries also confirmed that phenolic compounds including phenolic acid, flavonoids, and tannins are the major sources of antioxidants in blackberries (48). Moreover, the linear correlations between ACY and TPC (r=0.91), RSA (r=0.90) and FRAP (r=0.82) in fresh blackberries (Table 3.6), ACY and ORAC (r=0.86) in processed blackberries were found (Table 3.7), which can be explained as ACY is the major contribution to the total phenolics, providing antioxidant capacity in fresh blackberries (48).
Finally, moderately positive correlation between ORAC and FRAP ($r=0.63$) was observed in processed blackberry products over room temperature storage (Table 3.7), but not for fresh blackberry during refrigeration storage. Although both ORAC and FRAP are widely acceptable methods to measure antioxidant capacity of berries, ORAC assay basically relies on hydrogen atom transfer reaction to evaluate the capacity of donating hydrogen against peroxyl radicals other than reducing power which FRAP assay relies on (47). The sensitivity of antioxidants for specific assay, various compositions or proportions of antioxidants in two varieties of blackberries and interactions among several types of antioxidants may partly induce different ORAC and FRAP values used for evaluating antioxidant capacity (53). ORAC values showed no significant changes in ‘Marion’ during refrigeration storage, which rendered the narrow ORAC data range for ‘Marion’ (Figure 3.4). Therefore, it is difficult to identify the relationship between ORAC and FRAP in this study.

ACKNOWLEDGEMENTS

The authors acknowledge Scenic Fruit Co. (Gresham, OR, USA) for donating ‘Marion’ blackberries, Mr. Brian Yorgey for helping make canned blackberries and blackberry jams, Mr. Jeff Clawson for running the driers, and Ms. Deborah Hobbs for the analysis of ORAC and FRAP values of the samples.
LITERATURE CITED


(42) Brownmiller, C.; Howard, L.R.; Prior, R.L. Processing and storage effects on monomeric anthocyanins, percent of polymeric color, and antioxidant capacity of processed blueberry products. *J. Food Sci.* **2008**, *73*, H72-H78.


CHAPTER 4

General Conclusion

Refrigeration storage, processing technologies and subsequent room storage affected physicochemical parameters, contents of bioactive compounds, and antioxidant capacities of ‘Marion’ and ‘Evergreen’ blackberries. Fruit ripening and decay underwent in blackberries during post-harvest refrigeration storage, leading to only 9 days of shelf-life of ‘Marion’ and 7 days of shelf-life of ‘Evergreen’ at 2 ± 0.2 °C and 96 ± 2% RH in this study. Generally, refrigeration storage didn’t well retain the level of bioactive compounds investigated in this study, and had inconsistent influences on antioxidant capacity of two varieties. These results confirmed previous findings and suggested that other treatments along with refrigeration storage are necessary for prolonging shelf-life and retaining nutraceutical benefits of blackberries in fresh market.

Freeze drying increased total soluble solids and total phenolic content but didn’t significantly change ORAC or FRAP in both blackberry varieties. Hot-air drying enhanced total soluble solids while decreased total monomeric anthocyanin content in both varieties and reduced antioxidant capacities of ‘Marion’. Canning diminished total monomeric anthocyanins heavily in all the blackberry samples. Two types of canning
solutions, water and 20° Brix sucrose syrup exerted different effects on canned products. Canning-in-sucrose syrup doubled the loss of total monomeric anthocyanins compared with canning-in-water. Canning-in-water significantly increased, but canning-in-sucrose syrup decreased radical scavenging activities. Besides, the losses of TA and ORAC were observed in canned blackberries except canned-in-water ‘Evergreen’. Total phenolics, total monomeric anthocyanins, radical scavenging activities against DPPH, ORAC and FRAP consistently dropped in blackberry jams of both varieties.

Post-processing room storage didn’t change TA of canned and dried blackberries but decreased that of blackberry jams. Total monomeric anthocyanins in canned, hot-air dried blackberries and blackberry jams decreased significantly and only freeze dried blackberries well preserved total phenolics and monomeric anthocyanins. Antioxidant capacities of blackberry samples reduced or remained stable during room storage except minor increase of radical scavenging activities in ‘Marion’ jams, FRAP in canned-in-water ‘Marion’ and freeze-dried ‘Evergreen’. Our results showed that among all tested processing technologies in this study, freeze drying best retained bioactive compounds and antioxidant capacity in processed blackberries. In contrast, jam manufacturing was the most detrimental mainly due to the application of high temperature for a long time.

Moreover, cultivar variations were detected in original nutraceutical contents in fresh blackberries and their changes over post-harvest storage, processing and subsequent storage in this study. Therefore, more blackberry cultivars should be included in the future investigations.
Finally, the correlations among physicochemical properties, bioactive compounds and antioxidant activities were observed and discussed. Such correlations would need more data from other cultivars of blackberries to confirm.


Brownmiller, C.; Howard, L.R.; Prior, R.L. Processing and storage effects on monomeric anthocyanins, percent of polymeric color, and antioxidant capacity of processed blueberry products. *J. Food Sci.* **2008**, *73*, H72-H78.


Shahidi, F.; Naczk, M. Contribution of phenolic compounds to flavor and color characteristics of foods. In Phenolics in food and nutraceuticals; Shahidi, F.; Naczk, M., Eds.; CRC press: New York, 2004; Chapter 9, pp 443-469.


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APPENDIX
APPENDIX

Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions

Ruyi Wu\textsuperscript{1}, Daniel Lin\textsuperscript{1}, Bernadine Strik\textsuperscript{2}, and Yanyun Zhao\textsuperscript{1,3}

\textsuperscript{1}Department of Food Science & Technology, Oregon Sate University, Corvallis, OR 97331, USA.
\textsuperscript{2}Department of Horticulture, Oregon Sate University, Corvallis, OR 97331, USA.
\textsuperscript{3}Corresponding author: E-mail: yanyun.zhao@oregonstate.edu.

To be submitted to HortTechnology
113 South West Street, Suite 200, Alexandria, VA 22314
Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions

ADDITIONAL INDEX WORDS. Northern highbush blueberry, *Vaccinium corymbosum*, cultivar, shelf-life, fruit quality, firmness, desiccation, antioxidants, phenolics, ready-to-eat

SUMMARY. The edible coatings, Semperfresh™ (SF), chitosan (CH), and calcium caseinate (CC), were applied to ‘Duke’, and CC to ‘Elliott’ ripe fruit to evaluate effect on quality during storage. Fruit were washed in 200 ppm chlorinated water before applying coatings, then stored at 2 °C for 1 week, followed by storage at room temperature (20±3 °C) for up to 12 days for quality evaluation. Storage conditions were chosen to simulate the commercial situation of a brief cold storage period followed by display/sale under un-refrigerated conditions. CH coating helped reduce the decay rate of ‘Duke’ throughout the storage period. SF coating decreased weight loss of ‘Duke’ after 6 days of storage. CC coating significantly increased firmness of ‘Elliott’ after 6 days of storage. In general, ‘Duke’ fruit that were washed and coated had a higher antioxidant content (AC) and total phenolic content (TPC) than non-washed fruit. Washing and coating did not significantly affect AC and TPC of ‘Elliott’. Our results suggest that edible coatings have potential for retaining quality of pre-washed, ready-to-eat fresh blueberries under commercial storage conditions.
INTRODUCTION

Blueberries are well-known for having prominent antioxidant activities and a high content of phenolic compounds and anthocyanins (Kalt et al., 2001; Zheng and Wang, 2003). Consumption of blueberries has shown promise of health benefits, including reduction of cholesterol level (Abidov et al., 2006; Kahlon and Smith, 2007), anti-aging (Wilson et al., 2006), anti-inflammatory (Torri et al., 2007), anti-carcinogenic (Seeram et al., 2006; Yi et al., 2006), prevention of urinary tract infection and diabetes (Martineau et al., 2006), and improvement of eyesight and brain health (Willis et al., 2005).

Fresh highbush blueberries have been reported to have a shelf-life of 1 to 8 weeks depending on stage of fruit ripeness, method of harvest, presence of fruit disease, and storage conditions (temperature, relative humidity, and atmosphere; Ballinger et al., 1978; Beaudry et al., 1998; Galletta et al, 1971; Hancock et al., 2008). Post-harvest respiration and transpiration cause quality deterioration of fresh fruit, limiting shelf-life. In addition, bioactive compounds may degrade rapidly during post-harvest storage, partly due to the oxidation of polyphenolics with exposure to light and oxygen (Srivastava et al., 2007).

Several preservation technologies, including cold storage, UV irradiation, modified atmosphere packaging and ozonation, have been used to reduce deterioration, prolong shelf-life and retain the nutritional value of fresh blueberries (Chiabrando et al., 2006; Connor et al., 2002; Trigo et al., 2006; Zheng et al., 2003). Since the first use of wax as a coating material in the 12th to 13th century in China, edible coatings have been applied
for extending shelf-life of fresh produce (Baldwin et al., 1999; Han et al., 2004; Nimitkeatkai et al., 2006; Park, 1999; Ribeiro et al., 2007; Vargas et al., 2006). Along with increased interest in ready-to-eat and ‘invisible processed’ fruit with high quality and safety, edible coatings may provide a means to provide pre-washed, ready-to-eat blueberries. Edible coatings can possibly control the internal gas atmosphere of the fruit, minimizing fruit respiration rate (Park, 1999) and may serve as a barrier to water vapor, reducing moisture loss and delaying fruit dehydration (Baldwin et al., 1995). In addition, some edible coating materials, such as chitosan, have shown antimicrobial functions against the growth of certain microorganisms (Park et al., 2005; Zhang and Quantick, 1998).

Polysaccharides, proteins, lipids and their combinations may be used as coating materials for fresh produce (Baldwin et al., 1995). Chitosan (1, 4 - linked 2 - amino – 2 - deoxy – β – D - glucan), a derivative of chitin, has excellent film-forming and antimicrobial functions and has been successfully used to control quality loss of fresh strawberries and raspberries (Han et al., 2004; Park et al., 2005; Ribeiro et al., 2007; Vargas et al., 2006), sliced mango fruits (Chien et al., 2007), citrus (Fornes et al., 2005), fresh-cut water chestnut (Pen and Jiang, 2003), and many other fruits and vegetables (Lin and Zhao, 2007). Caseinate, a milk-protein based material, has excellent oxygen barrier properties and has been studied in carrots (Mei and Zhao, 2003), apples and potatoes (Letien et al., 2001), celery (Avena-Bustillos et al., 1997), and strawberries (Vachon et al., 2003) for controlling post-harvest respiration. Semperfresh™ is a commercial coating product of sucrose-fatty acid ester, and was reported to effectively...
decrease weight loss of hardy kiwifruit (Fisk et al., 2008), cherry (Yaman and Bayoindirli, 2002), and summer squash (Kaynas and Ozelkok, 1999), and extend shelf-life of pineapple for up to 5 weeks by preventing moisture loss (Nimitkeatkai et al., 2006).

The objectives of this study were to investigate the effectiveness of chitosan, calcium caseinate, and Semperfresh™ based coatings for enhancing the shelf-life and retaining the antioxidant properties of pre-washed, ready-to-eat highbush blueberry cultivars under commercial storage conditions.

MATERIALS & METHODS

FRUIT. Two cultivars of highbush blueberry, ‘Duke’ and ‘Elliott’, were hand harvested by a commercial picking crew from a farm in Sheridan, Ore. in mid-July and mid-Aug. 2006, respectively. Harvested fruit were immediately packed in a 6-fl oz (170 g blueberry weight) vented, plastic “clam-shell” containers (industry standard) and transported to the Food Science laboratory at Oregon State University, Corvallis, Ore.

COATINGS. Food-grade coating materials were used including: chitosan (CH: Vanson Inc., Redmond, Wash.; 89.8% deacetyelation) extracted from shrimp shells; calcium caseinate (CC: Alanate 385, NZMP, Santa Rosa, Cal.; 92.9% protein and 1.4% calcium); and Semperfresh™ (SF: AgriCoat Industries Ltd., England; distributed by Pace International, Seattle, Wash.) which is a mixture of sucrose esters of fatty acids, sodium carboxymethlcellulose, and mono-diglycerides of fatty acids. Other materials
used in the coating formulation were glycerol (Fisher Scientific Inc., Fairawn, NJ) and glacial acetic acid (Baker Adamson, Morristown, NJ).

A 2% (w/v) CH coating solution was prepared by dissolving CH in 1% aqueous acetic acid with 50% glycerol (w/CH dry weight), adding 0.15% Tween 20 (w/w), homogenizing (Polytron PT 10-35, Kinematica AG, Littau, Switzerland) for 90 sec at 3000g, and then storing overnight at room temperature. A 2% CC coating solution was prepared by dissolved 2% CC in de-ionized water with addition of 50% glycerol (w/CC dry weight) and 0.15% Tween 20 (w/w). The mixture was homogenized for 1 min at 3000g and shaken in a 60 ºC water bath for 30 min, followed by cooling to room temperature. The SF coating solution was prepared by diluting 50% SF concentrate with deionized water to 1%, and mixing with 50% glycerol (w/SF weight) and 0.15% Tween 20 (w/w).

TREATMENT OF FRUIT. The major goal of applying coatings on fresh blueberries is to develop ready-to-eat fresh fruit with a longer shelf-life. Hence, fruit were first sanitized by washing in 200 ppm chlorinated water prior to coating application. Washed ‘Duke’ fruit were randomly assigned to one of three coating treatments: CH, CC or SF. A non-washed SF-coated treatment was included for comparison. Washed ‘Elliott’ were coated with CC only, based on observations that CC-coated ‘Duke’ fruit from the earlier harvest season had a more natural looking “bloom” (natural waxy coating on un-washed fruit). A non-washed, CC-coated treatment was included for comparison. In both cultivars, a non-washed (natural waxy bloom present) and washed (in water, removing much of the bloom) control were used.
as controls. All treatment coatings were applied twice, by dipping fruit in the coating solution for 30 sec, draining on a stainless steel screen for 30 min, and then repeating the same procedure to achieve a uniform surface coating.

To simulate commercial storage conditions, coated samples were re-packaged in the vented plastic containers, stored in a cooler at 2 ± 1 °C and 88% relative humidity in the dark for 1 week, and then removed and placed at room temperature (retail display condition) at 20 ± 3 °C and 30% relative humidity under normal room light for 12 days and changes in quality monitored.

FRUIT QUALITY PARAMETERS. All fruit quality parameters were measured before cold storage and then at 0 (1 week after cold storage), 3, 6, 9 and 12 days at room temperature. Blueberries taken out from one clamshell container were used for one replication of quality measurement. Total soluble solid content (TSS), pH and titratable acidity (TA) were measured following the procedures as described by Fisk et al. (2008), where TA was reported as percent malic acid (mass/mass) on the basis of fresh weight of fruit. Three replications were conducted for each treatment.

Firmness (FN) of fresh blueberries was determined by measuring compression force using pre-calibrated BioWorks FirmTech2 Instrument (BioWorks, Inc., Wamego, Kan.). A sub-sample of fruit (25 berries) were set on the indentures on the turntable, and tested individually through the compression of the load cell with continuously counterclockwise rotation of turntable after 25 fruits at a time. The force thresholds were from 25 to 250 g. Percentage weight loss (WL) was calculated as weight change of fruit at each sampling time divided by the initial weight of the fruit. Decay rate (DR)
of the fruit was defined as percentage of fruit with a visible lesion. Measurements were conducted at storage times of 0, 3, 6, 9, and 12 days after room temperature with 3 replications per quality measurement.

EXTRACTION OF POLYPHENOLICS. The extraction of polyphenolics was carried out using a modified method of Rodriguez-Saona and Wrolstad (2001). Briefly, fruit were cryogenically powered with liquid nitrogen, a 5 g powered sample was mixed with 100% (v/v) aqueous acetone (EMD Chemicals Inc., Gibbstown, NJ), sonicated for 1 min and centrifuged for 5 min, and the remaining filtrate was re-extracted twice using 70% (v/v) aqueous acetone. The aqueous phase at the top was combined and transferred into a glass centrifuge bottle with 50 mL of chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ) added and mixed. After centrifugation at 3000g for 30 min, the aqueous phase was collected to a rotary evaporator (Brinkmann Instruments, Westbury, NY) to remove the residual acetone. The extract was diluted in de-ionized water to desired concentration and stored at -70 °C until analysis.

TOTAL ANTIOXIDANT CONTENT ANT AND PHENOLICS. Total antioxidant content (AC) was determined using DPPH assay (Brand-Williams et al., 1995). The sample extract was mixed with 1.5 mL DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Kasel Kogyo Co. Ltd, Tokyo, Japan) in a small screw-cap test tube, vortexed, and set at room temperature for 5 min. The water soluble antioxidant content was determined spectrophotometrically at 517 nm with the absorbance of ascorbic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ) used as a standard. Results with two replications were
expressed as mg of ascorbic acid equivalents (AAE) per gram fresh weight (FW) of sample.

Total phenolic content (TPC) was measured by a modified method from Singleton and Rossi (1965). A series of test tubes containing 7.5 mL de-ionized water and 0.5mL Folin-Ciocalteu reagent (Sigma Chemical Co., MO) were prepared. A 0.5 mL diluted extract was mixed with 0.5 mL of 50, 100, 150, 200 ppm gallic acid (Sigma Chemical Co., MO) solutions, respectively, and 0.5 mL de-ionized water was used as control. After the solutions were mixed by a vortexer and set at room temperature for 10 min, they were mixed with 3 mL 20% (w/v) Na$_2$CO$_3$, then placed in water bath at 40 °C for 20 min. The samples were immediately cooled to room temperature in ice bath for 3 min. The absorbance of samples and a standard were analyzed by spectrophotometer at 765nm. TPC was calculated as mg of gallic acid equivalents (GAE) per gram FW of sample. AC and TPC measurements were performed at storage times of 0, 6, 12 days for both cultivars with two replicates.

STATISTICAL ANALYSES. Data analyses were performed by ANOVA (analysis of variance) and GLM (general linear model) using SAS statistical software 9.01 (SAS institute, Cary, NC). Multiple comparisons among the treatments with significant differences tested in ANOVA were conducted by using LSD (least significant difference) at P<0.05.
RESULTS & DISCUSSION

Based on ANOVA results, during 1-week cold storage, AC and TPC of ‘Duke’ and pH and TA of ‘Elliott’ were affected (P<0.05) by coating treatments, while FN and TPC of ‘Duke’ and TA and TPC of ‘Elliott’ were affected (P<0.05) by storage time (Table 1).
Table 1. ANOVA results of ‘Duke’ and ‘Elliott’ fruit during cold storage

<table>
<thead>
<tr>
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<td>FN&lt;sup&gt;c&lt;/sup&gt;</td>
<td>AC&lt;sup&gt;g&lt;/sup&gt;</td>
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<sup>a</sup> Total soluble solids; <sup>b</sup> Titratable acidity, reported as % malic acid (mass/mass) on the basis of fresh weight of fruit; <sup>c</sup> Firmness, N; <sup>d</sup> Weight loss, %; <sup>e</sup> Decay rate, %; <sup>f</sup> Percentage of marketable fruits, %; <sup>g</sup> Antioxidant content, mg ACE/g FW fruit; <sup>h</sup> Total phenolic content, mg GAC/g FW fruit; * Significant interaction between coating treatment and storage time.

During room temperature storage, all the quality parameters measured in this study were affected (P<0.05) by storage time for both cultivars, and coating treatments impacted (P<0.05) WL and PMF in ‘Duke’ and all measured quality parameter except TSS and TPC in ‘Elliott’ (Table 2).
Table 2. ANOVA results of ‘Duke’ and ‘Elliott’ fruit during room temperature storage

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<th>Factors</th>
<th>Duke</th>
<th>Elliott</th>
</tr>
</thead>
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<td>pH</td>
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<tr>
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<td>Pr&gt;F</td>
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<sup>a</sup> Total soluble solids; <sup>b</sup> Titratable acidity, reported as % malic acid (mass/mass) on the basis of fresh weight of fruit; <sup>c</sup> Firmness, N; <sup>d</sup> Weight loss, %; <sup>e</sup> Decay rate, %; <sup>f</sup> Percentage of marketable fruits, %; <sup>g</sup> Antioxidant content, mg ACE/ g FW fruit; <sup>h</sup> Total phenolic content, mg GAC/g FW fruit.

pH, TOTAL ACIDITY AND TOTAL SOLUBLE SOLIDS. For ‘Duke’, pH, TA and TSS were not significantly (P>0.05) affected by coating treatment or cold storage time (Table 1), but pH tended to increase while TA and TSS decreased based on the overall means of all the ‘Duke’ samples pre- and post- 1-week cold storage (Table 3). Non-washed control (3.81, 13.67%) ranked the lowest mean in pH but highest in TSS over cold storage compared to that in Washed (3.94, 13.17%) and Washed coated ‘Duke’ (3.92-3.96, 12.50%-12.83%) (Table 3 and 4). The overall means of TA in ‘Duke’ over cold storage fell in a narrow range of 0.62 to 0.65% (Table 3).
Table 3. Overall means of main effects on pH, TA, TSS and FN of ‘Duke’ and ‘Elliott’ fruit during 1-week cold storage\(^a\)

<table>
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<tr>
<th>Coating Treatment</th>
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<td></td>
<td>pH</td>
<td>TA</td>
<td>TSS</td>
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<td>Non-washed(^b)</td>
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<td>TA</td>
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<td>Non-washed(^b)</td>
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\(^a\) Values are means (n=3); \(^b\) No washing and no coating served as a control; \(^c\) Washing but no coating; \(^d\) Coating of Semperfresh\(^\text{TM}\) without washing; \(^e\) Washing and coating of Semperfresh\(^\text{TM}\); \(^f\) Washing and coating of chitosan; \(^g\) Washing and coating of calcium caseinate; \(^h\) Coating of calcium caseinate without washing.
Table 4. Physicochemical and nutritional properties of ‘Duke’ and ‘Elliott’ fruit during 1-week cold storage

<table>
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<th>FN</th>
<th>AC</th>
<th>TPC</th>
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<td>Non-washed b</td>
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<td>Post-CS</td>
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<td>Pre-CS</td>
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<td>0.72±0.13</td>
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<tr>
<td>Washed SF e</td>
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a Values are means (n=3)
During room temperature storage of “Duke”, coating treatments didn’t show effect on TA, pH and TSS (P>0.05), but the mean of TA declined significantly (P<0.05) over time from the initial 0.62% to 0.43% at 9 days, mean of pH tended to increase during the first 3 days, then declined over time reaching 3.84 at 9 days, significantly lower than 4.20 at 3 days. In contrast, TSS tended to decrease during the first 3 days, but increased during the rest of storage, reached to 13.22% at 9 days which was significantly higher than 12.00% at 3 days (Fig. 1).
Fig. 1. Physicochemical properties of ‘Duke’ fruit during room temperature storage. Since no significant differences (P>0.05) in TSS, pH, TA and firmness among different treatments were identified, mean values of all treatments were reported.
Fig. 1. Physicochemical properties of ‘Duke’ fruit during room temperature storage. Since no significant differences (P>0.05) in TSS, pH, TA and firmness among different treatments were identified, mean values of all treatments were reported. (Continued)
For ‘Elliott’, Washed CC had significantly (P<0.05) lower pH (3.06, 3.08), but higher TA (1.24%, 1.33%) than Non-washed (3.20, 3.30; 1.08%, 1.11%) and Washed (3.21, 3.28; 1.05%, 1.13%) both pre-and post- cold storage (Table 3). The means of pH and TA increased from 3.18 to 3.22 and from 1.12% to 1.21%, respectively during cold storage (Table 3). During room storage, CC coating showed significantly effect on pH and TA, where Washed CC and Non-washed CC had significantly (P<0.05) lower pH and higher TA (mean of pH=3.27 and TA=1.13%) than those of Washed and Non-washed (mean of pH=3.36 and TA=0.99%), while no significant differences were observed in TSS among the treatments (Fig. 2). In respect to the storage time, pH stayed stable during the first 6 days of room temperature storage, but increased from 6 to 12 days, while TA gradually decreased throughout the storage period (Fig. 2). Similar to ‘Duke’, washing itself didn’t show significant impact on pH, TA and TSS of ‘Elliott’.
Fig. 2. Physicochemical properties of ‘Elliott’ fruit during room temperature storage. Since no significant differences (P>0.05) in TSS and weight loss among different treatments were identified, mean values of all treatments were reported.
Fig. 2. Physicochemical properties of ‘Elliott’ fruit during room temperature storage. Since no significant differences (P>0.05) in TSS and weight loss among different treatments were identified, mean values of all treatments were reported. (Continued)
During post-harvest storage, the acid metabolism as a result of fruit ripening continued by converting starch and acid to sugar, resulted in decrease in TA and increase in pH and TSS (Thompson, 1996; Verma and Joshi, 2000). The results on CC coated fruit confirmed previous studies that CC coating helped control the changes of posr-harvest physicochemical properties, such as pH and TA. This may be explained as the protein-based CC delayed post-harvest respiration of fruit by providing a strong gas barrier on the surface of fruit (Khwaldia et al., 2004; Letien et al., 2001; Lin and Zhao, 2007).

FIRMNESS. FN of all the ‘Duke’ samples except Non-washed control was reduced (p<0.05) after 1-week cold storage, with reduction of 5% for Washed, 1% for Non-washed SF, 7% for Washed SF, 9% for Washed CH, and 6% for Washed CC, respectively (Table 4). There were no significant differences (P>0.05) in FN of ‘Duke’ samples subjected to different treatments during both cold and room temperature storage (Table 2 and 3). Mean of FN in ‘Duke’ increased initially (P<0.05) during the first 3 days of room storage (from 1.67N to 1.82N), then remained stable afterwards (1.82-1.84N) (Fig. 1).

FN of ‘Elliott’ didn’t show significant (P>0.05) change after 1-week cold storage (Tables 1 and 3). Mean of FN of Non-washed control (1.94N) ranked higher than all other ‘Elliott’ samples (1.80-1.88 N) after 1-week cold storage. During room storage, CC coated ‘Elliott’ had significantly (P<0.05) higher FN (mean of 1.99 N) than those non-coated ones (mean of 1.75 N), and FN of coated samples slightly increased during the first 9 days of room storage, then dropped, while those uncoated ‘Elliott’
continuously decreased throughout the storage period (Fig. 2). Washing did not affect FN of ‘Elliott’ during room storage (P>0.05).

Fruit softening, one of the important quality deteriorations during post-harvest storage, is generally caused by the hydrolysis of starch to sugar and the degradation of pectin in the fruit cell wall associated with fruit ripening (Thompson, 1996). In contrast, water loss of fruit may lead to the hardening of fruit. Both fruit softening and hardening affect the measured fruit firmness. In this study, we demonstrated that CC coating helped maintain the firmness of ‘Elliott’ during room storage. Calcium caseinate (CC) has been shown to provide calcium ions which may be chelated by adjacent acidic pectin polymers in the cell wall through non-covalent linkage forming an “egg box” model at the biochemical level during storage (Seymour et al., 1993), thus enhanced the firmness of ‘Elliott’ fruit.

WEIGHT LOSS. Overall, weight loss of both blueberry cultivars increased (P<0.05) throughout the room storage period (Fig. 1 and 2). In ‘Duke’, no significant differences were observed at the first 3 days of room storage, but at 6 days, Washed (2.28%) and Non-washed SF (4.85%) had less weight loss than other ‘Duke’ samples (6.38-6.66%) (Fig. 1). Weight loss of Non-washed SF and Washed SF ‘Duke’ (9.83%, 9.31%) was significantly lower than other ‘Duke’ samples at 12 day (Fig. 1). Similar to ‘Duke’, WL of ‘Elliott’ increased during room storage, up to a mean of 13% at the end of 12 days (Fig. 2). Washed non-coated ‘Elliott’ (mean of 7.89 %) had the highest WL among all samples (mean of 6.82-7.19%) over the 12 days of room storage (P<0.06).
Migration of water from the fruit to the environment is the major cause of weight loss of fruit during storage. Our results were consistent with previous studies that a hydrophobic coating material, such as SF, had high barrier to water loss (Morillon et al., 2002), while CH and CC were hydrophilic coating materials with relatively high moisture permeability. Therefore, it may be necessary to incorporate lipids into hydrophilic coating formulae for better control of water loss of coated blueberries if this is a major goal when applying coatings.

DECAY RATE. Fruit decay rate in both blueberry cultivars increased along with the time under room temperature storage (Fig. 1 and 2). Although there was no overall treatment effects on decay rate of ‘Duke’ (P>0.05) (Table 2), Washed CH ‘Duke’ showed a lower value (9.98% at 12 days) than other samples (20.57%-30.55% at 12 days) throughout the room storage (P<0.069), and Washed CC had lower decay rate than Non-washed CC at 9 and 12 days (Fig. 1). In respect to ‘Elliott’, CC coating again showed significant (P<0.05) effect on decay rate (P<0.05), but a opposite way, where CC coated ‘Elliott’ showed higher value (mean of 34.82 at 12 days) than non-coated ones (14.73% at 12 days) throughout the 12 days of room storage, and Non-washed CC (24.67-37.66%) had higher number than Washed CC (17.92-29.97%) during 9-12 days (P<0.05) (Fig. 2).

The anti-fungal function of CH coating to prevent fruit decay has been well reported in several studies (Chien et al., 2007; Han et al., 2004; Park et al., 2004; Zhang and Quantick, 1998). However, this anti-fungal property may be limited by several factors. Dipping and washing in chlorinated water may remove the natural waxy layer
on the surface of blueberries, thus weakening adhesion and durability of coatings. Moreover, residual water on the surface of blueberries, after washing, possibly diluted or dissolved applied coating materials, made it difficult to form a uniform and durable layer of edible films on the fruit surface (Lin and Zhao, 2007). However, CC, as a protein based material, may provide additional nutrients for fungi to grow when the fruit is contaminated, potentially leading to increased decay rate. Washing using chlorine solution has shown the benefit of decreasing fruit decay.

ANTIOXIDANT CONTENT. AC of Non-washed ‘Duke’ (3.38 mg AAE/g FW) was significantly (P<0.05) higher than those of other ‘Duke’ samples (1.83-2.81 mg AAE/g FW) right after coating treatment (Table 4). After cold storage, AC degraded 22% in Non-washed ‘Duke’ (P<0.05) while kept stable in Washed, Non-washed SF, and Washed CH, but increased 32% and 25% in Washed SF and Washed CC, respectively (p<0.05) (Table 4). There was almost a linear increase in AC of ‘Duke’ over room storage, from initial 2.59 mg AAE/g FW to 3.29 mg AAE/g FW after 12 days (Fig. 3). Weight loss of ‘Duke’ (up to 14%) during room temperature storage might have caused the increase in AC calculated on the basis of fresh fruit weight. Washed CH and Washed CC ‘Duke’ (3.46 and 3.67 mg AAE/g FW, respectively) had significantly (P<0.05) higher AC than Non-washed (3.03 mg AAE/g FW) and Washed SF (3.25 mg AAE/g FW) at the end of storage.
Fig. 3. Antioxidant content and total phenolic content of ‘Duke’ fruit during room temperature storage. Since no significant differences (P>0.05) among different treatments were identified, mean values of all treatments were reported.
AC in Non-washed and Washed CC ‘Elliott’ declined 7% and 4%, respectively after cold storage (p<0.05) (Table 4), but showed no change in Washed and Non-washed CC. No differences were found among treated ‘Elliott’ with mean of 7.10 and 6.94 mg AAE/g FW pre- and post- cold storage, respectively. Connor et al. (2002) reported that fresh ‘Elliott’ harvested before turning fully blue showed an increase in antioxidant activity after harvest and during cold storage. Therefore, changes in antioxidant activities are likely affected by storage conditions and stage of fruit ripeness at harvest. AC of all ‘Elliott’ samples declined at the first 3 days of room temperature storage, from mean of 6.94 to 5.48 mg AAE/g FW, but kept stable from 6 to 12 days (Fig. 4).
Fig. 4. Antioxidant content and total phenolic content of ‘Elliott’ fruit during room temperature storage. Since no significant differences (P>0.05) in total phenolic content among different treatments were identified, mean values of total phenolic content were reported.
TOTAL PHENOLIC CONTENT. TPC in all ‘Duke’ samples decreased (P<0.05) during cold storage, resulting in 27-49% loss depending on specific treatment (Table 4). According to Verma and Joshi (2000) and Seymour (1993), the oxidation of phenolics in ‘Duke’ catalyzed by polyphenoloxidase and peroxidase during post–harvest storage is a major reason for the quick drop of TPC. However, TPC of ‘Duke’ increased at the begin and then kept stable during room temperature storage (Fig. 3). At the 12 days of room storage, Washed CH (3.20 mg GAE/g FW) and CC (3.48 mg GAE/g FW), and Non-washed SF (3.41 mg GAE/g FW) had significantly higher TPC than Non-washed control (2.94 mg GAE/g FW). This result indicated that the intermolecular binding effect of CH, CC and SF coatings on ‘Duke’ may help stabilize the phenolics, thus effectively slowing the degradation of phenolic compounds associated with fruit ripening at the end of room storage.

Unlike ‘Duke’, TPC of ‘Elliott’ didn’t change (P>0.05) in Non-washed (mean=5.26 mg GAE/g FW) and Washed control (mean= 5.91 mg GAE/g FW) during cold storage, but increased 25% in Non-washed CC and 14% in Washed CC (p<0.05) (Table 4), which agreed with the results on fresh ‘Elliott’ reported by Connor (2002). During room temperature storage, mean of TPC in ‘Elliott’ decreased from 0 day (6.19 mg GAE/g FW) to 6 days (5.39 mg GAE/g FW), but increased from 6 days to 12 days (6.90 mg GAE/g FW) (Fig. 4). Specifically, TPC of Non-washed increased over storage time and that of Washed remained stable and then increased significantly after 6 day. Whereas, coated ‘Elliott’ decreased initially and then increased at the end of storage.
Washed, uncoated ‘Elliott’ had higher TPC than other ‘Elliott’ samples (P<0.05) at the end of room temperature storage.

COMPARISON OF TWO BLUEBERRY CULTIVARS. Significant differences between the two blueberry cultivars in post-harvest quality and response to coating treatment were observed in this study. ‘Elliott’ is a late–season cultivar harvested starting in August, compared to the early-season ‘Duke’ where harvest starts at the end of June in the Willamette Valley, Oregon. ‘Elliott’ fruit had lower pH and higher TA than those of ‘Duke’, while ‘Duke’ had a larger fruit size. In comparison with other reported studies, the fruit pH of ‘Duke’ in our study, for Washed or Non-washed fruit was slightly higher than that reported by Zheng et al. (2003), but the pH of ‘Elliott’ was similar to that reported by Connor et al. (2002). TA and TSS of both cultivars were slightly lower and higher than those reported by Connor (et al., 2002) and Zheng (et al., 2003), respectively, which may have been a result of differences in macroclimate or harvest time.

In respect to antioxidant capacity of the 2 cultivars, AC of ‘Elliott’ was significantly higher than that of ‘Duke’. This result agreed with the previous report that the later harvested ‘Elliott’ had higher antioxidant activity than earlier harvested ones such as ‘Bluecrop’ and ‘Jersey’ (Connor, 2002).

CONCLUSIONS

In conclusion, different coatings had various effects on the post-harvest quality of fresh blueberries. Chitosan coating showed the potential to control the decay incidence
of ‘Duke’ during storage under room conditions. Semperfresh™ coating helped control weight loss of ‘Duke’, while calcium caseinate coatings tended to improve the firmness of ‘Elliott’ during room storage, but not weight loss and decay rate. In general, washing the fruit using chlorine solution showed the benefit of reducing decay incidence in both cultivars. All three coatings, Semperfresh™, chitosan and calcium caseinate demonstrated the potential to help retain antioxidant and total phenolic content of ‘Duke’ during room temperature storage, but calcium caseinate coating did not show this benefit on ‘Elliott’.

Results from this study indicated the possibility of using edible coatings to develop ready-to-eat fresh blueberries with extended shelf-life. The key for success is using an appropriate coating material, formulation, and the method of applying coating. New coating materials with enhance microbial barrier and antimicrobial functionality may be evaluated, or modification on the existing coating formulation may be studied to obtain high quality and nutritional value of fruit. The current dipping method of applying coating might have caused the loss of some coating benefits; thereby new coating application methods should be investigated. The use of electrostatic spraying for coating application is under the way to be evaluated by the authors for achieving more uniform coating and avoiding the removal of the natural waxy layer on the surface of blueberries for maximizing the benefits of coatings.
LITERATURE CITED


