A COMPARATIVE STUDY OF ANTIOXIDANT AND PHYSICOCHEMICAL

PROPERTIES OF BLACKBERRY AND KIWIFRUIT

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A COMPARATIVE STUDY OF ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF BLACKBERRY AND KIWIFRUIT

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A COMPARATIVE STUDY OF ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF BLACKBERRY AND KIWIFRUIT

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THESIS ABSTRACT

A COMPARATIVE STUDY OF ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF BLACKBERRY AND KIWIFRUIT

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Increased consumption of fresh fruit and vegetables has been associated with reduction in chronic diseases such as cardiovascular disease, cancer, diabetes, and neurological disorders. The protective effects are significantly related to the various antioxidants contained in them. Blackberry and kiwifruit contain significant concentrations of several antioxidants. In this study, the antioxidant profiles and physicochemical properties of Alabama grown blackberry and kiwifruit were determined.

Results of physicochemical properties were related to the antioxidant capacities in fruit tissue. A high correlation was found between total phenolics content and Vitamin C equivalent antioxidant capacities (VCEAC) determined by the ABTS radical scavenging assay in blackberries. Chlorophyll a, chlorophyll b, and β-carotene content were highly

correlated with VCEAC determined by the DPPH radical scavenging assay in kiwifruit. Cultivar differences in physicochemical properties and antioxidants were found among five cultivars of blackberries and five cultivars of kiwifruit. In general, the cultivars that were found to have the highest VCEAC and fruit quality indices in the present study are likely to be selected by consumers in the market place. Hence, they are the cultivars that offer the most potential for commercial production in Alabama.

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I. INTRODUCTION

The health status of Alabama's population ranks above the national average with respect to the prevalence of poor overall health indicators. A contributing factor is a lack of knowledge of the health benefits of fresh fruit and vegetables by consumers. Increasing epidemiological evidence associates diets rich in fruit and vegetables with reduced risk of heart disease, cancer, inflammation, arthritis, immuno-suppressed system decline, brain dysfunction and cataracts (Moyer et al., 2002[a], Reyes-Carmona et al., 2005, Astley et al., 2002, Leong and Shui, 2002, Xu et al., 2004). For optimal diet, a daily intake of at least five portions of fruit and vegetables is recommended (Netzel et al., 2002). Yet, the compositional and nutritional qualities of fruit are highly variable among states with different climate and soil. Variation may also be due to genotypic differences among cultivars (Thomas et al., 2005). Compositional and nutritional data of fresh fruit, which reflect Alabama growing conditions, is limited. The objective of this study was to collect physicochemical properties and develop a nutrient profile for two types of Alabama grown fruit: kiwifruit and blackberry.

II. LITERATURE REVIEW

Definition and Classification of Dietary Antioxidants

Antioxidants may be defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate in a chain reaction (Halliwell and Whitemann, 2004; Leong and Shui, 2002). Humans have evolved a highly complicated antioxidant protection system, which involves a variety of endogenous and exogenous compounds that are able to function interactively and synergistically to neutralize free radicals. These include antioxidant enzymes that catalyze free radical quenching reactions, metal binding proteins that sequester free iron and copper ions that are capable of catalyzing oxidative reactions, diet-derived antioxidants, and other low molecular weight compounds such as α-lipoic acid (Kaliora et al, 2005). Antioxidants have become a popular research topic because they cannot be generated by the human body and hence have to be consumed in the diet. Many fruit and vegetables have been found to be rich sources of antioxidants. Since a large portion of the human diet is based on fruit and vegetables, it is important to understand the biological and biochemical interactions between these dietary antioxidants and living systems.

A major benefit from diets rich in fruit and vegetables may be increased consumption of antioxidant vitamins such as ascorbate (vitamin C) and tocopherol (vitamin E), vitamin like compound (glutathione), and pigments such as phenolics

(e.g. flavonoids and anthocyanin) and carotenoids (Moyer et al., 2002; Reyes-Carmona et al., 2004). These compounds along with dissolved sugars, acids, salts, amino acids, and some water-soluble pigments are situated in large central vacuoles of parenchyma cells, the main structural unit of edible portion of most fruit and vegetables (Potter and Hotchkiss, 1996). They act as major cellular redox buffers that can effectively quench reactive oxygen species (ROS) by donating one or more electrons to ROS. Natural phytochemicals act synergistically to increase their antioxidant capacity such that the total antioxidant effect is greater than the sum of the individual antioxidant activities, and the isolation of one compound will not exactly reflect the overall reaction (Chun et al., 2005; Leong and Shui, 2002). Plants have a similar nonenzymatic ROS scavenging system including the ascorbate-glutathione cycle in chloroplasts, glutathione peroxidase cycle in peroxisomes, as well as tocopherol, flavonoids, alkaloids, and carotenoids. It has been demonstrated that *Arabidopsis* mutants with decreased ascorbic acid levels or altered glutathione content are hypersensitive to stress (Apel and Hirt, 2004).

Biological and Biochemical Significance of Vitamin C, Carotenoids, Phenolics, and Chlorophylls

Ascorbate (Vitamin C)

Vitamin C (ascorbate) is considered to be one of the most typical, least toxic natural antioxidants. It is water soluble and is found in high concentrations in many plant and animal tissues. Ascorbate has diverse biochemical and biological functions such as synthesis of collagen, hormones and neurotransmitters (Leong and Shui, 2002). Much research has focused on the radical scavenging activity in aqueous solution (citation). The

benefit of daily consumption of ascorbate from fruit and vegetables is reviewed more thoroughly in the section of Evidence of Nutritional Benefit in this Literature review. Ascorbate is rapidly depleted in human extracellular fluids under conditions of oxidative stress. When ascorbate encounters a hydroxyl radical, it forms a semidehydroascorbate radical and a water molecule. Two semidehydroascorbates combine to generate a molecule of ascorbate and a molecule of dehydroascorbate. Dehydroascorbate is further catabolized to other breakdown products if it is not being reduced to regenerate ascorbate (Halliwell et al., 1995). The reactions are listed as follows:

Ascorbate + $OH^{\bullet}$ → semidehydroascorbate radical + H_2O

2 semidehydroascorbate → ascorbate + dehydroascorbate

Dehdyroascorbate → oxalate, threonate, other breakdown products.

The ascorbate-glutathione cycle is an important free radical scavenging system in the chloroplast, emitting excess excitation energy as heat from the photosynthetic system. It is also the most effective antioxidant defensive system in human cells (Halliwell et al., 1995). Glutathione plays a central role in this system because reduced glutathione (GSH) act as an electron donor to regenerate ascorbate from the oxidized form (Tausz et al., 2004). The enzymes involved are 1) ascorbate peroxidase (E.C. 1.11. 1. 11), 2) MDA reductase (E.C.1.6.5.4.), 3) DHA reductase (E.C.1.6.5.4.), and 4) glutathione reductase (E.C.1.6.4.2.). Regeneration of ascorbate from semidehydroascorbate radical or monodehydroascorbate and dehydroascorbate is crucial. Reductases, enzymes that possess reducing ability, are found in most tissues to regenerate ascorbate (Apel and Hirt, 2004). This system is an excellent example of how enzymatic and non-enzymatic

components cooperate together to efficiently scavenge potential free radicals. The mechanisms are shown below:

1) Ascorbate + H_2O_2 → Monodehydroascorbate (MDA) + H_2O
2) MDA + NAD(P)H → Ascorbate + NAD(P)+
3) Dehydroascorbate (DHA) +GSH → Ascorbate + GSSG
4) GSSG + NAD(P)H → GSH + NAD(P)+

Other compounds including niacin and thiols such as dihydrolipoic acid and thioredoxin can also assist in vitamin C regeneration (Gropper et al., 2004). The reaction involving niacin is as follows:

2 semidehydroascorbate radicals + NAD(P)H + H^+ → Ascorbate + $NADP^+$

Dihydrolipoic acid provides hydrogens to the dehydroascorbate form of vitamin C to recycle ascorbate.

Dehydroascorbate + dihydrolipoic acid (hydrogen doner) → ascorbate + lipoic acid.

Thioredoxin (Trx-$(SH)_2$) a dithiol, also provides reducing equivalents to dehydroascorbate

Dehydroascorbate + Trx-$(SH)_2$ → Ascorbate + Trx-S_2

The ROS defensive system in vacuoles is dominated by peroxidase. When hydrogen peroxide diffuses into vacuoles, peroxidases is able to quench it by using phenolics as primary electron donors. The phenoxyl radical generated by this oxidation is reduced by the ascorbic acid present in the vacuoles. The resulting monodehydroascorbic acid is exported to the cytosol where it is enzymatically regenerated to ascorbate, then transported into vacuoles (Edreva, 2005).

Carotenoids

Carotenoids are a family of compounds of over 600 fat-soluble plant pigments, thus they are associated with membranes. They are responsible for the brilliant colors of many birds, insects, marine animals, and plants, hence they are found in many fruit and vegetables (Rodriguez-Bernaldo de Quiros and Costa, 2006, Gropper et al., 2005). Yellow, orange, and red fruit are known to contain higher concentrations of carotenoids, which accumulate during fruit ripening (McGhie and Ainge, 2002). Carotenoids often occur along with chlorophylls in the chloroplasts, but are also present in other chromoplasts. After consumption, carotenoids are taken up by the mucosa of the small intestine. β-Carotene and other provitamin A carotenoids are partly converted to vitamin A, primarily retinyl esters, in the intestinal mucosal cell, and both β-carotene and retinyl esters are incorporated into chylomicrons and secreted into lymph for transport to the liver. The carotenoids eventually become part of lipoproteins (Krinsky and Johnson, 2005). Approximately two dozen carotenoids are found in human blood and tissue, and two in the retina and lens of the eye. The major dietary carotenoids are α-carotene, β-carotene, lycopene and the xanthophylls, β-cryptoxanthin, lutein and zeaxanthin (Krinsky and Johnson, 2005; Potter and Hotchkiss, 1996). Carotenoids can be categorized into two main groups: 1) carotenes of hydrocarbon carotenoids only composed of carbon and hydrogen atoms and 2) xanthophylls that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. The conjugated double bonds in carotenoids are largely responsible for their chemical, biochemical and physical properties (Rodriguez-Bernaldo de Quiros and Costa, 2006).

Dietary carotenoids contribute to both the appearance and attractiveness of fruit as well as provide additional nutritional value in the form of dietary antioxidants (McGhie and Ainge, 2002; Montefiori et al., 2005). Carotenoids "bleach" or lose their color when exposed to the radicals and oxidizing species. Bleaching occurs when the conjugated double bond system is cleaved or by addition to one of the double bonds. There are three possible mechanisms for the reaction of carotenoids with radical species especially singlet oxygen ($^1O_2$) and peroxyl radicals ($ROO^{\bullet}$) including 1) electron transfer (Eqn. 5) 2) hydrogen abstraction (Eqn. 6) and 3) addition of a radical species (Eqn. 7) (Young and Lowe 2001; Diplock et al, 1998).

$ROO^{\bullet} + CAR \rightarrow ROO^{-} + CAR^{\bullet+}$ (Eqn. 5)

$ROO^{\bullet} + CAR \rightarrow ROOH + CAR^{\bullet}$ (Eqn. 6)

$ROO^{\bullet} + CAR \rightarrow (ROO\text{-}CAR)^{\bullet}$ (Eqn. 7)

The major source of β-carotene comes from green leafy vegetables and orange and yellow fruit and vegetables. Cooking, chopping and the presence of dietary fat enhance the bioavailability of β-carotene. Of the 50 different carotenoids that can be metabolized into vitamin A, β-carotene contains the highest provitamin A activity. β-carotene and lycopene tend to be localized predominately in the low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Their antioxidant activity is dependent on the type of radical they encounter. β-Carotene is more sensitive to singlet oxygen ($^1O_2$) but less active towards hydroxyl radical ($OH^{\bullet}$) and superoxide anion ($O_2^{\bullet-}$). Singlet oxygen transfers its excitation energy and returns to the ground state, while β-carotene receives the energy (photosensitizer) and enters an excited state. β-carotene then releases the

energy in the form of heat (Gropper et al, 2004). The energy quenching reaction is shown as follows:

$^1O_2$ + β-carotene → $^3O_2$ + excited β-carotene → β-carotene + heat

The protection from cardiovascular disease by carotenoids is proposed as trapping peroxyl radicals and quenching singlet oxygen in lipoproteins (Krinsky and Johnson, 2005; Young and Lowe, 2001). Interestingly, β-carotene is able to function synergistically with both ascorbate (Eqn. 11) and α-tocopherol (vitamin E) (Eqn. 10), forming ascorbyl radical (Eqn. 12) due to inhibition of lipid peroxidation. This system demonstrates both hydrophilic and lipophilic antioxidants in the plasma work synergistically in scavenging free radicals (Young and Lowe, 2001). The reaction is shown as follows with T = vitamin E and $ASCH_2$ as ascorbate.

$CAR + TOH^{\bullet+} \rightarrow TOH + CAR^{\bullet+}$ (Eqn. 10)

$CAR^{\bullet+} + ASCH_2 \rightarrow CAR + ASCH + H^+$ (Eqn. 11)

$CAR^{\bullet+} + ASCH^- \rightarrow CAR + ASCH^{\bullet-} + H^+$ (Eqn. 12)

Phenolics

The term "phenolics" encompasses approximately 8,000 naturally occurring compounds, all of which possess a phenol as their common structure (an aromatic ring with at least one covalently bonded hydroxyl group). Phenolics are classified into polyphenols and simple phenols. Polyphenols have at least two phenol subunits (classified as A and B ring), which are connected by an oxygen containing pyrene ring, and simple phenols have only one phenol group (Erlund, 2004). Flavonoids and tannins refer to those compounds possessing three or more phenol subunits. Approximately one third of plant phenolics are phenolic acids and the rest are mostly flavonoids (Kris-

Etherton et al., 2002). They are a type of organic acid that have a carboxylic acid functional group covalently bonded to a simple phenol. Hydroxycinnamic and hydroxybenzoic acids are two main groups of phenolic acids, both of which are derived from the nonphenolic molecules benzoic and cinnamic acid (Rababah et al., 2005). Although the basic structure is the same, the position of the hydroxyl groups on the aromatic ring varies thus creating various derivatives. The most common phenolic acids in plants are caffeic, p-coumaric, vanillic, ferulic and protocatechuic. They are physically dispersed throughout the plant in seeds, leaves, roots, and stems. Only a minor fraction exists as "free acids". The majority are linked to cellulose, proteins, and lignin or to large polyphenolics (flavonoids) or small organic molecules (eg. glucose) through ester, ether, or acetal bonds. Flavonoids receive special interest because of their high occurrence in foods, especially in fruit, vegetables, and green leafy vegetables and green tea. Furthermore, flavonoids are known to reduce coronary heart disease, and they have anticancer and antioxidant properties (Sellappan et al., 2002). The general metabolic functions of phenolics include nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components, and allelopathy. They also offer protection against ultraviolet radiation (Robbins, 2003).

Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus (Heim et al., 2002). To date, more than 6,000 flavonoids have been identified although only a small number have dietary importance. (Erlund, 2004). The flavonoid group can be divided into 13 classes based on hydroxylation of the flavan nucleus as well as the linked sugar (Rababah et al., 2005; Kris-Etherton et al., 2002). The form lacking sugar moieties is called "aglycon" which

occurs less frequently. At least 8 different monosaccharides or combinations of these (di- or trisaccharides) can bind to the different hydroxyl groups of the flavonoid aglycone. The most common sugar moieties include D-glucose and L-rhamnose (Erlund, 2004). Flavonoid compounds such as flavones, isoflavones, flavonones, catechins, and anthocyanins are suggested to be potent *in vitro* antioxidants (Moyer et al., 2002[a]). The most abundant flavonoid in fruit is flavonol (catechin) (Kris-Etherton et al., 2002).

In most studies, an association exists between flavonoid intake and risk of chronic diseases. The term flavonoid refers to flavonols and flavones, with quercetin being the most abundant (Erlund, 2004) and it is the widespread throughout the plant being found in fruit, vegetables, nuts, seeds, flowers, and bark (Kris-Etherton et al., 2002) The superiority of quercetin in inhibiting both metal and nonmetal-induced oxidative damage is partially ascribed to the free 3-OH substituent on the B phenolic ring, which is thought to increase the stability of the flavonoid radical. This antioxidant mechanism is shown as follows:

$$F\text{-}OH + R^{\bullet} \rightarrow F\text{-}O^{\bullet} + RH$$

The B phenolic ring that flavonols contain with a 3-OH is planar, while the flavones and flavanones, lacking this feature, are slightly twisted. Planarity permits conjugation, electron dislocation, and a corresponding increase in flavonoid phenoxyl radical stability. Removal of a 3-OH abrogates coplanarity and conjugation, thereby compromising scavenging ability (Heim et al., 2002). Polymerization of flavanol to tannins or proanthocyanidins lead to higher structural existence of flavonoids in association with (+)catechin and (-)epicatechin monomers. Increasing degree of polymerization enhances effectiveness of flavonoids against a variety of radical species (Heim et al., 2002).

Anthocyanins are the major component of the phenolics/flavonoid class. They are copigments in flowers that attract pollinating insects and are responsible for the characteristic red, blue, and violet colors of berries, plums, apples, eggplants, wines, and certain vegetables. Hence, they are major sources of flavonoids in human diet (Erlund, 2004; Heim et al., 2002). The color of anthocyanin depends on pH and they lose their color rapidly at pH values > 3 (Redus et al., 1999). Varying pH causes structural transformation that alters both color quality and intensity (Stintizing et al., 2002[a]). Thus, many of the anthocyanins are violet or blue in alkaline media yet become red upon addition of acid. For example, the color of red fruit and vegetables shifts toward violet and gray-blue if the pH becomes basic (Potter and Hotchkiss, 1996).

Chlorophylls

Chlorophylls are bright green natural pigments largely contained within the chloroplasts which have a primary role in the photosynthetic production of carbohydrates from carbon dioxide and water (Potter and Hotchkiss, 1996). Although chlorophylls are not considered dietary antioxidants, they are widely distributed among green fruit and vegetables (Ferruzzi and Schwartz, 2001) with chlorophyll a and b derivatives predominate in higher plants (Ferruzzi et al., 2002). All natural chlorophyll derivatives can be described as substituted tetrapyrrols with a magnesium ion bound in the center.

Chlorophylls have been excluded in most research despite being the most abundant pigments in nature. The scarcity of information could be attributed to the difficulty to obtain pure and stable chlorophylls and their derivatives. Chlorophylls and pheophytins (metal free chlorophyll derivative) have been reported to possess

antimutagenic activity and antioxidant activity by breaking the radical chain reaction caused by autoxidation of vegetable edible oils stored in the dark via a hydrogen donating mechanism. The intact chemical structure of porphoryin and its chelating metal are reported to be important for antioxidant activity. Metallo-derivatives have been observed to exhibit significant higher antioxidant capacity than metal-free derivatives (Ferruzzi and Schwartz, 2001). Generally, chlorophyll a is more abundant over chlorophyll b by a 3 to 1 ratio. The antioxidant capacity of native chlorophyll a was found to be significantly higher than chlorophyll b (Ferruzzi et al., 2002). The antioxidant capacity of chlorophyll a might be due to its high level found in plants thus it may play a role in protecting against lipid oxidation (Lanfer-Marquez et al., 2005). Despite their abundant distribution in nature, there is no RDA or requirement for chlorophyll and their derivatives.

Green flesh is a distinguishing feature of ripe 'Hayward' kiwifruit and the color is due to the retention of chlorophyll during ripening. Chlorophylls a and b were both present in 'Hayward' with chlorophyll a present at a greater concentration (McGhie and Ainge, 2002; Nishiyama et al., 2005). The orange color visible during senescence and ripening of many fruit is due to the accumulation of esterified carotenoids associated with the transition of chloroplast to chromoplasts. Since 'Hayward' does not contain any esterified carotenoids, it "stays green" as the transformation of chloroplast to chromoplast does not appear to occur (McGhie and Ainge, 2002).

Evidence of Nutritional Benefits

Brief overview of important chronic diseases associated with ROS

A major focus in current research on the nutritional benefits of dietary antioxidants is based on their prevention of chronic diseases related to aging such as cardiovascular diseases (CVD), neurodegenerative disease, and cancer. Lipid peroxidation has been shown to be the initiation step for many of these chronic diseases. Hence, a brief review is provided on lipid oxidation and their association with two examples of chronic diseases. Also, an overview of cancer formation is introduced. The nutritional benefits of dietary antioxidants are explained following this section.

Dietary fats from food sources are transported intracellularly via lipoproteins after digestion and absorption. Low-density lipoprotein (LDL) is the most cholesterol rich lipoprotein that contains approximately 2700 fatty acids/molecules, about half of which are polyunsaturated fatty acids (PUFAs) that are very sensitive to oxidation (Wilcox et al., 2004). Oxidative modification of low density lipoprotein (oxLDL) by increased formation of ROS is an important initial event for the pathogenesis of atherosclerosis and other cardiovascular diseases (Yoshida et al., 2003; Milne et al., 2005; Kaliora et al., 2005).

Cardiovascular disease is the primary cause of mortality in the US, Europe and Japan (Wilcox et al., 2004). Most cardiovascular events are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall. Oxysterols appear to be a possible reactive mediator of the structural and functional changes occurring in the vascular wall (Poli et al., 2004). Three types of pathological entities are generally recognized: foam cells, fatty streaks, and fibrous plaques. When the lumen of the vessel

becomes completely occluded, a stroke or myocardial infarction occurs. The pathological entities are related to products of lipid peroxidation (Willcox et al., 2004). In addition, people with diabetes have an increased risk of atherosclerosis that is partially accounted by an increased level of oxLDL. Higher plasma lipid peroxides and higher markers of oxidation have been demonstrated in diabetic patients (Willcox et al., 2004).

In-vitro experiments show that oxidized LDL causes endothelial cells to signal monocytes into the arterial wall (Willcox et al., 2004). The monocytes are then converted into macrophages that engulf the oxidatively modified LDL and eventually become foam cells, which composes the early-stage atherosclerotic plaques (Milne et al., 2005; Nordberg and Arnér, 2001). It is proposed that macrophages identifies oxLDL as a non-self component and engulfs it in an unregulated manner thereby transforming themselves into lipid-laden foam cells (Wilcox et al., 2004). Consequently, the fatty streak engendered from the foam cells becomes the earliest and most common atherosclerotic lesions in CVD (Willcox et al., 2004).

The primary targets of ROS are free long-chain fatty acids in the cytosolic compartment and membrane-bound lipids leading to the formation of lipid peroxides (Kaliora et al, 2005). The central nervous system (CNS) is especially vulnerable to ROS attack due to high concentrations of PUFAs in neuronal cell membranes and a high level of transition metals (Zatta et al., 2002, Cherubini et al., 2005). The brain consumes approximately 20% of body oxygen although it is poorly protected with antioxidant enzymes. Membrane lipid peroxidation in the brain is associated with neurodegenerative processes in brain injury. Chronic diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis are outcomes of this effect.

Increased ratio of cholesterol and phospholipids, particularly, in the membranes of the brain are observed during aging (Zatta et al., 2002). Thus, the membrane of the brain has a high propensity to become the initiation site of aging-related diseases.

One key mechanism proposed for the induction of cancer accounts for an equilibrium between cell proliferation and cell death. The most powerful effect of metals and ROS on signaling pathways has been observed in the mitogen-activated protein kinase (MAPK) pathway. ROS activate MAPK, which turns on its downstream mediators, the nuclear transcription factors (AP-1, NF-κB, p53, HIF-1, NFAT). These factors regulate the expression of protective genes that repair damaged DNA, power the immune system, block the proliferation of damaged cells, and stimulate apoptosis. Subsequent mutations in these proteins lead to production of an unique cell type that can replicate uncontrollably in an autonomous fashion. This cell type is resistant to destruction by the immune response and capable of invading other tissues (Tate et al., 2003). During cell proliferation, protein p53 plays a primordial role in checking the integrity of DNA. p53 triggers cell death or apoptosis if the DNA cannot be repaired due to excessive damage hence eliminates mutation. More than half of cancers have defects in upstream or downstream genes of p53 function. This leads to uncontrolled apoptosis and destruction of healthy cells. AP-1 is a group of proteins that behave as downstream signal transducers of MAPK. One effect of AP-1 in cancer experiments is their ability to increase cell growth and differentiation. The activation of nuclear transcription factor NF-κB is linked to the carcinogenesis process because of its role in differentiation, inflammation, and cell growth. Other transcription factors such as NF-κB have been expressed in tumor cells from blood neoplasms, colon, breast, and pancreatic cell lines

(Valko et al., 2006). For optimal cancer prevention, antioxidant compounds must be able to block mutagenesis not only from the initiation lesion, but also from evolution of the original tumor cell into a more aggressive phenotype (Tate et al., 2003).

Nutritional Benefits of Ascorbate, Carotenoids, and Phenolics

Ascorbate

The dietary benefit of vitamin C includes its possible preventative effect on diseases ranging from the common cold, cancer and heart diseases, to cataracts (Gropper et al., 2004). The role of ascorbate in disease prevention is believed to be from its ability to scavenge free radicals via the ascorbate-glutathione cycle in chloroplast of the plants and in mitochondria of animals. The cycle leads to scavenging of H_2O_2 which is detoxified to H_2O and O_2 (Edreva, 2005). The importance of vitamin C is due to its capability of regenerating tocopherol from a tocopheroxyl radical which is formed by inhibition of lipid peroxidation by vitamin E. This process allows for transport of a radical from a lipophilic compartment to an aqueous compartment where it is quenched by an efficient enzyme defense system (Diplock et al., 1998, Lapointe et al., 2006). Also, ascorbate protects LDL against copper mediated modification by decreased binding of Cu^{2+} to LDL. Thus, ascorbate effectively prevents initiation of oxLDL by acting as a co-antioxidant (Kaliora et al., 2005). Ascorbate is abundant in many fruit and is more prevalent in most fruit than is vitamin E (Leong and Shui, 2002) mainly due its water solubility. Vitamin E is most active in a lipid environment due to its hydrophobicity. In many studies, vitamin C protects against cell death triggered by various stimuli. Ascorbate may act as an effective antioxidant that is able to slow down the process of

uncontrolled cell proliferation in cancer by regulating the AP-1 complex (Valko et al., 2006).

The major dietary source of ascorbate is found in fruit, especially citrus, kiwifruit, cherries, tomatoes, melons, and potatoes (Diplock et al., 1998; Kaliora et al., 2005). The recommended dietary allowances (RDA) for adult men and women are 90 mg and 75 mg, respectively, with requirements estimated at 75 mg and 60 mg, respectively. Smokers are recommended to consume an extra 35 mg vitamin C daily (Gropper et al., 2004). Vitamin C is an important health component of kiwifruit, which is one of the reasons why kiwifruit are becoming increasingly popular. One study documented that the New Zealand kiwifruit purchased from the market has approximately 52.8 mg/100g FW of ascorbate (Leong and Shui, 2002). The nutritional data reported from the USDA (2005) of fresh, raw kiwifruit (*A. chinensis*) showed an average of 92.7 mg of total ascorbate in 100g of edible portion. Hence, the RDA of vitamin C can be fulfilled by simply consuming one or two kiwifruit per day. Based on the USDA database (USDA, 1986), vitamin C content of kiwifruit is greater than strawberry, orange, grapefruit, honeydew, melons, and tomato. Wang et al. (1996) demonstrated that the oxygen radical absorbing capacity (ORAC) of a fruit was usually less than 15% except for kiwifruit and honeydew melon. This suggests that the major source of antioxidant in kiwifruit may be contributed from vitamin C. Ascorbate has been shown to make only a minor contribution to the total antioxidant activity or capacity in blackberries; however, this feature is cultivar dependent (Thomas et al., 2004; Clark et al., 2002; Deighton et al., 2000). The USDA (2005) reported that the average total ascorbic acid of raw blackberry (*Rubus spp*.) is 21.0 mg/ 100g of edible portion.

Carotenoids

It has been suggested that carotenoids are the chemoprotective agents in fruit and vegetables (Nishiyama et al., 2005). Clinical evidence indicating it acts as a chemoprotective agent in fruit and vegetables comes from epidemiologic studies in which 11 out of 15 of the studies demonstrated a significant inverse relation of β-carotene intake and/or plasma level and risk of lung cancer; 10 out of 17 case-control studies found that a high intake of fruit and vegetables that are rich in carotenoids have been associated with decreased risk of cancer of the lung and stomach (Krinsky and Johnson, 2005; Young and Low, 2001). In cancer prevention, carotenoids can function as a provitamin A, which would have an effect on cellular differentiation and proliferation. However, β-carotene has been observed as a co-carcinogen in some experiments, especially in individuals exposed to cigarette smoke and other carcinogens found in industrial settings (Willcox et al., 2004). Other antioxidant functions of carotenoids include prevention of free radical induced damage to cellular DNA and other molecules. Immunomodulatory effects could enhance immune surveillance in tumorigenesis (formation of tumors) and enhanced cell-cell communication would restrict clonal expansion of initiated cells (Krinsky and Johnson, 2005). As mentioned, lutein and zeaxanthin have been suggested to be protective against certain eye diseases, because they are the only carotenoids found in the retina and lens. This region includes high visual acuity and contains the highest density of cone photoreceptors. Lutein and zeaxanthin prevent age-related macular degeneration and other ocular diseases by filtering blue light, which is particularly damaging to photoreceptors and to the retinal pigment epithelium color (Nishiyama et al., 2005). Recommendations for vitamin A intake is expressed as retinol activity equivalents (RAE).

1 RAE = 1µg of retinol = 12µg of β -carotene. This means, eating 12 µg of beta-carotene is equal to the consumption of 1µg of retinol. The requirements for vitamin A for adult men and women are 625 and 500µg RAE, respectively. The RDA for vitamin A for adult men and women are 900 and 700µg RAE, respectively (Gropper et al., 2004).

The yellow to orange color of carotenoids is an attractive index for consumers and also influences the health effects of the foods (Nishiyama et al., 2005). McGhie and Ainge (2002) reported that the carotenoids detected in 'Hayward' kiwifruit were those generally associated with chlorophyll-containing tissues and in unesterified form. The carotenoids detected included β-carotene, lutein, violaxanthin, and 9'cis-neoxanthin, with β-carotene concentration the highest (McGhie and Ainge, 2002; Nishiyama et al., 2005). 'Hort16A' kiwifruit are expected to contain greater concentration and possibility different types of carotenoids than the 'Hayward' cultivar, because of their yellow flesh color at maturity. They were found to contain carotenoids, however, in different forms than that of the 'Hayward' cultivar (McGhie and Ainge, 2002). The xanthophyll components were identified as 9'-cis-neoxanthin, violaxanthin, and lutein that accumulate in the yellow flesh of 'Hort16A' as esterified compounds. The accumulation of xanthophylls esterified with acyl fatty acids is associated with the conversion of chlorophyll-containing chloroplasts to carotenoid-containing chromoplasts during fruit ripening. The yellow color of 'Hort16A' is mainly due to the absence of chlorophylls in the flesh rather than a significant increase in carotenoid concentration (McGhie and Ainge, 2002).

Phenolics

Phenolic compounds are closely associated with the sensory and nutritional quality of foods, contributing directly or indirectly to desirable or undesirable aroma and taste. In low concentrations, phenolics may protect food from oxidative deterioration, thus they are good antioxidants and substrates for prevention of oxidative browning. However, at high concentrations, they (or their oxidative products) may participate in discoloration of foods, and interact with proteins, carbohydrates, and minerals (Imeh and Khokhar, 2002). The predominant mode of antioxidant activity is believed to be radical scavenging via hydrogen donation. Other radical quenching mechanisms are through electron donation and singlet oxygen quenching. Substituents on the aromatic ring affect the stabilization and therefore affect the radical-quenching ability of these phenolic acids (Robbin, 2003; Reyes-Carmona et al., 2004). Caffeic acid, one of most prominent naturally occurring hydroxycinnamic acids, can selectively block the biosynthesis of leukotrienes, components involved in immunoregulation diseases, asthma, and allergic reactions (Robbins, 2003). Other studies have shown caffeic acid and its ester derivatives might possess antitumor activity against colon carcinogenesis (Robbins, 2003). Phenolic acids have also been shown to inhibit AP-1 transcriptioanl activity thus inhibit potential tumor cell proliferation. Caffeic acid derivatives (e.g. dicaffeoylquinic and dicaffeolytartaric acids) have been shown to be potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase. This enzyme catalyzes the integration of viral DNA into the host chromatin (Robbins, 2003). The total dietary intake of polyphenols is about 1 g/day and is 10 times higher than that of vitamin C and 100 times higher than those of vitamin E and carotenoids (Scalbert et al., 2005). Total polyphenols

are typically consumed on a daily basis of 25mg-1g/day thus they are recognized as dietary antioxidants due to their ubiquitous presence in plant-based foods such as in fruit, vegetables, grains, teas, coffee, and spices (Robbins, 2003). There is no RDA currently available for total polyphenols.

By virtue of their capacity to inhibit LDL oxidation, flavonoids have demonstrated unique cardioprotective effects. Flavonoid-rich diets have been shown to reduce myocardial post-ischemic damage in rats. High flavonoid intake reflects lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men and reduced the risk of coronary heart disease by 38% in postmenopausal women (Heim et al, 2002). Quercetin has been studied more thoroughly than other flavonoids, not only because of its abundance, but because it has been reported to exhibit antioxidative, anticarcinogenic, anti-inflammatory, anti-aggregatory, and vasodilating effects (Erlund, 2004; Kahkonen et al., 2001). Other protective effects include inhibition of human immunodeficiency virus type 1 protease as well as antimicrobial and anticarcinogenic capacities (Chun et al, 2005). Epidemiologic evidence shows that food providing 16-24 mg/day of quercetin exhibits a protective effect against CVD (Kris-Etherton et al., 2002). The daily intake of total flavonoids in American diets is proposed to be 103 mg CE (catechin equivalence). Although there is no RDA currently available for flavonoids, studies have shown that approximately 30mg/day was associated with approximately 50% reduction in coronary heart disease mortality rate when compared with individuals who had <19 mg/day (Kris-Etherton et al., 2002).

Studies have shown that antioxidant capacities of red fruit are relatively much higher than those of other fruit. The anthocyanins in red fruit are strong contributers to

the total antioxidant capacity of fruit (Chun et al., 2005). Blackberries are of particular interest because of their high polyphenolic content especially anthocyanin, flavonols, and ellagic derivatives. Furthermore, blackberries ('Navaho',' Triple Crown', 'Arapaho', 'Kiowa', 'Hull', 'Chester', 'Chickasa', and 'Choctaw') have been reported to strongly suppress mutagenesis induced by carcinogens that interact with DNA (Tate et al., 2003). Hence, they are potent anti-cancer reagents; ellagic acid has been especially studied in this respect (Tate et al., 2003). Blackberry seeds and leaves also contain high antioxidant levels (Wang and Lin, 2000). Blackberry seeds account for approximately 5% by weight of the berry. The seeds are very rich in procyanidins, ellagic acid derivatives, and ellagitannins (Siriwoharn and Wrolstad, 2004). Researchers are currently linking anthocyanin activity to improved vision, controlling diabetes, improving circulation, preventing cancer, and retarding the effects of aging, particularly loss of memory and motor skills (Rababah et al., 2005). Cyanidin-3-glucoside, a primary anthocyanin in blackberry, has been reported to have the highest antioxidant capacity of 14 different anthocyanins tested (Elisia et al., 2006). Thus, blackberry and their seed are a potential source for nutraceuticals and natural antioxidants (Siriwoharn and Wrolstad, 2004). Fan-Chaing (1999) reported anthocyanin content ranged from 70 to 201 mg/100g FW with a mean of 137mg/100g for 52 blackberry selections collected from the United Sates and international sources. Moyer et al. (2002[b]) also reported the total anthocyanin content for blackberry cultivars ranged from 8 to 230 mg/100g FW based on a spectrophotometry method. The daily intake of anthocyanins in humans has been estimated to range from 180 to 215 mg/day, about 20% of the total polyphenols consumed per day in the United States (Scalbert et al., 2005). Hence, supplementing blackberries with a balanced diet

could be more effective and economical than consuming an individual antioxidant in protecting the body against various oxidative stresses (Wang and Lin, 2000).

Kiwifruit has not received much attention for their polyphenol content. Analysis of total phenolics in kiwifruit obtained from supermarkets indicates the level of phenolic compounds to be low in comparison with other fruit. Total phenolic concentrations reported was 61.21 mg GAE (gallic acid equivalence)/100 g FW, approximately 1/6 of plum and ¼ of strawberry; total flavonoids was 7.30 mg CE/100g FW, approximately 1/26 of plum and 1/7 of strawberry (Chun et al., 2005). This might be beneficial to kiwifruit processing at some point because the low activity and low levels of polyphenol do not cause browning readily on cut surfaces of kiwifruit. Instead, enzymatic browning in the presence of oxygen that occurs in kiwifruit juice is proposed to be due to acid-catalyzed ascorbate degradation in kiwifruit juice concentrate. Recently, a red-flesh kiwifruit of *A. chinensis* has been reported to contain anthocyanin (~14 mg/100g FW) (Montefiori et al., 2005); however, the concentration of anthocyanin is very low compared to that of blueberries (*Vaccinium ashei*) (Sellappan et al., 2002) and black raspberries (*Rubus occidentalis*) (Wada et al., 2002). It is therefore, unlikely that the presence of anthocyanins would make a significant contribution to antioxidant capacity of kiwifruit.

Chemistry of Reactive Oxygen Species

Reactive Oxygen Species (ROS)

Oxidants produced by cells are mainly derived from aerobic respiration and mitochondrial oxidative activity such as accidental leakage of superoxide ($O_2^{\bullet -}$) and

hydrogenperoxyl (HOO$^{\bullet}$) radicals from the electron transport chain (ETC) (Zatta et al., 2002). Interestingly, mammalian cells can process 10^{12} O_2 molecules per day and generate significant amount of hydrogen peroxide (H_2O_2) that is estimated to account for approximately 2% of the total oxygen uptake by the organism (Valko et al., 2006). Approximately 2 x 10^{10} superoxide and peroxide (created by one electron lost of H_2O_2) are leaked out of mitochondria (Willcox et al., 2004). The terminal enzyme in the ETC, cytochrome oxidase (E.C. 1.9.3.1), is responsible for neutralizing ROS by adding four electrons to an oxygen molecule to generate two water molecules as the final product. This step effectively prevents ROS from accumulating in mitochondria and leaking into other organelles. A stepwise generation of reactive oxygen species is able to occur. The generated ROS are randomly leaked out of the ETC (Zatta et al., 2002).

$$O_2 \xrightarrow{e^-} O_2^{\bullet -} \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e^-} H_2O$$

The net reaction is

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

In addition, microsomes and peroxisomes are sources of ROS. Microsomes are responsible for 80% of H_2O_2 concentration produced *in-vivo* at hyperoxia sites. Peroxisomes are known to generate H_2O_2, but not $O_2^{\bullet -}$. There are some ROS molecules found in the outer mitochondrial membrane that are not linked to respiration. ROS can be induced from external sources such as radiation from UV or X-rays. UV and X-rays are able to extract electrons from cellular water to form various ROS molecules. ROS can also be generated during metabolism of xenobiotics (foreign material to body or living organism such as drugs) and steroids. In order to successfully transport these large molecules into the cell, the electron transport chain localized in the smooth endoplasmic

reticulum (SER) hydroxylates different substrates and other liposoluble substances to make them more hydrophilic thus more readily removable. Superoxide radical ($O_2^{\bullet -}$) may be initiated by NADPH cytochrome P450 reductase (E.C.1.6.2.4) during substrate hydroxylation. ROS can be generated and inhaled with cigarette smoke (Poli et al., 2004; Diplock et al., 1998). Finally, ROS can be induced by neutrophils and macrophages in the presence of metal during inflammation (Valko et al., 2006). In plants, ROS are continuously produced as byproducts of various metabolic pathways localized predominantly in chloroplasts, mitochondria, and peroxisomes. The photosynthetic electron transport leads to the production of ROS in chloroplasts and peroxisomes under high light, drought, low temperature, and mechanical stress (Apel and Hirt, 2004). Plant mitochondria do not produce more ROS relatively to the mammalian mitochondria. One of the reasons could be the presence of the alternative oxidase (AOX) that catalyzes the tetravalent reduction of O_2 by ubiquinone in the ETC. The AOX competes with cytochrome bc_1 complex (Complex III) for electrons and thus reduce ROS production in mitochondria by preventing accidental leakage of electrons (Wager, 1995; Maxwell et al., 1999).

Reactive oxygen species (ROS) derived from the mitochondrial electron transport system have been traditionally viewed as toxic to human health. ROS reacts with lipids, proteins, sugars, and vitamins, producing undesirable volatile compounds, destroying essential fatty acids, amino acids and vitamins, and producing carcinogens. ROS changes the functionalities of proteins, lipids, and carbohydrates by forming oxidized dimers and trimers (Choe and Min, 2006). Cellular aging, mutagenesis, carcinogenesis, and coronary heart disease, possibly through destabilization of membranes, are observed as outcomes

of these undesirable reactions induced by excess ROS (Heim et al., 2002; Chun et al. 2005). The derivation and mechanism of ROS are focused in the following section.

The term oxidative stress has been defined very vaguely. Cherubini et al. (2005) define oxidative stress as the condition occurring when the physiological balance between pro-oxidants and antioxidants is disrupted in favor of the former with potential damage for the organism. Free radicals are generated during the process of cellular physiological imbalance. Wilcox et al. (2004) define a free radical as any chemical species capable of independent existence carrying one or more unpaired electrons. Thus, they can react readily because they acquire another electron to fill the orbital to become stable. ROS encompass both oxygen radical and non-radical derivatives (Choe and Min., 2006). Examples of possible reactive oxygen species are illustrated in Table 1. The oxygen radicals that will be discussed more in detail include superoxide anion, hydroxyl, peroxy, alkoxy, and hydroperoxyl radicals. These free radicals can readily attack but the non-radicals must acquire one or two unpaired electrons to become active. The nonradical derivatives are hydrogen peroxide, ozone, and singlet oxygen (Choe and Min, 2006).

Superoxide radical ($O_2^{\bullet -}$) is generated enzymatically and chemically from triplet oxygen. Triplet oxygen is the most stable and abundant form of oxygen and is the common oxygen that we breathe. When a singlet electron is added to one of the antibonding π^* orbital, the parallel spin is destroyed leaving one unpaired and one coupled π^* orbital. A superoxide radical is formed when triplet oxygen carries one unpaired electron at its π^* orbital. Hydroperoxy radical ($HOO^{\bullet}$) is a protonated form of superoxide radical and produced by the reaction of hydrogen peroxide and hydroxyl

radicals ($OH^{\bullet}$) (Choe and Min, 2006). Superoxide anion and hydrogen peroxide are moderately reactive with biological molecules, but in the presence of transition metals, such as iron or copper, they can generate a powerful reactive species, namely the hydroxyl radical ($OH^{\bullet}$), through the Fenton and Haber–Weiss reactions (Poli et al, 2004). A common misconception is that all the possible ROS listed are able to react with lipids immediately. Hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) are essentially non reactive with lipids (Halliwell and Whiteman, 2004) unless they have gained an electron from other sources to make $OH^{\bullet}$.

Transition metal ions are potent catalysts and capable of initiating processes in lipid peroxidation, especially in membranes. Fe^{2+} is oxidized to Fe^{3+} to generate a superoxide radical (Eq. 1). When it becomes oxidized with hydrogen peroxide a hydroxyl radical and a hydroxyl anion are produced (Fenton reaction) (Eq. 2) (Wilcox et al., 2004; Zatta et al., 2002). Singlet oxygen ($^1O_2$) is the high energy form of triplet oxygen. After it transfers its energy to the solvent via light emission or internal conversion, it returns to the triplet state (Choe and Min, 2006). Singlet oxygen participates in generation of ROS as shown below.

$Fe^{2+} + {}^1O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$ (Eq. 1)

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$ (Eq. 2, Fenton Reaction)

$Fe^{2+} + H_2O_2 \rightarrow Fe(OH)_3 + OH^-$ (Eq. 3)

The combination of equation 1 and equation 2 results in the Haber-Weiss reaction:

$O_2^{\bullet -} + H_2O_2 \rightarrow {}^1O_2 + OH^{\bullet} + OH^-$ (Eqn. 4)

Both $OH^{\bullet}$ and $Fe(OH)_3$ species are extremely reactive towards lipids, proteins, and nucleic acids (Zatta et al., 2002). As seen in equation 1, although singlet oxygen does not qualify as ROS due to no unpaired electrons, it can act as a potential generator of a

superoxide radical in the presence of transition metal ions. Therefore, effective quenching of singlet oxygen prevents the Harber-Weiss reaction, which prevents hydroxyl radicals from forming. Tocopherol, carotenoids, phenolics, ascorbate, urate and lipid can quench singlet oxygen.. Saturating fatty acids quench singlet oxygen by energy transfer from singlet oxygen to the vibrational sublevels of -CH- and COOH groups of fatty acids (Choe and Min, 2006).

Peroxy radical ($ROO^{\bullet}$), alkoxyl radical ($R_1O^{\bullet}$), and hydrogen peroxide are created during the process of lipid peroxidation. Lipid peroxidation is extremely harmful to living organism because once it is initiated it cannot be terminated until it is either scavenged by either enzymatic or non-enzymatic systems in the cell, or until it meets another free radical. It is the initiation step of cardiovascular diseases, neurodegenerative diseases, and it appears to be related to diabetes (Wilcox et al., 2004). The initiation step involves an oxygen radical ($OH^{\bullet}$ being most reactive) attacking a methylene group ($-CH_2-$) of a PUFA to abstract a hydrogen atom and an electron thus splitting the double bonds. This reaction creates a lipid radical, $R_1^{\bullet}$ (Negrelo Newton et al., 2004). With subsequent rearrangement, lipid epoxides, hydroperoxides (R_1OOH), alkoxyl ($R_1O^{\bullet}$), and peroxyl ($R_1OO^{\bullet}$) radicals are successively produced (Willcox et al., 2004; Zatta et al., 2002). The latter two species create a chain reaction by abstracting another hydrogen atom (Propagation Step), hence free radicals are continuously being propagated. Only when one free radical accidentally collides with another free radical is the chain reaction terminated (Termination step) (Zatta et al., 2002). PUFAs having two or more double bonds are increasingly susceptible to free radical attack as the number of double bonds

increases (Willcox et al., 2004). The mechanism of lipid peroxidation is proposed as follows.

Initiation	$R_1H + OH^- \rightarrow R_1^{\bullet}$
Propagation	$R_1^{\bullet} + O_2 \rightarrow R_1OO^{\bullet}$
	$R_1OO^{\bullet} + R_2H \rightarrow R_2OOH + R_2^{\bullet}$
Termination	$R^{\bullet} + R^{\bullet} \rightarrow R\text{-}R$
	$R^{\bullet} + R_1OO^{\bullet} \rightarrow ROOR$
	$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$

Flavor and Shelf Life

ROS in food is inevitable due to their biological nature. Furthermore, accumulation of ROS makes food products less acceptable to consumers by lowering the overall quality of foods during storage and marketing (Choe and Min, 2006). The most important ROS in foods during storage and processing are hydroxyl and singlet oxygen. Flavor is extremely affected by ROS generated during food storage and processing. Peroxidation of lipids is a major concern of food manufacturers because it can lead to the development of unpleasant "rancid" or "off" flavors as well as toxic end products. For example, singlet oxygen is able to illuminate riboflavin (i.e. transfer excited energy to a photosensitizer so that it can return to ground state oxygen) in milk making it spoil quickly (Halliwell et al., 1995). It is extremely important to control the formation of ROS in foods to preserve food quality (Choe and Min, 2006).

Other factors which contribute to decreased fruit flavor and shelf life include by products of polyphenol oxidase (PPO; E.C. 1.14.18.1). PPO catalyzes the formation of highly active quinines that react with amino or sulfhydryl groups in proteins or enzymes. Quinones lead to polymerization and condensation reactions between proteins and polyphenols, forming brown pigments. PPO activity may also be responsible for the loss

of red color of some fruit through the degradation of anthocyanin pigments (Gonzalez et al., 2000).

Physicochemical and Sensorial properties of Small Fruit

Titratable Acidity, Initial pH, Soluble Solids Content

Consumers demand high quality fruit that have an attractive appearance, high nutritional value, and good taste (Crisosto and Crisosto, 2001). The appearance and nutritional value are generally influenced by pigments and vitamins in fruit where as taste is primarily influenced by acidity, sweetness and volatiles. Two common ways to quantify the acid content of samples are determination of initial pH and titratable acidity (TA). pH is defined as the negative logarithm of the hydrogen ion concentration in solution where pH < 7 indicates acid, pH > 7 indicates base, and pH 7 is neutral. The magnitude of pH provides immediate or actual acidity (actual hydrogen ion concentration). Titratable acidity indicates the total or potential acidity such that it includes the total number of acid molecules, both protonated and unprotonated. Soluble solids content (SSC) includes molecules that are truly soluble in an aqueous sample. Fats, for example, are insoluble in water thus cannot be considered as soluble solids. In fruit juices, sugars are the predominant soluble solids, but many salts, acids, and proteins can also be classified as soluble solids (FS 310 Lab IV, University of Wisconsin, 2006). In commercial standards, the SSC of a ripe fruit is often used to indicate its sweetness (Crisosto and Crisosto, 2001). SSC is measured as units of Brix value that is defined as percent sucrose by weight. SSC has been shown to reflect the eating quality of ripe fruit (Burdon et al., 2004). Sweet and sour taste are correlated with SSC and TA at ripeness

(Fisk et al., 2006; Marsh et al., 2004). The ratio of sugar to organic acids (SSC/TA) has been related to flavor quality for a variety of fruit and indicate the optimum time for harvesting (Kafkas et al., 2005).

In California, the standard for harvest maturity of 'Hayward' kiwifruit is to have a minimum of 6.2-6.5% SSC with flesh firmness approximately 14 lb or 6.3 kg force (Crisosto and Crisosto, 2001). In Chile and New Zealand, a minimum average SSC of 6.2 % measured in 10 fruit is required for export. If 2 out of 10 fruit have less than 5.8% SSC, the orchard is not considered acceptable for export (Rushing, 2004; Crisosto and Crisosto, 2001). However, kiwifruit can be left on the vine until the SSC reaches 10-12% if marketed locally.

The ideal storage condition for fuzzy kiwifruit, *A. deliciosa* var. 'Hayward', harvested at 6.5% SSC is at 0 °C for up to 6 months for good fruit quality (Fisk et al., 2006). Refrigeration is known to delay the ripening process. The increase in SSC is anticipated because the fruit of *A. deliciosa* have been shown to contain sucrose phosphate synthase (SPS), a key enzyme in sucrose biosynthesis whose activity increases during ripening and in response to low temperature stress (MacRae et al, 1992; Lanerkämper et al., 1998). The decrease in TA in cooler climates such as New Zealand is due to the induction of vacuolar H^+-translocating inorganic pyrophosphatase (V-PPase; EC 3.6.1.1) (Marsh et al., 2004). In fruit, V-PPase supplies a driving force for loading sugars from the cytosol into the vacuole by generation of an electrochemical proton gradient across the vacuolar membrane (antiport system) (Hockema and Echeverria, 2000). V-PPase translocates proton and organic acids into the vacuole using energy released from ATP and pyrophosphate hydrolysis, respectively (Mitsudal et al, 2003;

Etienne et al, 2002). The low pH created by V-PPase is found to automatically cleave sucrose entering the vacuole at a rate dependent on the hydronium ion concentration and temperature. In citrus fruit, cleavage of sucrose increase osomoticum of the vacuole, maintaining turgor pressure while simultaneously increasing sink strength (Hockema and Echeverria, 2000). The tonoplast (vacuolar membrane) gradient created by proton accumulation provides energy which can be utilized via pyrophosphatase to produce ATP under anaerobic conditions or in the absence of other energy sources (Hockema and Echeverria, 2000). Thus, the decrease in pH and titratable acidity contributes to increased concentrations of ATP and higher SSC in fruit vacuoles. In contrast to fruit from warmer climates, the gradients are maintained until dissipation is triggered by ripening or cool storage (Marsh et al., 2004). Accumulation of TA during growth and delay in the decrease of TA during refrigeration contribute to the characteristic acidity in 'Hayward' fruit (Marsh et al., 2004). Consumers may interpret a high TA content as unacceptable flavor. California grown kiwifruit ('Hayward') are most desirable by consumers when ripened SSC (rSSC) > 11.6% (Crisosto and Crisosto, 2001). The same study showed that rSSC <11.6% could be altered by TA of the fruit, with too high a TA (>1.17%) making the fruit taste sour or astringent. On average, kiwifruit contained 0.9-2.5% titratable acidity, with 40-50% as citrate, 40-50% as quinate, and 10% as malate (Marsh et al., 2004). Quinate or quinic acid had a stronger impact on perception of acidity than citric, malic or ascorbic acids (Marsh et al., 2005)

In blackberries, initial pH among six genotypes ('Navaho', 'Arapaho', 'Apache', 'Triple Crown', 'Loch Ness', and 'Chester') ranged between 3.3-3.9 (Thomas et al., 2005; Perkins-Veazie et al., 2000). Highest pH values were obtained for 'Arapaho' and

'Navaho' and lowest pH values for 'Loch Ness' and 'Triple Crown'. TA for the six cultivars ranged from 0.16-0.34%. 'Loch Ness' was reported to have the highest TA and 'Arapaho' and 'Navaho' the lowest. High SSC to TA ratio indicates a higher eating quality of fruit (Perkins-Veazie and Collins, 2001). 'Navaho' was reported by Thomas et al. (2005) to have the best flavor due to its high SSC (11.6%) and low TA (0.19%) ratio. 'Loch Ness' was considered to have low flavor due to its low SS (9.1%) and a very high acid content (0.34%).

Carbohydrates

In all plants, sucrose is one of most important sugar because it is the photosynthate that is transported through the phloem. Sucrose is commonly used as primary table sugar and it is the world's main sweetening agent with about 10^8 tons produced annually (Winter and Huber, 2000). During fruit elongation and expansion, sucrose and other fixed carbons are required to provide growing tissues with energy for metabolism and to provide osmotic solutes to maintain cellular turgor. Sequestering and net degradation of sucrose to yield hexose sugar derivatives in vacuoles permit the sink cell to maintain a sucrose gradient between itself and the phloem, allowing the continuous movement of sucrose toward the sink (Hockema and Echeverria, 2000; Winter and Huber, 2000). High sugar concentration and low acid are considered as sweet (Perkins-Veazie and Collins, 2001). Consumers prefer fruit that have a sweet taste more than aromatic flavor.

There are two specific enzymes capable of cleaving sucrose into simple sugars (hexoses) in plant cells to maintain a continuous supply of sucrose. Sucrose synthase

(E.C.2.4.1.13) performs a reversible reaction using sucrose and UDP to yield UDP-glucose and fructose. Invertase (E.C.3.2.1.20) catalyzes the irreversible hydrolysis of sucrose into an equimolar mixture of D-glucose and D-fructose (Winter and Huber, 2000). D-glucose and D-fructose are monosaccharides that are classified as aldose and ketos, respectively. D-glucose belongs to the aldose family which contains an aldehyde carbonyl group that can be readily oxidized to a carboxylic acid group. Aldoses have been called "reducing sugars" and its concentration can be measured as they reduce the oxidizing agent present in the medium. Although ordinary ketone groups cannot be oxidized, various ketone groups such as the one in fructose have reducing power. Fructose is readily isomerized to aldose in a basic solution by a series of keto-enol tautomeric shifts. Hence it can also be considered as a reducing sugar in a basic solution (Cui, 2005). Glucose and fructose can be both classified as reducing sugars.

'Hayward' kiwifruit is described by consumers as having fresh sweet-acid flavor and 'Hort16A' is perceived as having sweet and fruity flavor. The sweetness of kiwifruit is the result of the SSC and sugar content. Kiwifruit have been demonstrated to have a high content of glucose (~3 to 4%) and fructose (4 to 5%) in dry matter (Marsh et al., 2005). Sucrose is approximately 1% of the dry matter in both 'Hayward' and 'Hort16A'.

Wang et al., (2004) characterized the soluble sugars and acids of 17 representative blackberry varieties planted in the Pacific Northwest of the United States using high performance liquid chromatography. Glucose ranged from 22.6 to 45.8 mg/g and fructose ranged from 22.6 to 40.3 mg/g. 'Navaho' was reported by Kafkas et al. (2005) to contain the highest total sugar, fructose, glucose, and sucrose content out of five cultivars. 'Loch Ness' was the cultivar containing the highest levels of malic and ascorbic acid. Glucose

content in blackberry is important because it metabolically may serve as a precursor to ascorbic acid synthesis (Smirnoff et al., 2001). Hence, those cultivars identified with high hexose accumulation could possess high ascorbic acid content (Thomas et al., 2005).

III. THE ANTIOXIDANT PROFILE OF THORNLESS BLACKBERRIES (*RUBUS* SPP.) HARVESTED AT FULL RIPE STAGE

Abstract

Five erect and thornless cultivars of *Rubus* hybrid blackberries ('Loch Ness', 'Navaho', 'Arapaho', 'Apache', and 'Triple Crown') were analyzed for their antioxidant capacity and radical scavenging activity expressed as vitamin C equivalent antioxidant capacity (VCEAC), total phenolic (TPH), flavonoid (TF) and anthocyanin (ACY) were determined. ABTS and DPPH methods were highly correlated (R=0.897). Cultivar differences were observed with respect to antioxidant capacity, TPH and ACY ($P < 0.05$). The antioxidant capacity determined by the ABTS and DPPH methods ranged from 559.5 to 698.5 and 347.0 to 464.2 mg VCE/100g FW ,respectively, TPH from 221.2 to 342.1 mg gallic acid equivalent (GAE)/100g FW, TF from 53.6 to 71.4 mg catechin equivalent (CE)/100g FW and ACY from 38.3 to 72.3 mg cyanidin 3-glucoside equivalents (CGE)/100g FW. In general, 'Loch Ness' had the highest TPH (342.1 mg GAE/100g FW), TF (71.4 mg CE/100g FW), ACY (74.1 mg CGE/100g FW), antioxidant capacity (698.5 mg VCE/100g FW) and radical scavenging activity (464.2 mg VCE/100g FW) respectively. The results of this study indicates that phenolic compounds contribute significantly to the overall antioxidant capacities of Alabama grown blackberries. Such information will assist Alabama fruit growers and consumers in regards to the health

benefiting qualities of fresh fruit consumption as a daily dietary source of natural antioxidants.

Keywords: *Rubus spp.*, hybrid blackberry, phenolics, flavonoids, anthocyanins, antioxidant capacity, ABTS, DPPH, VCEAC.

Introduction

Blackberry is a favorite fruit in the southern United States due in part to its flavor and nutritional properties. Blackberries have a high commercial value due to their use in ice cream, juice, jam, marmalade, cakes, and many more food products. The potential health benefits of berries are being increasingly known (Beattie et al., 2005). High concentrations of bioactive compounds such as phenolics, flavonoids, and anthocyanin have been found in blackberries. Hence, they are also considered useful as dietary supplements (Kafkas et al., 2005; Moyer et al., 2002a). Detailed clinical and epidemiological studies have provided convincing evidence of the nutritional benefits of diets rich in fruit and vegetables in diminishing the occurrence of certain forms of cancers (Zhang et al., 2000; Kaur and Kapoor, 2001), cardiovascular disease (CVD) (Kris-Etherton et al., 2002; Lopackzynski and Zeisel, 2001; Lee et al., 2004), diabetes, cataracts, and inflammatory disease (Wilcox et al., 2004). Accordingly, approximately 20% or more of all cancers may be prevented by the inclusion of five daily servings of fruit and vegetables.

Considerable data illustrate that total phenolics (TPH), total monomeric anthocyanins (ACY), total flavonoid (TF), Vitamin C, and Vitamin E contribute

substantially to the antioxidative capacity of blackberry fruit (Wang and Lin, 2000; Clark et al., 2002; Sellapan, et al., 2002; Reyes-Carmona et al., 2005; Elisia et al., 2006). Genetic and environmental factors, such as cultivar, maturity, UV light exposure, and harvesting methods, influence in berry composition. Phenolic compounds and antioxidant capacity of blackberries are influenced by maturity at harvest and there is a pronounced variation among cultivars (Siriwoharn et al., 2004). Blackberry may be a better alternative and potential crop with high market value for farmers in Alabama. However, only limited information on fruit composition of specific cultivars grown in Alabama can be found. The objective of this study was to compare TPH, TF, ACY, and antioxidant capacities using ABTS and DPPH radical scavenging assay among five cultivars of *Rubus spp.* blackberries.

Material and Methods

Fruit source

Sample Collection. A blackberry cultivar trial was established in 1999 at the Gulf Coast Research and Extension center at Fairhope, Alabama, USA (30^{o}, 33 N latitude) to study yield and fruit quality traits (Himelrick and Nesbitt, 2002). The blackberry cultivars included 'Apache', 'Arapaho', 'Chester', 'Loch Ness', 'Navaho', and 'Triple Crown'. A randomized complete block design with four replications was used. In the 2002 harvest season, commercially ripe-dull black fruit, Stage VII as described by Perkins-Veazie et al., (2000) were hand-harvested from each replication at the peak harvest period for each cultivar by 10:00 am, placed in plastic zip lock bags and stored in an ice chest during transport to Auburn University's Department of Horticulture Postharvest

Physiology laboratory within 6 hours of harvest. Samples were segregated based on size and freedom from blemish or damage.

Chemicals

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; ABTS) as diammonium salt, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Wako Chemicals USA Inc. (Richmond, VA, USA). All other chemical reagents, solvents or standards were either purchased from Sigma / Aldrich Chemical Co. (St. Louis, MO), or Fischer Scientific (Fisher Scientific, Raleigh, NC) and were either of high-performance liquid chromatography (HPLC) or analytical grade quality. Ultrapure Milli-Q water was used throughout this study and had electrical conductivity of 18.2 MΩ cm^2 obtained through a Millipore Direct-Q™ 5 filter system (Millipore Corp., Bedford, MA).

Extraction of crude phenolic compounds

Care was taken to exclude any direct sunlight during extraction and quantification procedures with all sample operations being performed under amber fluorescent lighting conditions (GE F40/G0, 40W). Five grams of frozen fruit tissue was homogenized in 25 mL of extraction solvent (400 mL of acetone/400 mL of methanol/200 mL of water/10 mL of acetic acid) as described by Rababah et al., (2005) in a Virtis Shear homogenizer (Virtishear, model 225318, Gardiner, NY) at a speed setting of 70 for 1 min. The homogenate was transferred into a 50 ml Oak Ridge Centrifuge Tube (Nalge Nunc International Corporation, Rochester, NY), purged with nitrogen and sealed. Samples

were incubated in a water bath at 60 °C for 1 h followed by a 3 min sonication (Branson, model 5510 Branson Ultrasonic Corporation, Danbury, CT). Sonicated samples were clarified by centrifugation (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 rpm for 15 min at 4°C, then filtered with Miracloth (Calbiochem, La Jolla, CA) and diluted to a final volume of 50 mL. Samples were purged again with nitrogen and stored in a -80 °C freezer until analyzed.

Determination of total phenolics (TPH)

TPH content was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Appropriately diluted methanolic extracts (200 µL) or gallic acid standard solutions were mixed with 2.6 mL of Milli-Q water. Generation of a standard curve was achieved by constructing five different concentrations of gallic acid (20, 40, 60, 80 and 100 mg/L). A blank was prepared using Milli-Q water instead of sample. Subsequently, a 200 µL of Folin-Ciocalteu's Reagent (FCR-1:5 dilution with Milli-Q water) was mixed with methanolic sample, standard or the blank. The reaction mixture was allowed to stand at room temperature for 6 min to permit the FCR reagent to react completely with oxidizable substrates or phenolates. Following incubation, 2.0 mL of 7% Na_2CO_3 solution was added to each mixture and allowed to stand at room temperature for 90 min. Absorbance was measured at 750 nm using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). Results are expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight based on four replications per sample or standard.

Determination of total flavonoids (TF)

TF content was determined by the colorimetric method of Zhishen et al., (1999). Briefly, aliquots (1.0 mL) of appropriately diluted methanolic berry extract samples or catechin standard solutions were pipetted into 15-mL conical Pyrex™ graduated tubes containing 0.3 mL 5% $NaNO_2$ at zero min. Generation of a standard curve was achieved by constructing five different concentrations of catechin (20, 40, 60, 80 and 100 mg/L). A blank was prepared using Milli-Q water instead of sample. After 5 min, 0.3 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added to the reaction mixture followed by the addition of 2 mL of 1 M NaOH one min later. The solution was diluted with 2.4 mL of Milli-Q water and vortexed for 30 sec. Absorbance was determined immediately at 510 nm. Results are expressed as mg catechin equivalent (CE) per 100 g fresh weight based on four replications per sample or standard.

Determination of total monomeric anthocyanins (ACY)

ACY content was determined according to the pH-differential method of Giusti and Wrolstad, (2001). Briefly, aliquots of appropriately diluted methanolic crude extract (200 μL) were mixed with either 3.8 mL of 25 mM potassium chloride buffer (pH 1.0) or 400 mM sodium acetate buffer (pH 4.5). The absorbance was measured at 510 and 700 nm against a blank consisting of Milli-Q water. Total monomeric anthocyanin content (ACY) was calculated as:

$$A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$$

Monomeric anthocyanin pigment (mg/L) = A x MW x DF x 1000/ (ε x 1)

Where A = absorbance, MW= molecular weight (449.2); DF = dilution factor, ε = molar absorptivity (26,900). The concentration of samples was expressed as mg cyanidin 3-glucoside equivalents per liter (mg CGE/L). The final concentration of anthocyanin (mg CGE/100 g fresh weight) was calculated according to the total volume of extract and weight of sample and based on four replications per sample.

Antioxidant properties

Extraction and determination of antioxidant capacity (VCEAC) assay using ABTS Radical

The antioxidant capacities of blackberry fruit tissue were extracted by homogenizing 5g of frozen fruit in approximately 25 mL of 80% HPLC grade ethanol in a Virtis Shear homogenizer (Virtis shear, model 225318, Gardiner, NY) at a setting of 70 for 1 min. Samples were transferred into 50 mL Oak Ridge Centrifuge Tube (Nalge Nunc International Corporation, Rochester, NY) and sealed after purging with nitrogen gas. Homogenized samples were further sonicated (5510 Branson, Branson Ultrasonic Corporation, Danbury, CT) for 3 min to obtain a uniform consistent sample. Sonicated samples were clarified by centrifugation (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 rpm for 15 min at 4°C, then filtered with Miracloth (Calbiochem, La Jolla, CA) and diluted to a final volume of 50 mL. Samples were purged again with nitrogen and stored in a -80 °C until time of analysis.

Antioxidant capacity was measured using the blue/green ABTS radical as developed by Kim et al., (2002). This method is based on the ability of antioxidants to quench the long-lived ABTS radical anion, a blue/green chromophore in comparison to

that of Trolox, a water-soluble vitamin E analogue or directly with L-ascorbic acid standard (Vitamin C). Briefly, 0.25 mM of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] were mixed with 0.1 mM of AAPH [2,2'-azobis-(2-amidinopropane) HCl] in 100 mL phosphate-buffered saline (PBS) solution [100mM potassium phosphate buffer (pH 7.4) containing 150mM NaCl]. The ABTS radical solution was heated in 68 °C water bath for one hour with frequent agitation and filtered at reduced pressure through a ZAPCAP®-CR filter unit (Whatman Inc., Florham Park, NJ). The blue-green ABTS radical solution was adjusted with fresh PBS to an absorbance of 0.650 ± 0.020. Four µL of sample solution or blank (80% HPLC grade methanol) was mixed with 196 µL of ABTS radical solution. The sample or standard were read every minute for a duration of 30 min in a microplate reader at 734 nm at 37 °C. Generation of a standard curve was achieved by constructing five different concentrations of L-ascorbic acid (20, 40, 60, 80 and 100 mg/L). Samples were analyzed in triplicate. The ABTS radical anion scavenging capacities of sample extracts were expressed on the fresh weight basis as mg Vitamin C equivalent (VCE) $100g^{-1}$ fresh weight.

Antioxidant radical scavenging activity

Antioxidant radical scavenging activity was measured according to the modified method described by Brand Williams et al., (1995) as follows: 0.02 mmol 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) was prepared in 200 mL of 80% HPLC grade methanol. The radical solution was stirred at room temperature for 20 min and then adjusted to 0.650 ± 0.020 absorbance value at 517 nm. Generation of a standard curve was achieved by constructing five different concentrations of L-ascorbic acid (20, 40, 60, 80 and 100

mg/L) with 6.67 µL of clarified methanolic extract obtained for the quantification of antioxidant capacity, blank, or L-ascorbic acid standard were mixed with 193.33 µL of DPPH radical solution. The decrease in absorbance of the resulting solution was monitored at 517 nm for 30 min duration in a microplate reader. The DPPH radical scavenging activity of methanolic blackberry extracts was expressed as Vitamin C equivalent (VCE) 100 g^{-1} fresh weight.

Statistical analysis

Statistical analyses were performed using The SAS System for Windows V9.1 (SAS Institute, Inc., Cary, N.C., 2004 – 2005). Analysis of variance and protected least significant difference (LSD) test were conducted via One-way Analysis of Variance (ANOVA) to identify differences among means, while Pearson Correlation test was conducted to determine the correlations among means. T-test was performed to identify differences among total flavonoids and total monomeric anthocyanins content. Statistical significance was indicated at $P \leq 0.05$.

Results and Discussion

Total Phenolics (TPH)

The TPH content in five different cultivars of blackberries varied from 221.2 to 342.1 mg GAE/100g FW (Table 2). The mean of TPH for the five blackberry cultivars (290.2 mg GAE/100g FW) was similar to that of reported by Wang and Lin (2000) (226 mg GAE/100g FW) . A mean of TPH value of 566.5 mg GAE/100g FW was calculated from the total phenolic content of 'Apache', 'Arapaho', 'Navaho', and 'Triple Crown'

reported by Clark et al (2002). Results reported in the present study were 2/3 lower than that of Moyer et al. (2002a) (478 mg GAE/100g FW), Sellappan et al. (2002) (487 mg GAE/100g FW), and half the content reported by Cho et al. (2005) (386 mg GAE/100g FW) and Moyer et al. (2002b) (434 mg GAE/100g FW). The discrepancy in reported TPH content could be due to several factors such as genetic differences, maturity at harvest, environmental conditions, different extraction and laboratory methods employed, and tissue type (whole fruit vs. puree or juice extract). In addition, the selection of standards utilized may contribute to varied results of others and those reported within the present study. In previous blackberry studies of Clark et al. (2002), TPH content was expressed as chlorogenic acid equivalents (CAE). In order to compare on an equivalent basis, the mean values reported by Clark et al. 2002 was divided by a conversion factor 1.8 in order to transform the TPH content based on gallic acid equivalents (GAE), the standard utilized within the present study. Following conversion, 314.7 mg GAE/100g FW was the final TPH content, which was in accordance with our results. Lastly, the over estimated values of TPH content in crude sample preparations may be due to non-phenolic reducing compounds such as sugars, organic acids, proteins, and pigments, reacting with the FCR in fruit extract (Cho et al., 2005; Kahkonen et al., 2001; Kim et al., 2002). In such instances, TPH values may be masked by these interfering reductive substances, which act in either inhibitory, additive or augmenting manner (Singleton et al., 1999). Inhibitory modifications are the result of oxidants competing with the FCR which may be negligible and corrected by matching appropriate standards and blanks with associated samples. Additive modifications are due to unanticipated phenols or enols which are present and may be associated with microbial contamination and

metabolites which may be directly associated with altered cellular protein and carbohydrate metabolism of samples in question. Assay conditions with modest amounts of carbohydrates at room temperatures apparently contribute to minor alterations. In contrast, elevated relative concentrations of sugars greater than 2% in combination with high temperatures may produce unreliable results (Waterhouse, 2005). This type of effect is associated with strong alkaline conditions of enediol reductones from the presence of carbohydrates especially with the presence of fructose. In addition, under acidic assay condition (pH 3), ascorbic acid is reactive with polyphosphotungstan in FCR resulting in the appearance of blue color generated prior to the addition of alkali reagent in this assay. Augmentation effects are noted when the amount of FCR reacts with phenols present by reducing quinones as they form thus prolonging the reaction. Collectively, these three reactions mask TPH content. In such instances, solid-phase extraction (SPE) technique is commonly used to fractionate and remove undesired components from bioactive compounds of interest (Robbins, 2003). For example, a 4-fold over-estimated value of TPH content in crude non-fractionated extracts from citrus fruit peels has previously been reported (Smith and Hossain, 2006). In the lemon cultivar 'Meyer', a TPH value of 156.37 GAE/100 g FW of crude non-fractionated lemon peel extract and 37.47 GAE/100g FW of fractionated lemon peel extracts were reported ,respectively. Hence, further purification or fractionation may be required to obtain a more accurate approximation of TPH content in fruit extracts.

'Loch Ness', 'Arapaho' and 'Apache' had a higher amount of total phenolics than 'Navaho' and 'Triple Crown' (Figure 1A). Similar results were found by Cho et al. (2005) and Clark et al. (2002). Phenolic compounds are an essential daily dietary

component of fresh fruit and vegetables which aids in the protection and function of essential cellular constituents against oxidative damage associated with various etiologies of neurological and chronic diseases (Scalbert et al., 2005). The average daily intake of phenolics is estimated at 1g/day (Scalbert and Williamson, 2000). The results from this study indicate the consumption of 100g of blackberries could contribute approximately 22 to 32% of TPH per day. Hence, it could be an alternative source of TPH compared to orange which contributed to highest amount of TPH (117.1 mg GAE/individual /day) in the American diet (Chun et al., 2005).

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Total Flavonoids (TF)

TF content of the five blackberry cultivars varied from 53.6 to 71.4 mg CE/100g FW and the mean for the 5 cultivars was 62.2 mg CE/100g FW (Table 1). The flavonoid content in the five blackberry cultivars were not different (Figure 1B). 'Loch Ness' contained the highest concentration of TF followed by 'Apache' and 'Arapaho'; 'Triple Crown' and 'Navaho' had the lowest concentrations. Limited information is available with regards to blackberry flavonoid composition. However, flavonols, catechin, and anthocyanins have been reported as predominant flavonoids in blackberries (Cho et al., 2004; Sellappan et al., 2002). The TF and ACY content were compared for each cultivar to identify if anthocyanins were the most abundant flavonoids in blackberries. 'Navaho' and 'Arapaho' showed significant differences between TF and ACY. 'Navaho' had a lower TF content than ACY ($P = 0.029$) and 'Arapaho' had a higher TF content than ACY content ($P < 0.001$). The rest of the cultivars had similar TF and ACY concentrations. The higher TF content in 'Arapaho' indicates that it may have higher

flavonol, catechin, or other types of anthocyanins than the other blackberry cultivars. Interestingly, the hybrid blackberry cultivars from the University of Arkansas breeding program ('Apache', 'Arapaho', and 'Navaho') appeared to be unique in their flavonol components. They contained only derivatives of quercetin but did not contain myricetin which is also an effective scavenger of free radicals (Kim et al., 2006). Hence, these blackberries did not have the genetic capacity to synthesize the enzyme flavonoid 3', 5'-hydrolyase (E.C. 1.14.13.88), which converted dihydrokampferol to dihydromyricetin, a key step in the biosynthetic pathway for myricetin (Cho et al., 2005). Flavonoids are potent antioxidants which exert their effects *in-vivo* in a variety of ways. The daily intake of flavonoids in the German population is estimated to be 1-2 grams (Havsteen, 2002). However, in the American diet the average daily intake of TF content derived from fruit and vegetable consumption has recently been reported to be 41.42 and 2.75 mg CE/100 g, respectively (Chun et al., 2005).

Total Monomeric Anthocyanins (ACY)

ACY varied significantly between among cultivars ($Pr > F = <0.0001$) (Table 2, Figure 1C). 'Loch Ness' and 'Navaho' had higher ACY content than 'Arapaho' and 'Triple Crown' which contained the lowest ACY content. This trend was supported by Cho et al. (2004) who reported 'Apache' (241 mg/100g FW) had a higher anthocyanin concentration than 'Navaho' (182 mg/100g FW) and 'Arapaho' (180 mg/100g FW). Clark et al. (2002) reported 'Triple Crown' and 'Navaho' were approximately five fold higher in ACY content than 'Apache' (130 mg/100g FW) and 'Arapaho' (141 mg/100g FW). The mean ACY content of the five blackberry cultivars (58.6 mg CGE/100g FW)

was lower than the TPH content reported by Moyer et al. (2002b) (80-230 mg/100g FW), and Mazza and Miniati (1993) (83-326mg/100g FW). It was approximately 2 folds lower than reported by Moyer et al. (2002a) (138 mg/100g FW), Perkins and Collins (2002) (108 mg/100g FW), and Sellappan et al. (2002) (117 mg/100g FW); approximately 3 folds lower than the results previously reported by Elisia et al. (2006) (176 mg/100g FW), Siriwoharn and Wrolstad (2004) (190 mg/100g FW), Cho et al. (2004) (173 mg/100g FW), and Wang and Lin (2000) (153 mg/100g FW).

Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins (Strack and Wray, 1993). Non-acylated anthocyanins such as cyanidin 3-glucoside may constitute approximately 80 to 87.5% of the ACY found in blackberry (Serraino et al., 2003; Elisia et al., 2006). However, there are some minor pigments such as acylated anthocyanins that are detected in blackberry fruit (Stinzing et al., 2002a). Most anthocyanins loose their color and/ or are converted into blue quinonoid forms in aqueous solution in a pH dependent manner. At pH values > 3, color intensity declines due to the addition of water to C-2 of the flavylium cation, converting it into a colorless hemiacetal (Redus et al., 1999). Acylated anthocyanins had greater stability and were resistant to color change at pH values > 3. One of these pigments was identified as cyanidin 3-glucoside acylated with malonic acid (Stintizing et al., 2002b). We observed a slight pinkish color during the experiment when the buffer (pH 4.5) was added to the fruit extract, which may indicate that the low total monomeric anthocyanin concentrations obtained was due to the acylated anthocyanins. Sapers et al. (1986) reported one of the cyanidin derivatives that is substituted with dicarboxylic acids is the main pigment (50.5%) in unripe blackberries of the cultivar 'Hall Thornless' (*Rubus sp. L.*). Moreover,

acylated anthocyanins decreased dramatically during ripening process. This could be due to the transport of anthocyanins into the vacuole during pigment accumulation (Hopp and Seitz, 1987). The low TPH content reported when compared to previous studies may be due to maturity at harvest and cultivar difference (Parr and Bolwell, 2000). ACY content are known to increase in blackberry, raspberry, and strawberry during maturation (Wang and Lin, 2000). Although blackberries in our study were harvested at commercially ripe stage, some variations in fruit maturity at harvest could have occurred since in blackberry maturity is variable at harvest (Clark et al., 2002).

Antioxidant capacity determined by ABTS and DPPH radical scavenging assays

The vitamin C equivalent antioxidant capacity (VCEAC) values of five blackberry cultivars varied significantly ($Pr > F = 0.0137$) (Table 3, Figure 1D). 'Loch Ness' and 'Apache' possessed the highest antioxidant capacity (698.5 mg VCE/100g FW) and 'Triple Crown', 'Navaho' and 'Arapaho' possessed the lowest antioxidant capacity (559.5 mg VCE/100g FW). The oxygen radical absorbance capacity (ORAC) assay has most often been used in previous reports to determine antioxidant capacity and therefore due to the difference in standards employed no direct comparison could be made from our results to other studies utilizing the ORAC procedure. In terms of ORAC assay, Clark et al. (2002) ranked 'Apache', 'Navaho', 'Triple Crown', and 'Arapaho' in descending order of ORAC values. Based on results of our study using ABTS radical scavenging assay and Clark et al. (2002) results using ORAC procedure, 'Apache' ranks highest in terms of antioxidant capacities. The results of this study indicate that 'Apache' would be an excellent blackberry cultivar for commercial production in Alabama. In Clark et al.

(2000), the omission of 'Loch Ness' did not allow the ranking of this cultivar in their study. Although 'Loch Ness' had the highest antioxidant capacity of the cultivars evaluated in this study, further analyses need to be conducted to identify its antioxidant profile.

The DPPH assay underestimated antioxidant capacity approximately 34% relative to the ABTS assay (Table 3, Figure 2). The underestimation of the ABTS by the DPPH method might be due to the interference of other absorbing compounds at 517 nm i.e. secondary reaction products between the chromogen and the samples being analyzed (Arnao, 2000). TPH and ACY are good indicators of antioxidant capacity. The underestimation could be due to a difference in reactivity of phenolic compounds with the free radicals in aqueous and organic phase. In general, the ABTS radical chromogen utilized in the ABTS method were able to be dissolved in both aqueous phases and organic phases, where as the DPPH radical chromogen could be solubilized only in the organic solvents. As a result, the ABTS radical chromogen was able to measure the total antioxidant activities (either lipophilic or hydrophobic) whereas the DPPH radical chromogen could only measure the antioxidant activity in a hydrophobic environment (Kim et al., 2002). Nevertheless, the DPPH method could be more advantageous when antioxidants being tested are more soluble in organic solvents (Ozgen et al., 2006).

The antioxidant capacity of the five blackberry cultivars varied from 347.05 to 464.2 mg VCE/100g FW. 'Loch Ness' had the greatest antioxidant capacity as determined by the DPPH free radicals scavenging assay while 'Triple Crown' had the least capacity. The radical scavenging capability of 'Loch Ness' was not significantly different than that of 'Apache'. In this respect, 'Triple Crown' was not significantly

different from ‘Arapaho’ which also had a low antioxidant capacity. ‘Navaho’ possessed intermediate free radical scavenging ability. The Pr > F = 0.0822, which may indicate that the antioxidant capacity among the five blackberry cultivars may not be different (Figure 1E). Thus, the antioxidants in the organic phase (such as cellular membranes) have similar free radical quenching ability for the five blackberry cultivars examined in this study. The antioxidant in the aqueous phase (i.e. Vitamin C) of plant cells might be a better indicator for cultivar selection.

We have previously determined cultivar differences of Vitamin C content in Alabama-grown blackberries (Thomas et al., 2005). Interestingly, the Vitamin C content reported previously corresponded to the antioxidant capacity determined in this study. ‘Loch Ness’ had the highest Vitamin C content followed by ‘Apache’, ‘Triple Crown’, ‘Araphao’, and ‘Navaho’. ‘Apache’ and ‘Triple Crown’ had very similar Vitamin C concentration. Vitamin C may be a potent water-soluble antioxidant contributing to the ABTS radical scavenging activity in blackberries.

Correlations

A high positive correlation was detected between the ABTS and DPPH radical scavenging assays (r = 0.897) (Table 4, Figure 3A). The high correlation may partly result from a similar hydrogen donating mechanism of antioxidants in both assays (Leong and Shui, 2002). VCEAC values from the ABTS assay were highly correlated with TPH (r = 0.893) (Figure 3B), and TF (r = 0.895) (Figure 3D) but not the ACY (Figure 3F). Results were supported by Kim et al. (2002) and Wang and Lin (2000). Both research groups observed a high correlation between the ABTS or ORAC values and TPH content.

There was a strong correlation between TPH and TF with the ABTS values, and TPH and TF were strongly correlated to each other (r = 0.916) (Figure 3H). Hence, TF contributed a large portion of the TPH thereby contributing greatly to the antioxidant capacity as measured by the ABTS method. ACY was highly correlated with the DPPH radical scavenge activity (r = 0.899) (Figure 3G). Cho et al., (2004) found acylated anthocyanins contributed more to the antioxidant capacities measured by ORAC than monoglucosides. Hence, the high correlation between ACY and DPPH could be due to the acylated anthocyanins in the fruit that are more non-polar or hydrophobic than the anthocyanin monoglucosides. TPH and ACY, and TF and ACY were poorly correlated (r = 0.372, r = 0.273) (Figure 3I and 3J). Thus, flavonoids other than the ACY contributed greatly to the antioxidant capacity. Clark et al. (2002) reported similar findings but Reyes-Carmona et al. (2005) reported that the TPH and ACY were strongly correlated (r = 0.92). Both authors utilized ORAC method to measure antioxidant capacity.

Conclusion

The phenolic compounds, especially flavonoids, contributed to the high antioxidant capacities as measured by the ABTS radical scavenging assays. ABTS and DPPH methods were highly correlated; however, the ABTS method was better for analyzing the overall antioxidant capacity. The DPPH method was highly correlated with the ACY content thus acylated anthocyanins were suspected to be the major contributor of the antioxidant capacity more than anthocyanin monoglucosides. Cultivar differences were found in TPH, ACY, and antioxidant capacity as determined by the ABTS method. Results from the ABTS assay were most apparent between ‘Loch Ness’ and ‘Triple

Crown' because the former had the highest concentration in all antioxidant assays evaluated and the latter had the lowest concentration in most assays determined. This difference was most likely due to genetic variation but other factors such as maturity at harvest, climatic conditions, environmental factors, and antioxidants contributed by seeds may also be involved (Prior and Cao., 2000; Clark et al., 2002b). Overall, 'Loch Ness' and 'Apache' are two blackberry cultivars that have potential for production in Alabama due to their high concentrations in TPH, TF, and ACY. However, more work needs to be conducted to determine the antioxidant mechanisms and capacities of 'Loch Ness'. Based on the results of this research, future studies should emphasize the *in-vivo* and *in- vitro* antioxidative mechanisms, especially the chemical composition and contribution of specific polyphenolic components that contribute to the bioactive potential of blackberry cultivars. Relating antioxidative properties *in-vivo* would greatly enhance our knowledge of dietary antioxidants in potential health benefits and disease management.

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IV. THE ANTIOXIDANTS AND PHYSICOCHEMICAL PROPERTIES OF KIWIFRUIT AT COMMERICAL HARVEST MATURITY AND CONSUMPTION STAGE

Abstract

In this study, two cultivars of green fleshed kiwifruit (*Actinidia deliciosa* var. 'Hayward' and 'AU Fitzgerald') and three cultivars of yellow fleshed kiwifruit (*Actinidia chinenesis var.* 'Golden Dragon', 'Golden Sunshine', and 'Hort16A') were compared for physicochemical and antioxidant properties at two stages of ripeness (at-harvest and consumer eating quality). The physicochemical comparisons consisted of pH, titratable acidity (TA), soluble solids content (SSC), SSC /TA ratio and ethanolic soluble sugar content. Antioxidants determined included β-carotene, chlorophyll a and b, antioxidant capacities as determined by 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (expressed in terms of VCEAC), total phenolics (TPH) and flavonoids (TF). SSC varied from 5.90 to 14.1% and were highly variable among cultivars ($Pr > F = <0.0001$). TA varied significantly among the five cultivars ($Pr > F = <0.0001$). Green fleshed cultivars had lower SSC content than the yellow fleshed cultivars. 'AU Fitzgerald' contained the highest TA (0.606%) while 'Hort16A' contained the lowest (0.326%); 'Hayward',

‘Golden Sunshine’, and ‘Golden Dragon’ contained intermediate levels of TA. SSC/TA ratios in this study ranged from 12.1 to 37.1. ‘Hort16A’ had the highest SSC/TA ratio with ‘Golden Sunshine’ (29.21) and ‘Golden Dragon’ (28.84) intermediate and ‘Hayward’ (12.25) and ‘AU Fitzgerald’ (12.12) the lowest SSC/TA ratios. The yellow flesh cultivars had higher reducing sugars (RS) and total sugars (TS) concentrations than the green flesh cultivars. β-Carotene concentrations were lower in the yellow fleshed cultivars than the green fleshed cultivars. Results of the ABTS anion radical and DPPH radical scavenging activities revealed similar trends in cultivar variation in that ‘Golden Sunshine’ and ‘Golden Dragon’ had the highest antioxidant capacities followed by ‘Hort16A’. The green flesh kiwifruit, ‘Hayward’ and ‘AU Fitzgerald’, were not significant different in terms of antioxidant capacities as determined by the ABTS and DPPH methods. TPH content was higher in yellow fleshed than in the green fleshed kiwifruit cultivars. ‘Hort16A’ contained the highest TPH concentration (155.5 mg GAE/100g FW) and ‘AU Fitzgerald’ contained the lowest (88.5 mg GAE/100g FW). TF did not vary significantly among cultivars ($Pr > F = 0.1581$) ‘Golden Sunshine’ had the highest TF content (39.5 mg CE/100g FW) while ‘Hort16A’ was found to have the lowest (26.1 mg CE/100g FW).

Keywords: *Actinidia deliciosa, Actinidia chinenesis,* soluble solids, pH, titratable acidity, reducing sugars, total sugar, phenolics, flavonoids, antioxidant capacity, VCEAC, ABTS, DPPH.

Introduction

The most common edible kiwifruit in the Western world is *Actinidia deliciosa*, a perennial deciduous vine of Actinidiaceae. The cultivar 'Hayward' is a green flesh kiwifruit that has become a mainstream commercial crop on most Western markets by the mid-1990s (Jaeger et al., 2003). Since then it has been widely used as the standard kiwifruit cultivar for various research and database construction (Rushing, 2004, Montefiori et al., 2005). The seed of this cultivar was imported into New Zealand from China (Jaeger et al., 2003). In tropical or sub-tropical regions with mild winters, 'Hayward' was found to have inadequate bud break, leading to low flower numbers and extended flowering (Sibley et al., 2005). Hence, it is important to find new cultivars that can sustain and prosper in a sub-tropical environment such as in Southern Alabama.

'AU Fitzgerald' has been indicated as a potential cultivar that can be grown in Alabama's warm weather conditions (Sibley et al., 2005). 'AU Fitzgerald' produces more fruit than 'Hayward' due to better flowering, flowers per stem, and flower retention (Sibley et al., 2005). Both 'Hayward' and 'AU Fitzgerald' possess green flesh whereas the other three cultivars of *Actinidia chinensis*, 'Hort16A', 'Golden Sunshine', and 'Golden Dragon', have yellow flesh. There is an increase in popularity in the yellow flesh cultivars because they have been reported to have more consumer preferred characteristics ie. less acidic and sweeter (Rushing, 2004; McGhie and Ainge, 2002). 'Hort16A' is sold under the trade name of "Zespri^{TM} Gold" kiwifruit, thus a cultivar that is more familiar to the consumers. The color difference in the flesh contributes to the uniqueness in their appearance and attractiveness, as well as providing nutritional value in the form of dietary antioxidants. For example, the yellow flesh color of Zespri

kiwifruit is due to the accumulation of carotenoids where as the green-flesh color of 'Hayward' is mainly due to the presence of chlorophylls (Montefiori et al., 2005).

If flesh color is to be used as a visual to guide selection of new cultivars, sugars, acids, and other physicochemical components must also be found desirable by consumers (Marsh et al., 2004). Our goal was to explore all these properties in both green and yellow flesh kiwifruit. Hence, commercially ripened kiwifruit (*Actinidia deliciosa* var. 'AU Fitzgerald', and 'Hayward', and *Actinidia chinenesis* var. 'Hort16A', 'Golden Dragon', and 'Golden Sunshine') at their harvest and consumption stages were compared for fruit quality and antioxidant properties.

Material and Methods

Plant Material

Kiwifruit from two different cultivars of *Actinidia deliciosa*, 'Hayward', and 'AU Fitzgerald', and two cultivars of *Actinidia chinensis,* 'Golden Sunshine' and 'Golden Dragon', were harvested from the Chilton Area Horticultural Research and Extension Center located at Clanton, AL in 2002, at the normal stage of maturity for commercial harvest. 'Hort16A', an *A. chinensis* cultivar, was purchased from a local supermarket in year 2004. The kiwifruit samples were harvested again in 2004 and 2005. 'Hayward' and 'Fitzgerald' were stored at 0°C for 30 days to attain eating quality. At the end of the storage period, 30 fruit for each cultivar were quartered into sections and divide equally into quart sized freezer bags and stored at -80°C freezer.

Chemicals

All solvents were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Solvents included ethanol, methanol, acetone, hexane, o-phosphoric acid, acetic acid, and hydrochloric acid. For carbohydrate analysis, enzyme invertase (E.C. 3.2.1.26) (practical grade) was obtained from Sigma Chemical Co (St. Louis, MO, USA; Catalog # I-9253). For VCEAC and DPPH assays, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS) as diammonium salt , 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Wako Chemicals USA Inc. (Richmond, VA, USA). Both potassium monobasic phosphate, potassium dibasic phosphate, and sodium chloride that constituted potassium phosphate buffer were purchased from Fisher Scientific with A.C.S. certified grade. For assays to determine total phenolics and total flavonoids, Folin-Ciocalteu Reagent, sodium carbonate, sodium nitrite, aluminum chloride, and the two standards, gallic acid and (+)catechin, were purchased from Sigma Chemical Co. All reagents used were of A.C.S. certified grade and standards were of HPLC grade. Sodium hydroxide (A.C.S. certified grade) was purchased from Fisher Scientific. Ultrapure water (18.2 MΩ cm) or Milli-Q water was prepared by using a Millipore system (Millipore Corp., Bedford, MA).

Carbohydrate Extraction and Analysis

Care was taken to exclude any direct sunlight during all extraction and quantification procedures with all sample operations being performed under amber fluorescent lighting conditions (GE F40/G0, 40W). Five grams of frozen fruit sample

from each cultivar were homogenized in 25 mL of 80% HPLC grade ethanol using a Virtis Shear homogenizer (Virtishear, model 225318, Gardiner, NY) for 1 min at a setting of 70. The shaft was rinsed with ethanol and combined with the homogenate. The homogenized extracts were incubated in a water bath at 90 °C for 15 min and centrifuged (Beckmann Centrifuge, model J2-21, San Antonio, TX) at 13,000 rpm for 15 min at 4 °C. The clarified ethanol soluble supernatant was filtered through four layers of Miracloth (Calbiochem, La Jolla, CA) and diluted to a final volume of 50 mL with 80% HPLC grade ethanol. All extractions were stored in -80 °C freezer until further analysis. Total soluble sugars and reducing sugars were determined, respectively, by the methods of Wood, (1984) and Somogyi-Nelson (Nelson, 1944). The amount of non-reducing sugars was determined by calculating the difference between the two methods respectively. To one set of test tubes aliquots (25 µL) from ethanol-soluble sugars were retained for measurements of reducing sugars and combined with 475 µL of 50 mM sodium acetate buffer (pH 4.7). To a parallel set of test tubes, 25 µL of ethanol soluble sugars were incubated with 100 units of invertase (E.C. 3.2.1.26) (Sigma, St. Louis, MO; Catalog # I-9253) similarly dissolved in 475 µL of sodium phosphate buffer (pH 4.7). Soluble sugar levels were determined colorimetrically by measuring the absorbance at 500 nm using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). Absorbance was compared with a standard curve comprised of seven glucose concentrations (0, 10, 20, 40, 60, 80, and 100 µg/mL) for quantification and results were expressed as mg/100g FW. Three independent determinations were performed for each cultivar.

Determination of soluble solids content (SSC), titratable acidity (TA), and pH

Ten grams of frozen fruit tissue were homogenized in pre-chilled 40 mL of Milli-Q water using a Virtis Shear homogenizer (Virtishear, model 225318, Gardiner, NY) at a setting of 70 for 1 min. The homogenate was centrifuged at 10,000 rpm at 13,000 rpm for 15 min at 4 °C, in a Beckman Centrifuge model J2-21, (Beckman Instruments, San Antonio, TX), and filtered with four layers of Miracloth (Calbiochem, La Jolla, CA). Soluble solids content (SSC), were measured by adding four drops of clarified extract onto a digital refractometer (Leica Mark II Plus, Model 110494; Leica, Buffalo, NY) calibrated in °Brix (grams of sucrose equivalent per 100 g of juice), and expressed as a percentage. Initial pH and titratable acidity were determined using an automated titrimeter (Metrohm Titrino Model 751 GPD and Metrohm Sample Changer, Metrohm Corp., Herisau, Switzerland). The automated titrimeter was housed in a Fisher Scientific refrigerated chromatography chamber maintained at 10 ^{o}C, Fisher Scientific model Isotemp Laboratory Refrigerator Model 13-986-1276 (Fisher Scientific, Raleigh, NC.) Ten 10 mL of clarified kiwifruit fruit sample were placed into a sample cup and titrated to the endpoint of pH 8.1 using 0.1 N sodium hydroxide. The results were expressed as % citric acid equivalent. SSC/TA ratio was calculated by dividing soluble solids values by the titratable acidity values of each cultivar.

Antioxidant properties

Extraction and determination of chlorophyll a, b and β-carotene

The extraction and determination of fruit pigments were performed according to Nagata and Yamashita (1992). Five grams of frozen fruit tissue were homogenized in

4 ^{o}C HPLC grade acetone: hexane (2:3) using a Virtis Shear homogenizer (Virtishear, model 225318, Gardiner, NY) at a speed setting of 70 for 1min. The homogenate was centrifuged at 13,500 rpm for 15 min at 4 °C, in a Beckman Centrifuge model J2-21, (Beckman Instruments, San Antonio, TX), filtered with four layers of Miracloth (Calbiochem, La Jolla, CA) and diluted with HPLC grade acetone: hexane (2:3) to a final volume of 50 mL. The absorbance of supernatant was measured spectrophotometrically at λ = 663, 645, 505, and 453 nm using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont) for the estimation of β-carotene, chlorophyll a and b contents. The contents of β-carotene, chlorophyll a and b were estimated by the following equations.

β-carotene (mg/100mL of extract) = $0.2160\ A_{663} - 1.220\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}$

Chlorophyll a (mg/100mL of extract) = $0.999\ A_{663} - 0.0989\ A_{645}$

Chlorophyll b (mg/100mL of extract) = $-0.328\ A_{663} - 1.77\ A_{645}$

Extraction of crude phenolic compounds

Care was taken to exclude any direct sunlight during extraction and quantification procedures with all sample operations being performed under amber fluorescent lighting conditions (GE F40/G0, 40W). In addition all glassware was shielded with aluminum foil. Five (± 0.2) grams, of frozen fruit tissue were homogenized in 25 mL of extraction solvent (400 mL of acetone/400 mL of methanol/200 mL of water/10 mL of acetic acid) as described by Rababah et al., (2005) in a Virtis Shear homogenizer (Virtishear, model 225318, Gardiner, NY) at a speed setting of 70 for 1min. The homogenate was transferred into a 50 ml Oak Ridge Centrifuge Tube (Nalge Nunc International

Corporation, Rochester, NY), purged with nitrogen and sealed. Samples were incubated in a water bath at 60°C for 1h followed by a 3 min sonication (Branson, model 5510, Branson Ultrasonic Corporation, Danbury, CT). Sonicated samples were clarified by centrifugation (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 rpm for 15 min at 4°C, then filtered with Miracloth (Calbiochem, La Jolla, CA) and diluted to a final volume of 50 mL. Samples were purged again with nitrogen and stored in a -80 °C until time of analysis.

Determination of total phenolics (TPH)

TPH content was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Appropriately diluted methonolic extracts (200 µL) or gallic acid standard solutions were mixed with 2.6 mL of Milli-Q water. Generation of a standard curve was achieved by constructing five different concentrations of gallic acid (20, 40, 60, 80 and 100 mg/L). A blank was prepared using Milli-Q water instead of a sample. Subsequently, 200 µL of Folin-Ciocalteu Reagent (FCR-1:5 dilution with Milli-Q water) were mixed with the methanolic sample, standard or blank. The reaction mixture was allowed to stand at room temperature for 6 min to permit the FCR reagent to react completely with oxidizable substrates or phenolates. Following incubation, 2.0 mL of 7% Na_2CO_3 solution were added to each mixture and allowed to stand at room temperature for 90 min. The absorbance was measured at 750 nm using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). Results are expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight based on four replications per sample or standard.

Determination of total flavonoid content (TF)

TF content was determined by the colorimetric method of Zhishen et al., (1999). Briefly, aliquots (1.0 mL) of appropriately diluted methanolic kiwifruit extract samples or catechin standard solutions were pipetted into 15-mL conical Pyrex™ graduated tubes containing 0.3 mL 5% $NaNO_2$ at zero min. Generation of a standard curve was achieved by constructing five different concentrations of catechin (20, 40, 60, 80 and 100 mg/L). A blank was prepared using Milli-Q water instead of a sample. After 5 min, 0.3 mL of 10% $AlCl_3 \cdot 6H_2O$ solution were added to the reaction mixture followed by the addition of 2 mL of 1 M NaOH one min later. The solution was diluted with 2.4 mL of Mili-Q water and vortexed for 30 sec. The absorbance was read immediately at 510 nm using a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). Results were expressed as mg catechin equivalent (CE) per 100 g fresh weight based on four replications per sample or standard.

Extraction and determination of antioxidant capacity (VCEAC) assay using ABTS Radical

The antioxidants of kiwifruit fruit tissue were extracted by homogenizing 5g (± 0.2) of frozen fruit in approximately 25 mL of 80% HPLC grade ethanol in a Virtis Shear homogenizer (Virtis shear, model 225318, Gardiner, NY) at a setting of 70 for 1 min. Samples were transferred into 50 mL Oak Ridge Centrifuge Tube (Nalge Nunc International Corporation, Rochester, NY) and sealed after purging with nitrogen gas. Homogenized samples were further sonicated (5510 Branson, Branson Ultrasonic Corporation, Danbury, CT) for 3 min to obtain a uniform consistent sample. Sonicated

samples were clarified by centrifugation (Beckman Centrifuge, model J2-21, San Antonio, TX) at 13,000 rpm for 15 min at 4 °C, then filtered with Miracloth (Calbiochem, La Jolla, CA) and diluted to a final volume of 50 mL. Samples were purged again with nitrogen and stored in a -80 °C until time of analysis.

Antioxidant capacity was measured using the blue/green ABTS radical as developed by Kim et al., 2002. This method is based on the ability of antioxidants to quench the long-lived ABTS radical anion, a blue / green chromophore in comparison to that of Trolox, a water-soluble vitamin E analogue or directly with L-ascorbic acid standard (Vitamin C). Briefly, 0.25 mM of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] were mixed with 0.1 mM of AAPH [2,2'-azobis-(2-amidinopropane) HCl] in 100 mL phosphate-buffered saline (PBS) solution [100mM potassium phosphate buffer (pH 7.4) containing 150mM NaCl]. The ABTS radical solution was heated in a 68 °C water bath for one hour with frequent agitation and filtered at reduced pressure through a ZAPCAP®-CR filter unit (Whatman Inc., Florham Park, NJ). The blue-green ABTS radical solution was adjusted with fresh PBS to an absorbance of 0.650 ± 0.020. 4 µL of sample solution or blank (80% HPLC grade methanol) were mixed with 196 µL of ABTS radical solution and immediately read at 734 nm at 37 °C for a duration of 30 min in a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). Generation of a standard curve was achieved by constructing five different concentrations of L-ascorbic acid (40, 80, 120, 160, and 200 mg/L). Samples were analyzed in triplicate. The ABTS radical scavenging capacities of sample extracts were expressed on the fresh weight basis as mg Vitamin C equivalent $100g^{-1}$ fresh weight (VCEAC).

Antioxidant radical-scavenging activity

Antioxidant radical scavenging activity was determined according to a method described by Brand-Williams et al., (1995) with some modifications. The modified protocol involved dissolving 0.02 mmol of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) in 200 mL of 80% HPLC grade methanol. The radical solution was stirred at room temperature for 20 min and then adjusted to 0.650 ± 0.020 absorbance values at 517nm. Generation of a standard curve was achieved by constructing five different concentrations of L-ascorbic acid (40, 80, 120, 160, and 200 mg/L). To determine the antioxidant capacity, 6.67 µL of clarified methanolic extract, blank, or L-ascorbic acid standard were mixed with 193.33 µL of DPPH radical solution. The decrease in absorbance of the resulting solution was monitored at 517 nm for 30 min duration in a microplate reader (Synergy HT, BIO-TEK Instruments, Inc., Winooski, Vermont). The DPPH radical scavenging activity of methanolic kiwifruit extracts was expressed as Vitamin C equivalent $100g^{-1}$ fresh weight (VCEAC).

Statistical analysis

Statistical analyses were performed using The SAS System for Windows V9.1 (SAS Institute, Inc., Cary, N.C., 2004 – 2005). Data are reported as means ± SD. Analysis of variance and protected least significant difference test (LSD) were conducted to identify differences among means, while Pearson Correlation test was conducted to determine the correlations among means. Statistical significance was indicated at $P \leq 0.05$.

Results and Discussion for Kiwifruit

Physicochemical Properties

Five kiwifruit cultivars were categorized according to their flesh color. 'Hayward' and 'AU Fitzgerald' at commercial maturity are green in flesh color whereas 'Hort16A', 'Golden Sunshine', and 'Golden Dragon' are yellow flesh in appearance at commercial maturity (Table 5). pH values of the five cultivars were significantly different ($Pr > F = 0.0052$). 'AU Fitzgerald' was found to have lower pH values when compared to 'Hort16A', 'Golden Sunshine', and 'Gold Dragon' but did not differ from 'Hayward' (Figure 4A). TA varied significantly among the five cultivars ($Pr > F = <0.0001$) (Figure 4B). 'AU Fitzgerald' contained the highest TA (0.606%) while 'Hort16A' contained the lowest (0.326%); 'Hayward', 'Golden Sunshine', and 'Golden Dragon' contained intermediate levels of TA. These results indicate 'AU Fitzgerald' has a more acidic flavor than the other cultivars and pH and TA are known to significantly contribute to desirable consumer perception and sensory (MacRae et al., 1989).

Soluble solids content (SSC) ranged from 5.90 to 14.1% and were highly variable among cultivars ($Pr > F = <0.0001$) (Figure 4C). 'Golden Dragon' (14.1%) and 'Golden Sunshine' (13.6%) had the highest SSC content followed by 'Hort16A' (12.0%). Hence, the yellow fleshed cultivars had a sweeter flavor than green fleshed cultivars in this study. SSC content of 'AU Fitzgerald' (7.40%) was higher than the SSC of 'Hayward' (5.9%). The SSC/TA ratio is usually cited as an indice of maturity and quality predictor in fruit (Kafkas et al., 2005; Perkins-Veazie and Collins, 2001). SSC/TA ratio in *A. chinensis* was higher than that of *A. deliciosa* in our study, ranging from 12.12 to 37.13 (Figure 4D). 'Hort16A' had the highest SSC/TA ratio, which indicates a more desirable flavor.

'Golden Sunshine' (29.21) and 'Golden Dragon' (28.84) had similar SSC/TA ratios which were higher than the SSC/TA ratios of 'Hayward' (12.25) and 'AU Fitzgerald' (12.12). SSC content of the five kiwifruit cultivars correlated strongly with reducing sugars and total sugar content (Table 11). SSC content was, therefore, possibly influenced by the metabolism of sucrose. High SSC in *A. chinensis* is suggestive of increased sucrose phosphate synthase (EC 2.4.1.14) and acid invertase activities (EC 3.2.1.26). SPS is a key enzyme in sucrose biosynthesis whose activity increases during ripening (Fisk et al, 2006). Invertase in plants is responsible for cleaving sucrose into D-glucose and D-fructose irreversibly (Winter and Huber, 2000).

Total sugar, reducing sugars, and soluble solids content correlated significantly with pH (Table 11). The pH of kiwifruit is known to increase with maturation. Reducing sugars content strongly correlated with total sugar and soluble solids contents, which indicates that hexoses were the major sugars accumulated during ripening.

Soluble Sugars Content

Soluble carbohydrate content plays an important role in cell enlargement of *A. deliciosa* (Boldingh et al., 2000). Accumulated soluble sugar content was suggested to serve as a primary source of osmotica to maintain cell turgor during rapid periods of the plant cell expansion phase. The sugar content of the five kiwifruit cultivars is summarized in Table 6. In our study, the yellow flesh cultivars had higher RS and TS concentrations than the green flesh cultivars. 'Golden Sunshine' (16,025 mg/100g FW) had a higher RS concentration than 'Golden Dragon' (12,786 mg/100g FW) and 'Hort16A' (10,697 mg/100g FW). The green fleshed cultivars 'AU Fitzgerald' (2,587

mg/100g FW) and 'Hayward' (1,601 mg/100g FW) had the lowest RS content. 'Golden Dragon' (16,234 mg/100g FW) and 'Golden Sunshine' (14,458 mg/100g FW) had higher total sugars content than 'Hort16A' (10,488 mg/100g FW). 'AU Fitzgerald' (3,746 mg/100g FW) and 'Hayward' (3,464 mg/100g FW) had the lowest but similar total sugar content. Sucrose content in 'AU Fitzgerald' (1,159 mg/100g FW) and 'Hayward' (1,863 mg/100g FW) were less than 'Golden Dragon' (3,448 mg/100g FW). We were unable to detect any sucrose in 'Hort16A' and 'Golden Sunshine'. In contrast, 'Golden Dragon' had the highest sucrose concentration among the five kiwifruit cultivars.

As fruit of *Actinidia* species begin to ripen, net starch hydrolysis commences and reducing sugars such as glucose and fructose accumulate rather than sucrose. In 'Hayward', glucose constitutes approximately 60% of the total sugar upon ripening (Boldingh et al., 2000). Reducing sugars content of 'Hayward' was slightly higher than previously reported (965 mg/100g FW) (MacRae et al., 1989a). MacRae et al., (1989a) performed their analysis on 'Hayward' kiwifruit at harvest maturity with a soluble solids content of 5.8 % w/v. The total sugar/carbohydrate and sucrose level for 'Hayward' in their study was 8,020 mg/100g FW and 248 mg/100g FW.

Total sugar content of 'Hayward' (3,464 mg/100g FW) was approximately half of the reported value and the sucrose concentration (1,863 mg/100g FW) was 7.5 times higher than the reported value (MacRae et al., 1989a). These results could be due to high activity of SPS thus yielding high sucrose accumulation during development. It has been demonstrated that SPS activity increases in kiwifruit at the consumption stage with an increased amount of sucrose while the activity of other sucrose metabolizing enzymes, acid invertase, sucrose synthase, and neutral invertase, remained constant as fruit matured

(Hubbard et al., 1991). The high level of total sugars observed by MacRae et al. (1989a) was suspected to contain a high concentration of *myo*-inositol, a soluble carbohydrate that could reach more than 30% total sugar at the mid-point of kiwifruit development (Bieleski et al., 1997). *myo*-Inositol is a sugar alcohol commonly found in higher plants. Its functions include osmoprotection and a substrate for synthesis of cell wall precursors (Boldingh et al, 2000). *myo*-Inositol was found to be 10% in *A. deliciosa* and 20% in *A. chinensis* of the total sugars accumulated. The two cultivars of *A. deliciosa* were similar in total sugars, reducing sugars and sucrose content. Hence, metabolism of carbohydrates especially sucrose in 'Hayward' and 'AU Fitzgerald' were controlled similarly.

In general, the three cultivars of *A. chinensis* were higher in TS, RS, and sucrose content than the two cultivars of *A. deliciosa.* Although the fruit of *A. chinensis* were found to have a similar seasonal pattern of carbohydrate accumulation as that of *A. deliciosa* (Boldingh et al., 2000), the enzymes involved in sucrose synthesis and metabolism should have a higher catalytic efficiency or present in a high concentration because high level of total sugars, reducing sugars, and sucrose were detected. The high sucrose concentration in 'Golden Dragon' indicated its ability to store a higher amount of soluble sugar since sucrose is composed of glucose and fructose together (Klann et al., 1993). The level of sucrose was treated as zero for 'Hort16A' and 'Golden Sunshine' because the reducing sugars content was greater than the total sugars content. This is suggestive of high acid invertase activity toward sucrose hydrolysis hence every sucrose molecule had been hydrolyzed to hexoses.

β-Carotene and Chlorophyll a and b

β-Carotene, chlorophyll a, and b have been shown to be important compounds that could influence flesh color of kiwifruit (McGhie and Ainge, 2002). The concentrations of β-carotene, chlorophyll a (CHL a), chlorophyll b (CHL b), and chlorophyll a+b (CHL a+b) varied significantly among cultivars (Table 7, Figure 7). Green flesh kiwifruit generally had higher concentrations of β-carotene and chlorophylls than the yellow flesh kiwifruit (Figure 9). 'Hayward' and 'AU Fitzgerald' are two green fleshed kiwifruit cultivars. 'Hayward' contained a total combined value of 1.44 mg/100g FW chlorophylls. The combined chlorophyll content was inclusive of 0.909 and 0.53 mg/100g FW of CHL a and CHL b, respectively. These values were in agreement with Nishiyama et al. (2005) but slightly higher than that of McGhie and Ainge (2002). The chlorophyll a and b contents of 'AU Fitzgerald' were similar to that of 'Hayward'. 'Hort16A' possessed the lowest content of CHL a+b (0.164 mg/100g FW). The low chlorophyll content in cultivars of *A. chinensis* was considered to reflect degradation of the pigment during fruit maturation, which induced a transition of chloroplasts to chromoplasts (Nishiyama et al., 2005).

Surprisingly, β-carotene concentrations in the three yellow flesh kiwifruit cultivars were lower than that in the green cultivars. It was anticipated that the yellow flesh color was due to higher concentrations of β-carotene in the pulp. 'Hayward' accumulated the highest β-carotene content (0.421 mg/100g FW) and 'Golden Sunshine' had the lowest (0.186 mg/100g FW). β-carotene concentration of 'Hayward' was three times higher than that reported by McGhie and Ainge (2002) who showed that there was no difference in β-carotene content between 'Hayward' and 'Hort16A'. In contrast,

Nishiyama et al. (2005) reported that 'Hort16A' was 25% lower in β-carotene concentration than 'Hayward'. Our results indicated 'Hort16A' was 46% lower in β-carotene content than 'Hayward'. Hence, the yellow flesh color of *A. chinensis* was mainly due to the absence of chlorophylls from the fruit instead of an abundance of the carotenoids (Nishiyama et al., 2005; McGhie and Ainge, 2002).

In plants, CHL a is the predominate form of chlorophyll at a 3 to 1 ratio (Ferruzzi and Schwartz, 2001). We observed a 2 to 1 ratio in the five kiwifruit cultivars (Figure 8). Ferruzzi et al. (2002) observed the antioxidant capacity of CHL a was significantly higher than that of CHL b. The correlation between antioxidant capacity and CHL a was also slightly higher than that of CHL b in our study (Table 10). The antioxidant capacity of CHL a may be due to its higher concentration found in plants thus it may play an important role in protecting against lipid oxidation (Lanfer-Marquez et al., 2005). The chlorophylls correlated significantly with the VCEAC values determined by the DPPH radical scavenging method, which indicate more lipophilic chlorophylls were being extracted. The organic solvents commonly used to extract lipophilic chlorophylls, including acetone and ether, cannot completely extract water-soluble derivatives because they remain in the aqueous phase (Ferruzzi and Schwartz, 2001).

β-Carotene had a positive linear relationship with CHL a and CHL b (Table 11). The slope is positive which indicates that β-carotene content increased along with chlorophyll accumulation. The high correlation was expected because β-carotene is known to react with triplet-excited chlorophyll molecules to prevent formation of singlet oxygen (Wahid and Ghazanfar, 2006). Thus, the strong linear relationship between chlorophylls and beta-carotene could be a stress tolerance strategy for kiwifruit. Since β-

carotene and chlorophyll content were linearly correlated, it is possible that both chlorophyll and carotenoid content depend mainly on the chloroplast density in the kiwifruit. Nishiyama et al. (2005) suggested that the β-carotene content in green flesh kiwifruit could be partially estimated by a visual inspection of the depth of the green color of the flesh. Our linear slope was constructed by the data collected from both yellow and green flesh kiwifruit cultivars.

In green flesh kiwifruit, 'Hayward' and 'AU Fitzgerald', the carotenoids were present in an unesterified form as is usual for carotenoids associated with chlorophyll-containing tissues. Thus, the composition of chlorophylls and carotenoids from the flesh of *A. deliciosa* appeared to be comparable to those found in the chloroplasts of normal photosynthetically active tissues (McGhie and Ainge, 2002). Although the carotenoid composition in *A. deliciosa* closely resembled that of *A. chinensis*, some of the carotenoids accumulated in *A. chinensis* were esterified. β-Carotene and xanthophylls, such as 9'-cis-neoxanthin, violaxanthin, and lutein, were esterified with acyl fatty acids and were often found in chlorophyll-containing chloroplasts that were transformed to carotenoid-containing chromoplasts during fruit ripening (McGhie and Ainge, 2002). β-Carotene correlated significantly with antioxidant capacities determined by both ABTS and DPPH radical scavenge assays (Table 10, Figure 10B), especially with the DPPH radical scavenge assay. It is assumed that the unesterified β-carotene contributed mostly to the ABTS radical scavenging activity and the esterified β-carotene contributed to the DPPH radical scavenging activity. Because β-carotene tends to localize in cell membranes, it is able to function synergistically with both ascorbate and α-tocopherol in the inhibition of lipid peroxidation. Naturally, higher antioxidant capacities would be

detected by the DPPH radical scavenge assay because the DPPH radicals were more soluble in organic solvents i.e. hydrophobic environments such as in the membrane.

β-Carotene and chlorophyll concentrations were found to be negatively correlated with antioxidant capacity. An increase in carotenoid content might result in the formation of carotenoid cation radicals or adducts at a level beyond which the tocopherol/ascorbate pool could effectively repair, resulting in prooxidant effects (Young and Lowe, 2001). At low oxygen pressure (pO_2), antioxidant effectiveness of β-carotene has been reported to decrease, but true prooxidant effects were not observed (Burton and Ingold, 1984; Palozza et al., 1995). Autooxidation of high levels of carotenoids are capable of stimulating increased levels of oxidative products (such as epoxides) which have been indicated to possess procarcinogenic effects (Wang and Russell, 1999). The low antioxidant activity observed at high concentrations of chlorophylls might be due to their high chemical instability in a medium that contained unsaturated fatty acids (Lanfer-Marquez et al., 2005). The unsaturated fatty acids were able to stimulate the decomposition of chlorophylls thereby reducing their antioxidant capacities (Lanfer-Marquez et al., 2005). Furthermore, bulky structures and lipophilic properties of chlorophylls might impair their ability to interact freely with the test radicals, especially the ABTS radicals, resulting in a potential depression of VCEAC values (Ferruzzi et al., 2002).

Antioxidant Capacity

Antioxidant capacities were measured via the ABTS or DPPH radical scavenging assays (Table 9). Both assays showed similar trends in cultivar variation in 'Golden

Sunshine' and 'Golden Dragon' which had the highest antioxidant capacities followed by 'Hort16A'. The green flesh kiwifruit, 'Hayward' and 'AU Fitzgerald', were not significantly different in antioxidant capacities as determined by the ABTS method. The antioxidant capacity as determined by the ABTS radical scavenge assay ranged form 68.5 to 161.1 mg VCEAC/100g FW. Vitamin C (ascorbate) was suspected to contribute a large portion of the antioxidant capacities because it is one of the most effective antioxidants in fruit and vegetables. The Vitamin C content of cultivars of *A. deliciosa* ranged from 30 to 400 mg/100g FW and 'Hayward' typically contained between 80 to 120 mg/100g FW (Rassam and Laing, 2005). *A. chinensis* is usually reported to have relative high concentrations of Vitamin C (Rassam and Laing, 2005). Thus, the high antioxidant capacities of the cultivars of *A. chinensis* detected in our study might be partially contribute to a high Vitamin C content. Both ABTS and DPPH radical scavenge assays were highly correlated with soluble solids, total sugars, and reducing sugars (Table 10). Smirnoff et al. (2001) suggested that the accumulated glucose in blackberry was important because it may metabolically serve as a precursor to ascorbic acid synthesis. Hence, fruit cultivars identified with high hexose accumulation could equivalently possess high antioxidative properties (Thomas et al., 2005).

Antioxidant capacity as measured by the ABTS radical scavenge assay were lower than that of the DPPH radical scavenging assay by approximately 40% (Figure 6). The ABTS radical method is an excellent tool for determining the ability of antioxidants to quench free radicals both in aqueous and organic phase (Kim et al., 2002; Leong and Shi, 2002). High free radical quenching activities associated with DPPH radicals indicates the antioxidants were more lipid soluble and had preference toward the lipid

peroxyl radicals. As a result, the antioxidant scavenging capacities determined via the DPPH radical scavenging method were higher than that of the ABTS radical scavenging method.

Cultivars that had high antioxidant capacities in the ABTS radical scavenging assay also had high antioxidant capacities in the DPPH radical scavenge assay (Table 9, Figure 10A). This high correlation might result partially from a similar mechanism of antioxidants (hydrogen donating ability to free radicals) and also a similar solubility in aqueous/ethanol systems (Leong and Shui, 2002). The similar mechanisms of antioxidants is clearly seen in the correlations between ABTS or DPPH radical scavenge activity and CHL a and CHL b (Figure 10C and 10D).

Antioxidant capacity determined by the ABTS radical scavenge assay for 'Hort16A' (124.1 mg VCE/100g FW) (Table 8) was in agreement with results reported by Leong and Shui (2002) and Chun et al. (2005). Leong and Shui, (2002) further indicated that the DPPH radical scavenging activities were lower in cultivars of *A. chinensis*. More DPPH radicals were quenched than ABTS radicals. Different extraction methods and solvent systems utilized could contribute to the varied results observed. Whole fruit samples which included the seed, pulp, and peel, were utilized; however, Leong and Shui (2002) and Chun et al. (2005) used pulp and peel portions of kiwifruit fruit to assess the antioxidant capacity. Guo et al. (2003) showed that the peel of kiwifruit is approximately 2.5 times higher in antioxidant activities than the pulp using ferric reducing/antioxidant power assay (FRAP assay). Thus, the antioxidants in the peel of the five kiwifruit cultivars used in our study may contribute to the high DPPH radical scavenging activities observed.

Total phenolics and Total Flavonoids

The concentration of total phenolics (TPH) and total flavonoids (TF) of five kiwifruit cultivars determined at the fully ripened stage are presented in Table 8. TPH content was greater in the yellow fleshed kiwifruit cultivars than green fleshed kiwifruit cultivars (Figure 6A). 'Hort16A' contained the highest TPH concentration (155.5 mg GAE/100g FW) and 'AU Fitzgerald' contained the lowest (88.5 mg GAE/100g FW). The mean TPH content of all the kiwifruit cultivars (122.1 mg GAE/100g FW) was twice the value previously reported by Chun et al. (2005) (61.21 mgGAE/100g FW), but approximately 40% of the value reported by Imeh and Khokhar, (2002) (302.8 mg GAE/100g FW). Chun et al. (2005) and Imeh and Khokhar (2002) reported TPH values varied considerably, this in part may be due to fruit samples that were purchased from local markets in which cultivar, stage of maturity or growing conditions were unknown.

TPH content was negatively correlated with β-carotene and chlorophyll a/b content (Table 11, Figure 11A). In contrast, TPH content increased concomitantly with pH (Figure 11C) while β-carotene, CHL a, and CHL b content declined (Figure 11B). These intricate relationships revealed interconnections between normal maturation physiologies and the defensive system in kiwifruit. Flavonoids, such as anthocyanins, are known to accumulate in shade-adapted plants and provide protection during periods of exposure to high solar radiation (Majumdar et al., 1991; Wahid and Ghazanfar, 2005). Kiwifruit generally possess low anthocyanin content thus other flavonoids may contribute to *in-vivo* protection during certain abiotic stress. During certain environmental stress TPH are known to increase to protect chloroplasts from photo-oxidative damage by absorbing UV-B (Wahid and Ghazanfar, 2005). Increased cellular pH is assumed to be

the result of specific H^+-antiporters located on the tonoplast when the TPH content increased. The specific H^+-antiporters were found to be involved in sequestration of flavonoids into mesophyll vacuoles in barley leaves. The pH gradient generated by the vacuolar proton pumps was the driving force for the uptake of the flavonoids that were often glycosylated (Frange et al., 2002). Hence, as more flavonoids were transported into the vacuoles, the pH of the vacuole increased due to more protons transported outside of the vacuole.

TF did not vary significantly among the cultivars ($Pr > F = 0.1581$) (Table 8, Figure 9B). The mean TF content for the five kiwifruit cultivars was 29.6 mg CE/100g FW) which was four times higher than reported by Chun et al. (2005). The difference could be due to different extraction methods and solvent systems utilized. We used an aqueous acetone mixture. In similar studies, Chun et al. (2005) used 80% aqueous methanol as the extraction solvent. Extraction solvents such as aqueous acetone have been reported to be superior extraction solvents than aqueous methanol in extracting phenolics in berries and apples (Kahkonene et al., 2001). Hence, it is logical that we obtained higher concentrations of TF for the five kiwifruit cultivars studied due to the choice of extraction solvent employed. In the present study, both TPH and TF contents did not correlate with antioxidant capacities determined by the ABTS and DPPH radical scavenging assays (Figure 10E, 10F). TPH and TF were determined for fruit commercially ripened and the antioxidant scavenging capacities (ABTS and DPPH) were determined at the initial harvest maturity stage. This difference in stage of maturity at the time of antioxidant assessment most likely reflects different antioxidant mechanisms *in-vivo*.

Anthocyanins were not detected in the five kiwifruit cultivars in this study (Data not shown). Montefiori et al. (2005) reported reduced or low concentrations in the range of micrograms of anthocyanins per gram fresh weight. Hence, it is unlikely that the presence of anthocyanins would make a significant contribution to the antioxidant capacity of kiwifruit.

Comparison between yellow flesh and green flesh kiwifruit

For each variable, the data collected from the five kiwifruit cultivars was organized into two groups based on flesh color for comparative purpose. 'Hayward' and 'AU Fitzgerald' became one group and 'Hort16A', 'Golden Sunshine', and 'Golden Dragon' became the second group. Significant differences were observed for each variable between the yellow and green flesh kiwifruit with the exception of TF (Table 12). Interestingly, the flavonol composition reported in the juice of 'Hayward', includes the presence of quercetin and kaempferol-3-rhamnoside and is largely unchanged after brief high temperature treatment and was indicated as the best identifier of kiwifruit juice (Dawes and Keene, 1999). Since the TF content were not significantly different between cultivars, the flavonoid compounds may be set up genetically to act as pigments as well as baseline protectors against heat and sunlight.

Conclusion

Kiwifruit are an excellent source of dietary phytonutrients such as Vitamins C and E, β-carotene and polyphenolics. In summary, the physicochemical and antioxidant properties of five kiwifruit cultivars were highly correlated. Based on results of SSC, pH,

TA and ethanol soluble carbohydrate content, the yellow fleshed kiwifruit ('Hort16A', 'Golden Sunshine' and 'Golden Dragon') were sweeter, less acid and therefore more appealing in terms of consumer preference than the green fleshed kiwifruit fruit ('Hayward' and 'AU Fitzgerald'). Based on antioxidant capacity as measured by ABTS and radical scavenging activity (expressed in terms of VCEAC), the yellow fleshed kiwifruit ('Hort16A', 'Golden Sunshine' and 'Golden Dragon') had significantly higher cellular protection from free radicals than the green fleshed kiwifruit ('Hayward' and 'AU Fitzgerald'). Both assays were highly correlated suggesting similar antioxidative mechanisms may be present in fruit extracts. In general, the results confirm that among the five cultivars of kiwifruit, the most health promotive effects associated with consumption of kiwifruit are with the yellow fleshed fruit ('Hort16A', 'Golden Sunshine' and 'Golden Dragon') when compared to the green fleshed cultivars ('Hayward' and 'AU Fitzgerald'). The commercial production of yellow fleshed kiwifruit in Alabama would therefore provide an excellent source of natural dietary antioxidant to meet the general public's daily nutrient requirement.

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APPENDICES

APPENDIX A: TABLES

Table 1. Nomenclature of all possible reactive oxygen species (ROS). Adapted from Wilcox et al. 2004.

Free Radicals	Nonradicals
Superoxide, $O_2^{\bullet -}$	Hydrogen Peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Hypobromous acid, HOBr
Hydrogenperoxyl, $HO_2^{\bullet}$	Hypochlorous acid, HOCl
Peroxyl, $RO_2^{\bullet}$	Ozone, O_3
Alkoxyl, $RO^{\bullet}$	Singlet oxygen, O_2^1
Carbonate, $CO_3^{\bullet -}$	Organic peroxide, ROOH
Carbon dioxide, $CO_2^{\bullet -}$	Peroxy nitrite, $ONOO^-$
	Peroxynitrous acid, ONOOH

Table 2. Total phenolic (TPH), total flavonoid (TF), and total monomeric anthocyanin (ACY) content of five blackberry cultivars measured at the full ripe stage.

Cultivar	TPH (mg GAE/ 100g FW)	TF (mg CE/100g FW)	ACY (mg CGE/ 100g FW)
Apache	306.2a	64.0	72.3a
Arapaho	328.7a	65.6	40.3b
Loch Ness	342.1a	71.4	74.1a
Navaho	252.6b	53.6	68.2a
Triple Crown	221.2b	56.5	38.3b
Pr > F	0.0007	0.0604	<0.0001

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); FW, fresh weight; Values are the mean of four determinations

Table 3. Antioxidant capacity determined by ABTS and DPPH radical scavenging capacity assay of five blackberry cultivars measured at the full ripe stage.

Cultivar	ABTS (mg VCEAC/100mg FW)	DPPH (mg VCEAC/100mg FW)
Apache	650.5ab	440.4
Arapaho	621.0bc	396.7
Loch Ness	698.5a	464.2
Navaho	590.5bc	419.2
Triple Crown	559.5c	347.0
Pr > F	0.0137	0.0822

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); VCEAC, vitamin C equivalent antioxidant capacity; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FW, fresh weight; Values are the mean of four determinations

Table 4. Linear regression correlation coefficient (R) of different variables measured among five blackberry cultivars.

Variable	ABTS	TPH	TF	ACY
DPPH	0.897*	0.748	0.614	0.899*
ABTS		0.893*	0.895*	0.670
TPH			0.916*	0. 372
TF				0.273

TPH, total phenolics; TF, total flavonoids; ACY, total monomeric anthocyanins
* Significant at $p \leq 0.05$

Table 5. Physicochemical properties of five kiwifruit cultivars measured at the commercial harvest stage.

Cultivar	Flesh color	pH	TA%	SSC (%)	SSC/TA
Hayward	Green	3.89ab	0.491b	5.90d	12.3c
AU Fitzgerald	Green	3.83b	0.606a	7.40c	12.1c
Hort16A	Yellow	3.97a	0.326c	12.0b	37.1a
Golden Sunshine	Yellow	3.98a	0.467b	13.6a	29.2b
Golden Dragon	Yellow	3.98a	0.492b	14.1a	28.8b
Pr>F		0.0052	<0.0001	<0.0001	<0.0001
LSD Values		0.0841	0.075	1.481	4.580

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); TA, titratable acidity; SSC, soluble solids content. Values are the mean of four determinations.

Table 6. Sugar content of five kiwifruit cultivars measured at the commercial harvest stage.

Cultivar	Total Sugars (mg/100 g FW)	Reducing Sugars (mg/ 100g FW)	Sucrose[a] (mg/ 100g FW)
Hayward	3,464c	1,601c	1,863
AU Fitzgerald	3,746c	2,587c	1,159
Hort16A	10,488b	10,697b	0*
Golden Sunshine	14,458a	16,025a	0*
Golden Dragon	16,234a	12,786b	3,448
Pr > F	<0.0001	<0.0001	

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); N/A, not available; Values are the mean of three determinations.

a. Sucrose level was calculated by subtracting reducing sugar content from the total sugar content from the mean value presented for each cultivar.

* Sucrose level was treated as zero because reducing sugar content was greater than the total sugar content.

Table 7. Chlorophylls and β-carotene concentrations of five kiwifruit cultivars measured at the commercial harvest stage.

Cultivar	β-carotene (mg/ 100g Fw)	β-carotene ratio to Hayward	CHL a (mg/ 100 g FW)	CHL b (mg/ 100g FW)	CHL a+b (mg/ 100g FW)	Chl a+b ratio to Hayward
Hayward	0.421a	1.00	0.909a	0.527a	1.44a	1.00
AU Fitzgerald	0.377a	0.897	0.974a	0.526a	1.50a	1.05
Hort16A	0.226b	0.538	0.115b	0.049b	0.164b	0.114
Golden Sunshine	0.186b	0.441	0.186b	0.118b	0.305b	0.212
Golden Dragon	0.196b	0.465	0.106b	0.064b	0.170b	0.118
Pr > F	0.0001		<0.0001	<0.0001	<0.0001	

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); CHL, chlorophyll. Values are the mean of three determinations

Table 8. Total phenolic (TPH) and total flavonoid (TF) concentrations of five kiwifruit cultivars measured at the consumption stage.

Cultivar	TPH (mg GAE/100g FW)	TF (mg CE/100g FW)
Hayward	95.2b	27.0
AU Fitzgerald	88.5.b	28.9
Hort16A	155.5a	26.1
Golden Sunshine	135.7a	39.5
Golden Dragon	135.7a	26.3
Pr > F	0.0010	0.1581

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); GAE, gallic acid equivalence; CE, catechin equivalence; FW, fresh weight. Values are the mean of four determinations

Table 9. Antioxidant capacities of five kiwifruit cultivars measured at the harvest stage.

Cultivar	ABTS (mg VCEAC/100g FW)	DPPH (mg VCEAC/100g FW)
Hayward	68.5c	129.6d
AU Fitzgerald	92.0c	168.9c
Hort16A	124.1b	240.3b
Golden Sunshine	177.9a	319.0a
Golden Dragon	161.6a	295.2a
Pr > F	<0.0001	<0.0001

Different letters in the same column indicate significant difference using LSD ($p \leq 0.05$); ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; VCEAC, vitamin C equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FW, fresh weight. Values are the mean of four determinations

Table 10. Linear regression coefficient of determination (R-square) between VCEAC, DPPH, and other dependent variables.

Variable	ABTS	Pr > F	DPPH	Pr > F
DPPH	0.994*+	0.002		
β-carotene	0.907*-	0.0124	0.948*-	0.0051
CHL a	0.730	0.0654	0.794*-	0.043
CHL b	0.713	0.0721	0.780*-	0.047
TPH	0.520	0.170	0.595	0.126
TF	0.310	0.330	0.260	0.380
SSC	0.921*+	0.0096	0.956*+	0.004
TS	0.912*+	0.0114	0.933*+	0.0076
RS	0.950*+	0.0048	0.970*+	0.0023
pH	0.652	0.098	0.701	0.0767
TA	0.0717	0.663	0.111	0.584

ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; CHL, chlorophyll, TP, total phenolics, TF, total flavonoids; TA, titratable acidity; SSC, soluble solids content; TS, total sugars; RS, reducing sugars.

* Significant at $p \leq 0.05$

+ positive correlation

- negative correlation

Table 11. Linear regression coefficient of determination (R-square) of different variables measured among five kiwifruit cultivars.

Variable	TA	RS	TS	SSC	TPH	TF	β-Carotene	CHL a	CHL b
pH	0.510	0.810*+	0.813*+	0.782*+	0.838*+	0.0567	0.796*-	0.918*-	0.879*-
TA		0.223	0.152	0.201	0.703	0.0125	0.259	0.475	0.478
RS			0.909*+	0.940*+	0.707	0.268	0.959*-	0.862*-	0.842*-
TS				0.963*+	0.641	0.103	0.923*-	0.863*-	0.839*-
SSC					0.736	0.108	0.988*-	0.910*-	0.907*-
TPH						0.0104	0.793*-	0.932*-	0.943*-
TF							0.131	0.0345	0.258
β-carotene								0.929*+	0.930*+
CHL a									0.995*+

CHL, chlorophyll; TPH, total phenolics, TF, total flavonoids; TA, titratable acidity; RS, reducing sugars; SSC, soluble solids content; TS, total sugars.

* Significant at $p \leq 0.05$

+ positive correlation

- negative correlation

Table 12. Comparisons between green flesh and yellow flesh kiwifruit.

Variable	Green	Yellow	Pr > F	Variable	Green	Yellow	Pr > F
pH*	3.86	3.98	0.0003	β-carotene*	0.399	0.216	< 0.0001
TA*	0.549	0.428	0.0058	CHL a*	0.942	0.136	< 0.0001
SSC*	6.65	13.2	< 0.0001	CHL b*	0.527	0.077	< 0.0001
SSC/TA*	12.2	31.7	< 0.0001	CHL a+b*	1.47	0.213	<0.0001
RS*	2,094	13,169	<0.0001	TS*	3,605	8,912	<0.0001
TPH*	91.9	142.3	< 0.0001	DPPH*	149.3	284.8	< 0.0001
TF	28.0	30.6	0.5232	ABTS*	80.3	154.5	< 0.0001

ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; CHL, chlorophyll, TPH, total phenolics; TF, total flavonoids; TA, titratable acidity; SSC, soluble solid content; TS, total sugars. Values of green are averages of cultivars of *A. deliciosa* and values of yellow are averages of cultivars of *A. chinensis*.

* Significant at $p \leq 0.05$

APPENDIX B: FIGURES

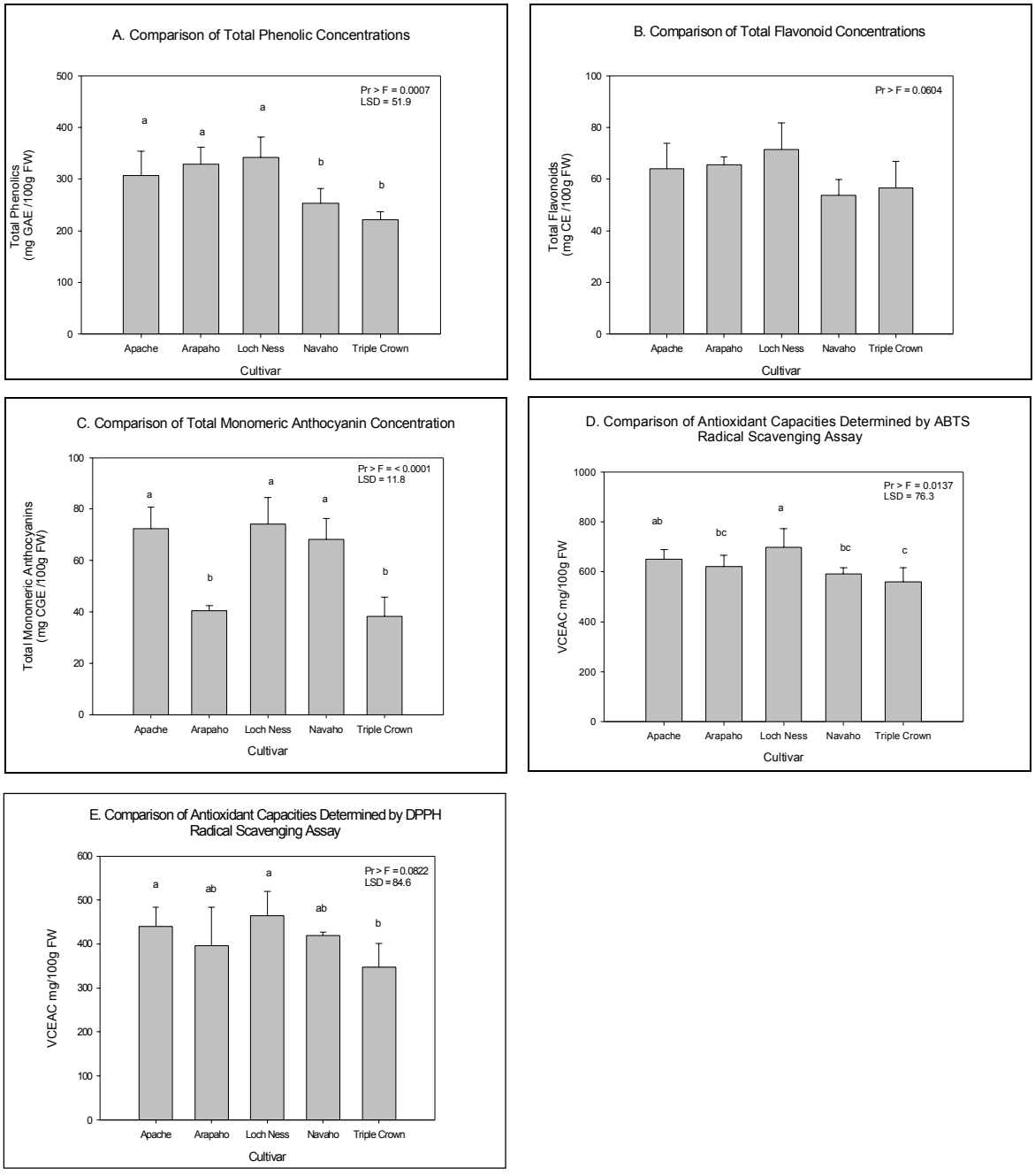

Figure 1.Comparison of significance between means of five blackberry cultivars in different assays harvest at the full ripe stage. Different letters indicate significant difference using LSD ($p \leq 0.05$)

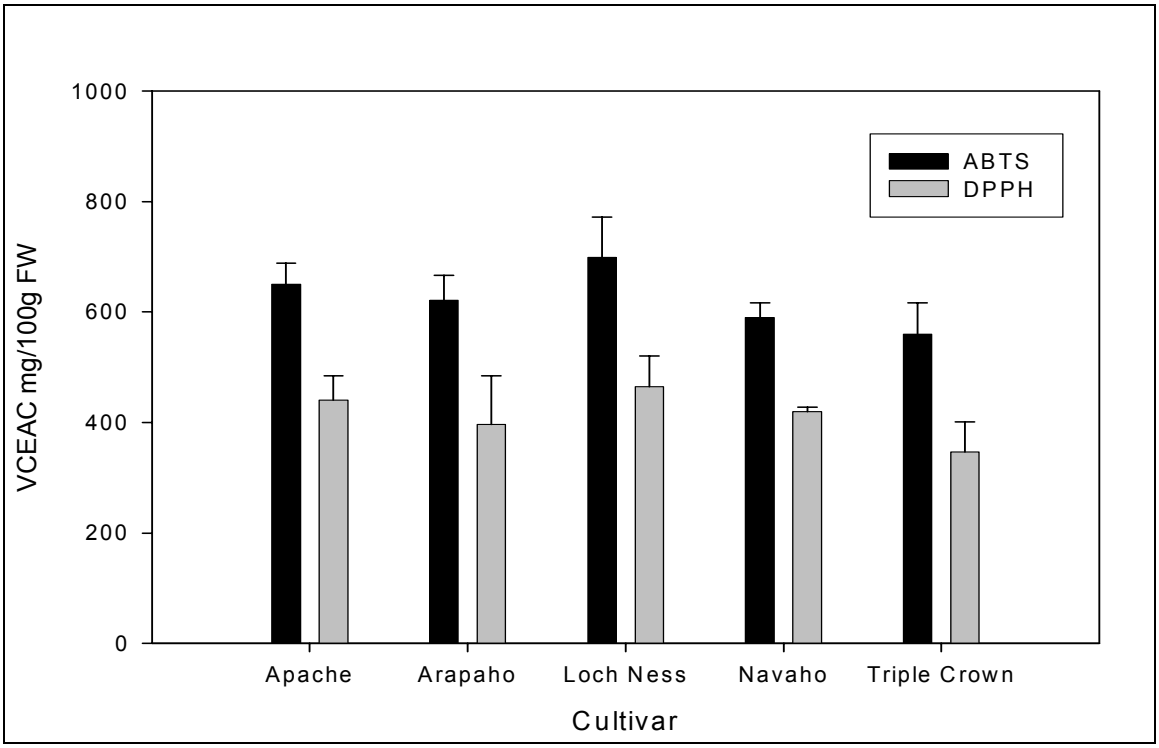

Figure 2. Comparison of antioxidant capacities measured by ABTS and DPPH radical scavenging assays among five blackberry cultivars at the full ripe stage. The DPPH radical scavenging activity underestimated antioxidant capacity approximately 34%.

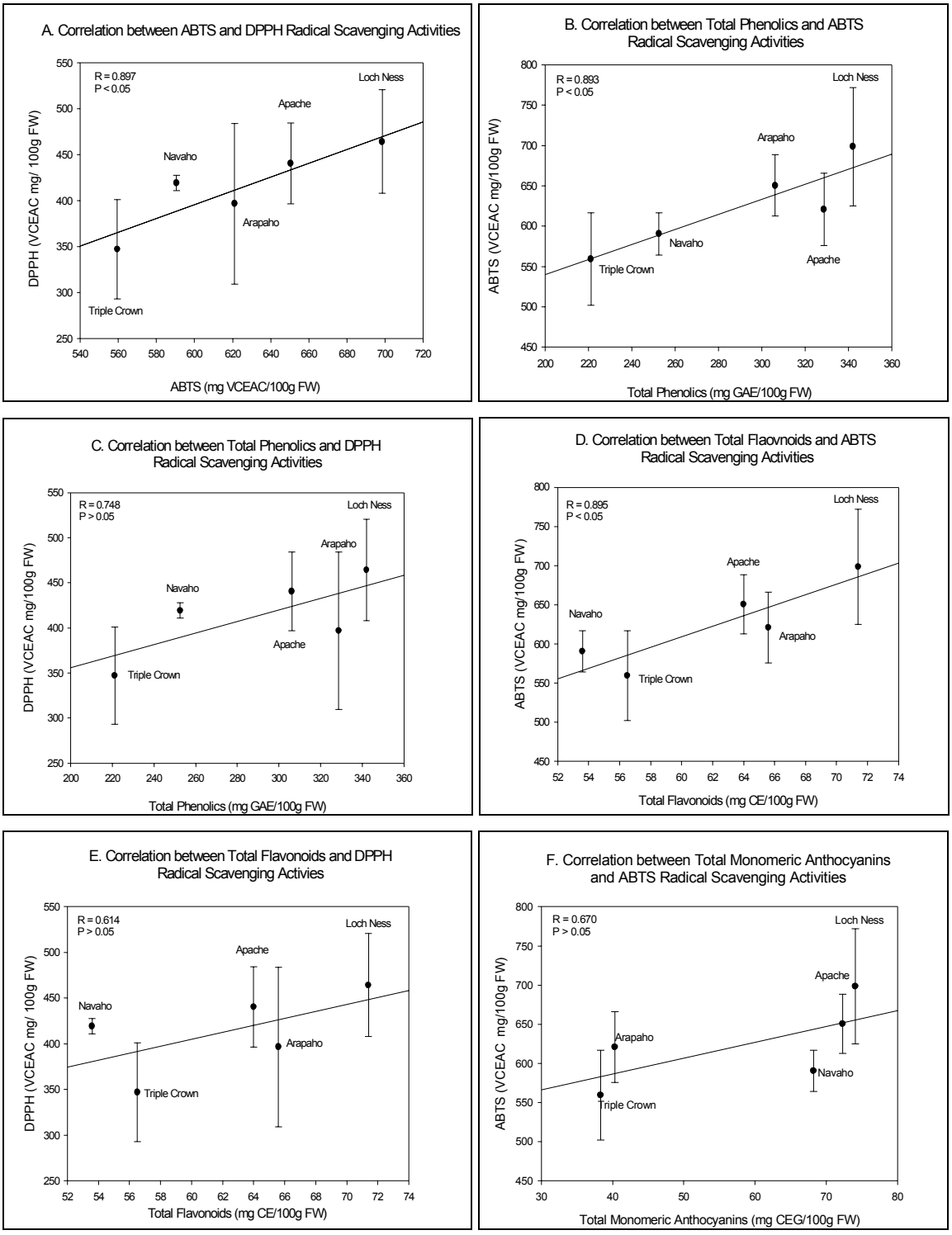

Figure 3. Correlations between antioxidant assays, and between bioassays and polyphenolic compounds, and between different types of polyphenolic compounds for blackberries harvested at the full ripe stage.

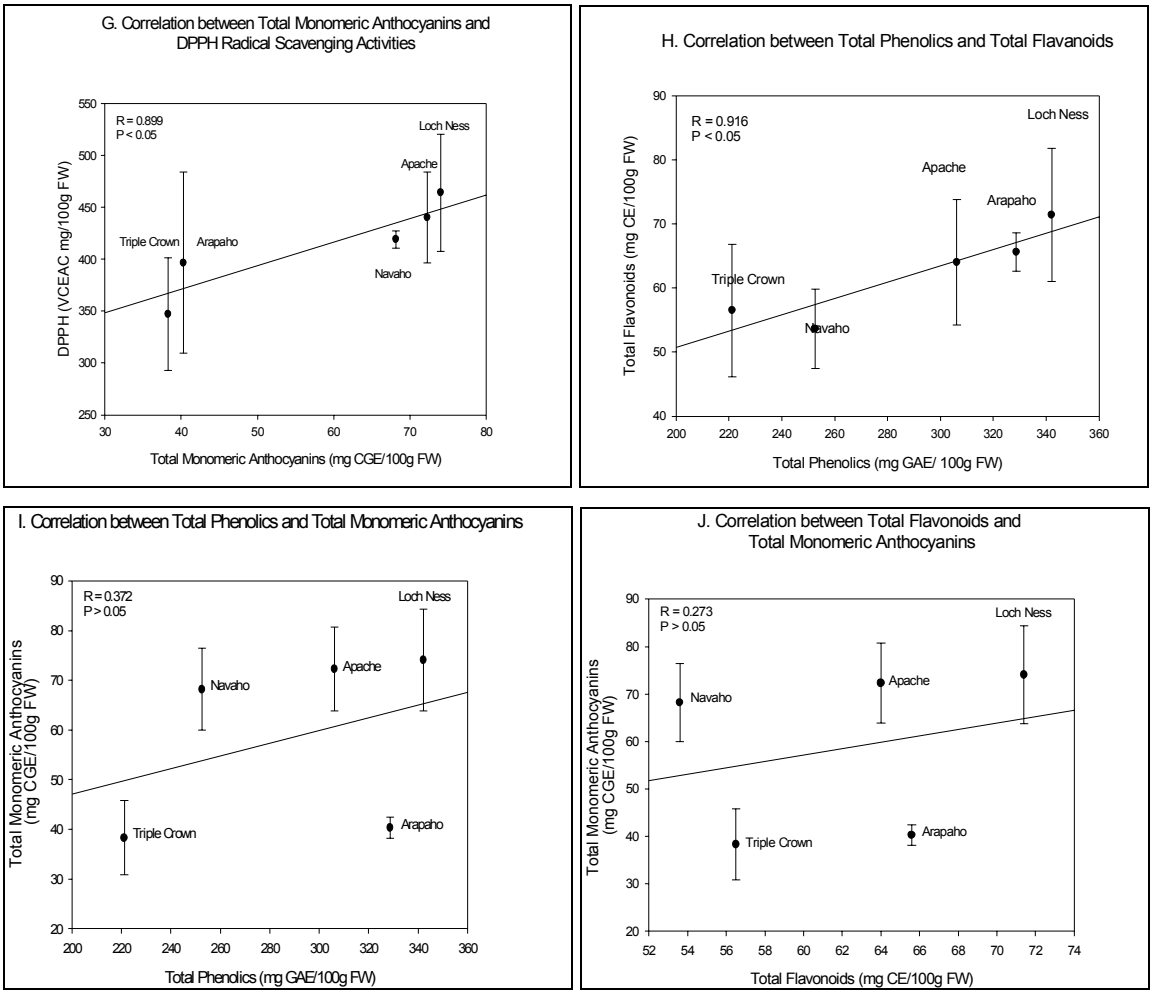

Figure 3. Correlations between antioxidant assays, and between bioassays and polyphenolic compounds, and between different types of polyphenolic compounds for blackberries harvested at the full ripe stage.

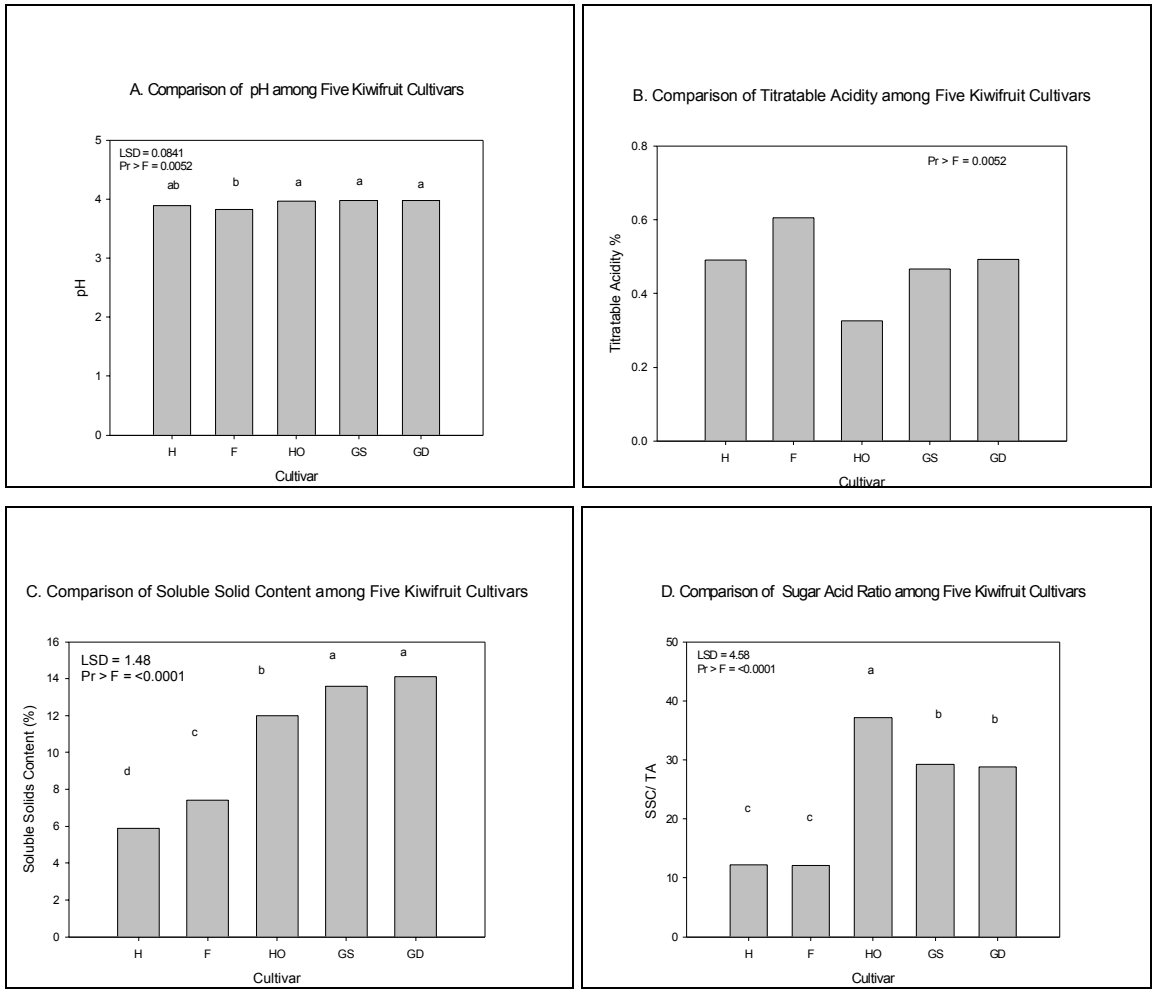

Figure 4. Comparison of significance between means of five kiwifruit cultivars for different physicochemical properties at commercial harvest stage. Different letters indicate significant difference ($p \leq 0.05$); SSC, soluble solids content; TA, titratable acidity; FW, fresh weight; H, Hayward; F, AU Fitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon. Values are the mean ± SD (standard deviation) of three determinations.

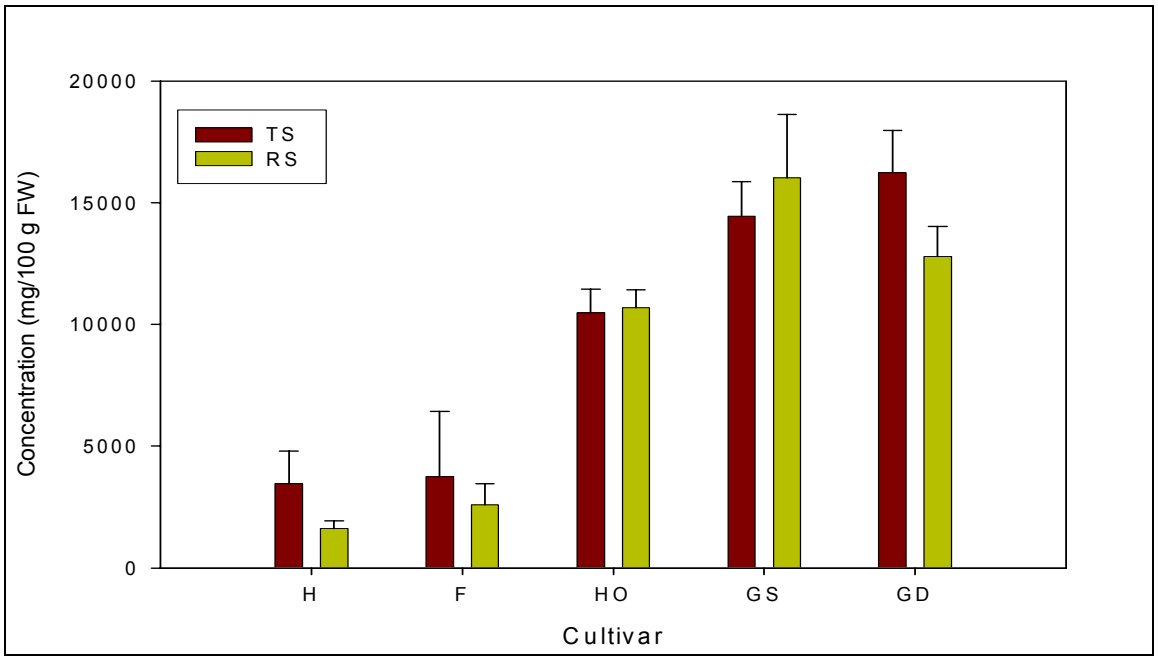

Figure 5. Total sugar and reducing sugar content of the five kiwifruit cultivars at commercial harvest maturity. Sucrose level was determined from the difference of total sugar and reducing sugar. The total sugar content for 'Hort16A' and 'Golden Sunshine' were treated as zero. TS, total sugars; RS, reducing sugars; FW, fresh weight; H, Hayward; F, AU Fitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon

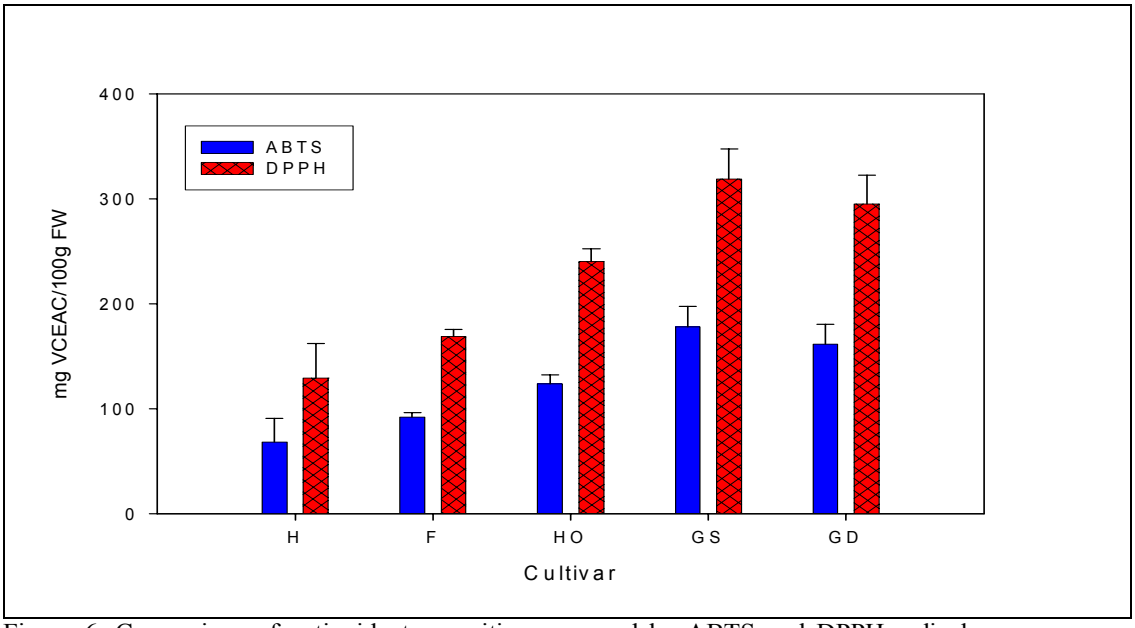

Figure 6. Comparison of antioxidant capacities measured by ABTS and DPPH radical scavenge assays between five kiwifruit cultivars at commercial harvest stage. The ABTS radical scavenge activity underestimated antioxidant capacity approximately 40% to the DPPH radical scavenge activity. ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; VCEAC, vitamin C equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FW, fresh weight; H, Hayward; F, AU Fitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon.

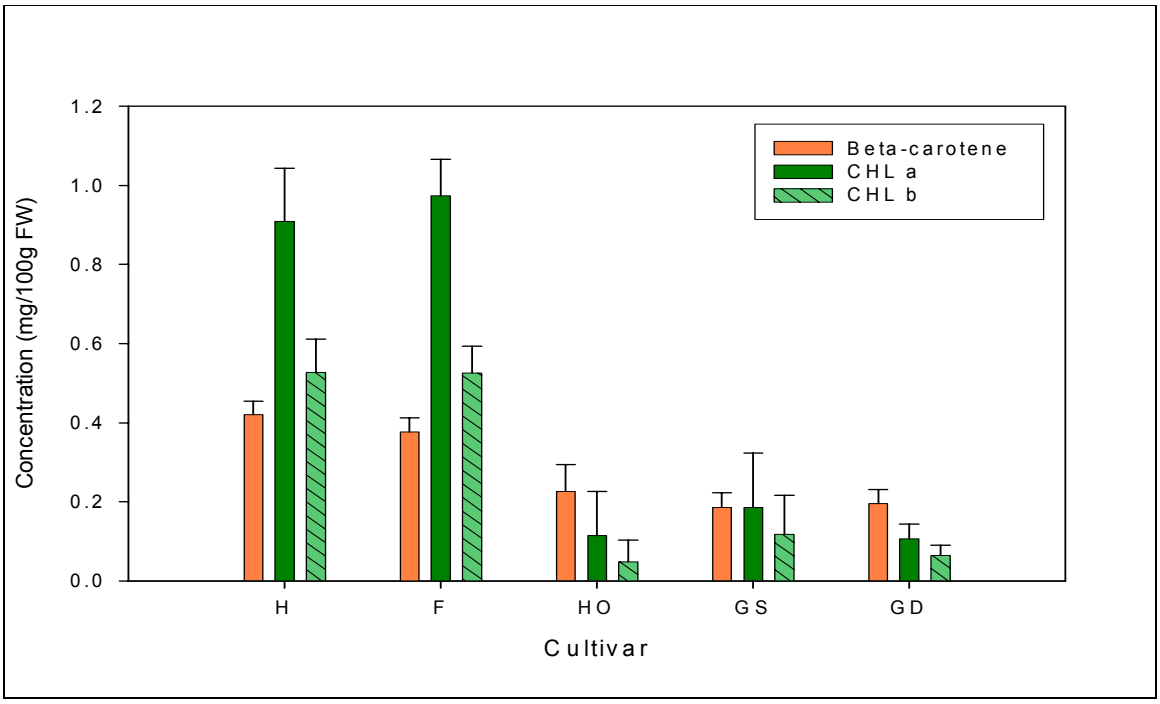

Figure 7. Comparison of β-carotene and chlorophyll a/b between five kiwifruit cultivars at commerical harvest stage. The green flesh kiwifruit, 'Hayward' and 'Fitzgerald', had high levels of β-carotene and chlorophyll a/b compared to the yellow flesh kiwifruit, 'Hort16A', 'Golden Sunshine', and 'Golden Dragon'. H, Hayward; F, AU Fitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon; FW, fresh weight.

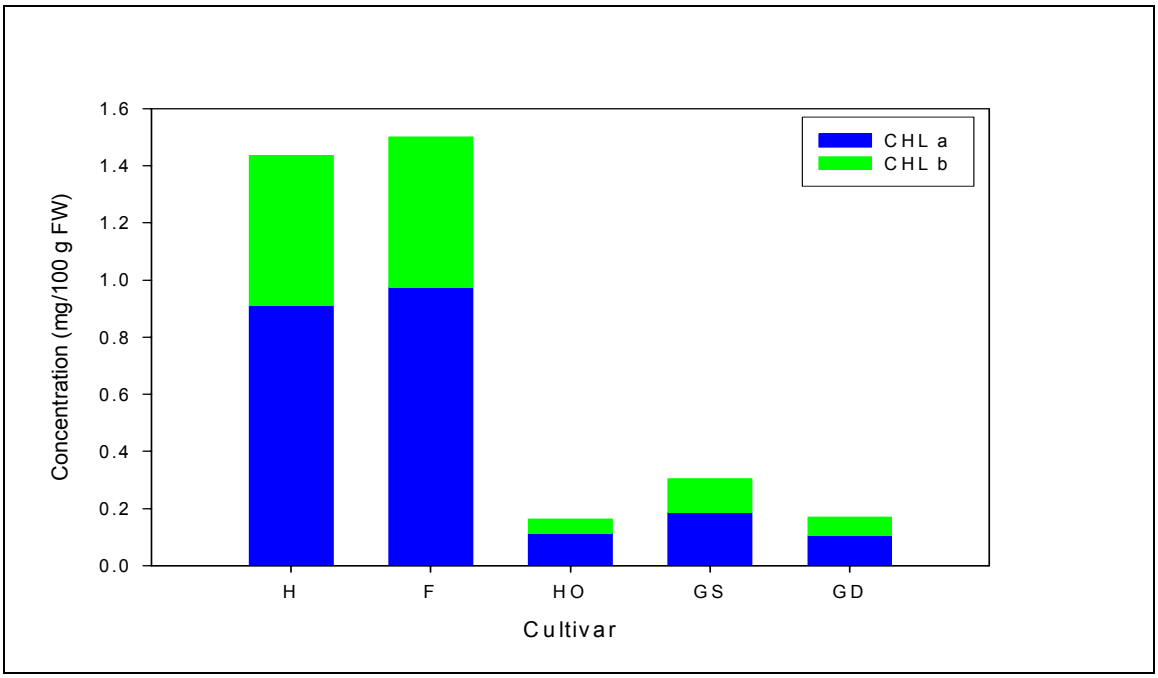

Figure 8. Comparison of chlorophyll a, b, and a+b between five kiwifruit cultivars at commercial harvest stage. Chlorophyll a dominated over chlorophyll b by a 2 to 1 ratio. CHL a, chlorophyll a; CHL b, chlorophyll b; FW, fresh weight; H, Hayward; F, AUFitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon.

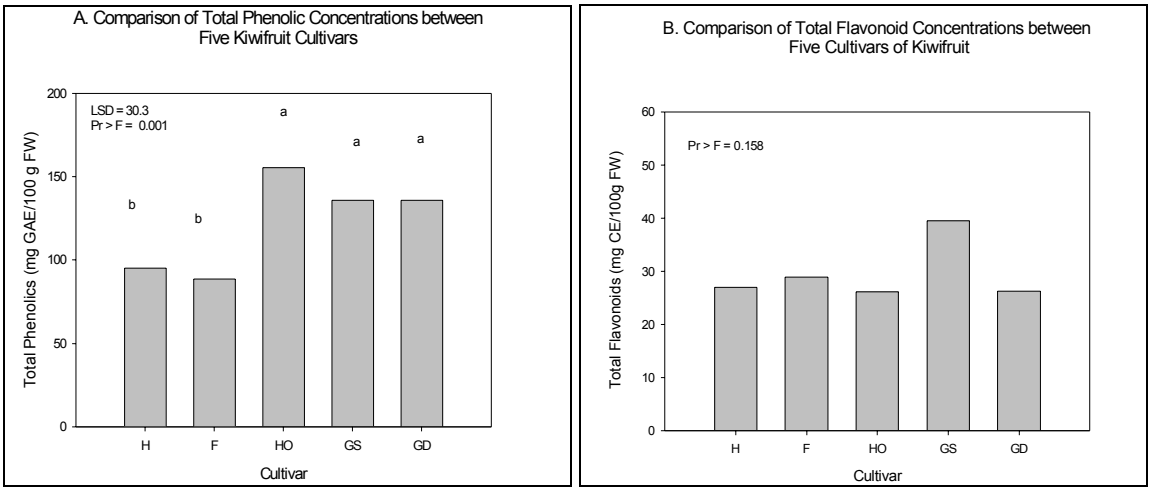

Figure 9. Comparison of polyphenols among five kiwifruit cultivars at consumption stage. Different letters indicate significant difference ($p \leq 0.05$); GAE, gallic acid equivalence; CE, catechin equivalence; FW, fresh weight; H, Hayward; F, AU Fitzgerald; HO, Hort16A; GS, Golden Sunshine; GD, Golden Dragon.

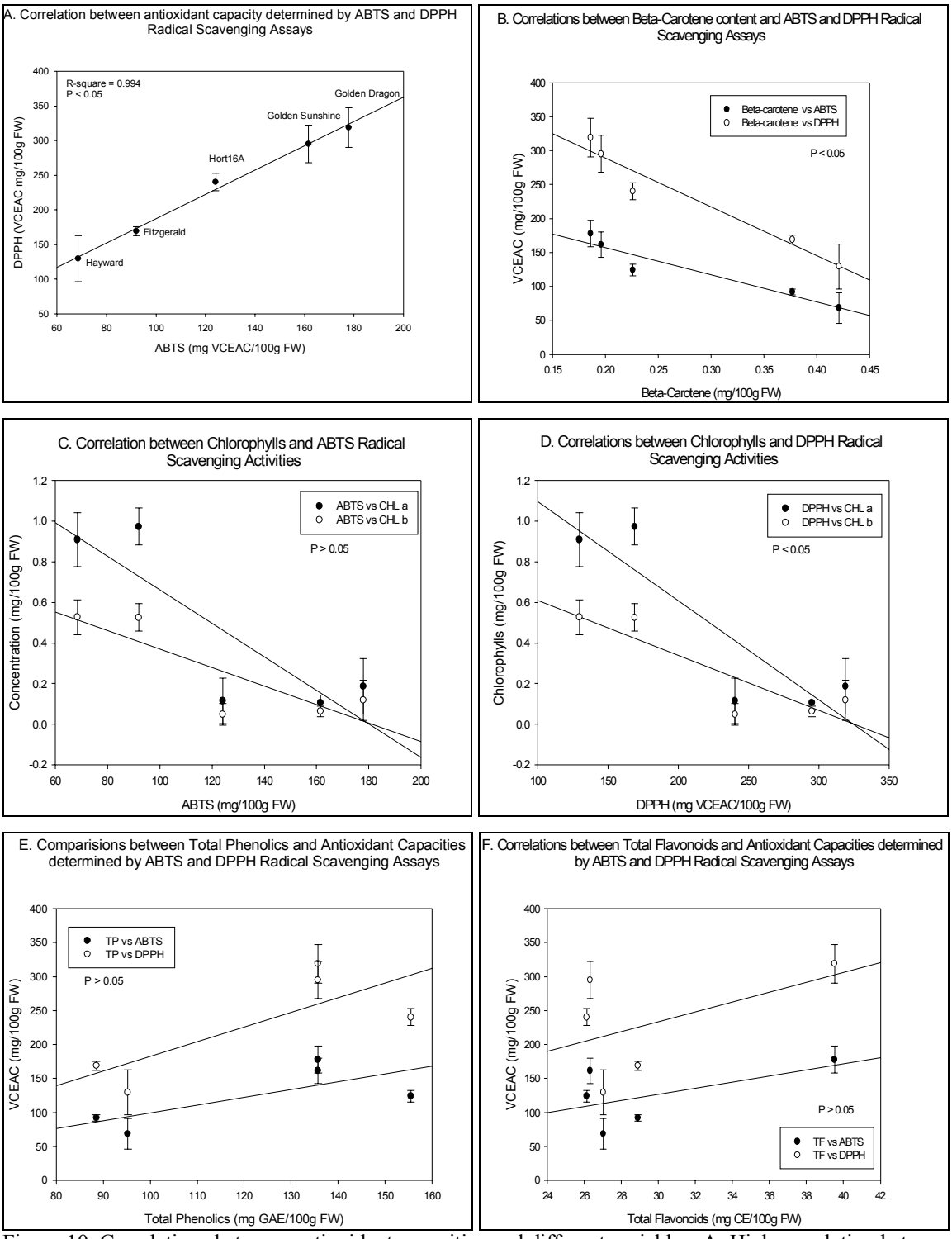

Figure 10. Correlations between antioxidant capacities and different variables. A. High correlation between the ABTS and DPPH radical scavenge assays. Linear relationship is significant at $p \leq 0.05$ CHL, chlorophyll; TP, total phenolics; TF, total flavonoids; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt; VCEAC, vitamin C equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; CE, catechin equivalence; GAE, gallic acid equivalence; FW, fresh weight.

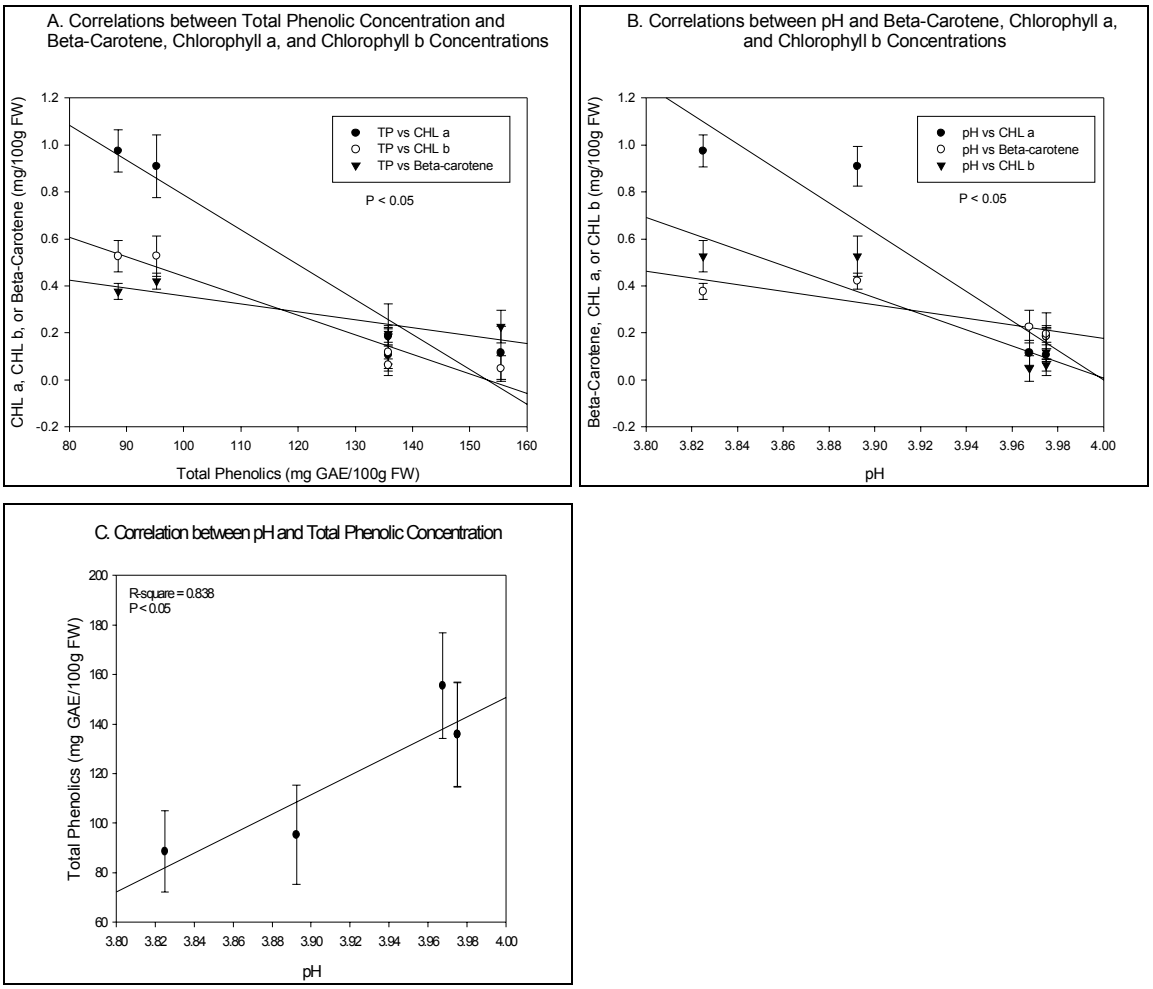

Figure 11. Linear correlations between different variables. A. Negative correlations were shown between total phenolic and β-carotene, chlorophyll a, and chlorophyll b content; B. Negative correlations were shown between pH and β-carotene, chlorophyll a, and chlorophyll b content; C. Positive correlations was shown between pH and total phenolic concentration. Linear relationship is significant at $p \leq 0.05$.

APPENDIX C: STANDARDIZED SUMMARY COMPARISON OF METHODS FOR DETERMINATION OF BIOACTIVE COMPOUNDS IN BLACKBERRY AND KIWIFRUIT.

Antioxidant Method	BASIS FOR SELECTION Advantage	BASIS FOR SELECTION Disadvantage
ABTS Radical Anion Scavenging Activity	Inexpensive and easy to use, based on the scavenging ability of antioxidants, rapid, applicable over wide pH ranges, soluble in both aqueous and organic solvents; total antioxidant value can be estimated.	Many compounds have low redox potentials and can thus react with $ABTS^{\bullet-}$, reaction may not be the same for slow reactions, and thus may take long time to reach endpoint.
DPPH Radical Scavenging Activity	Simple, needs only a UV-vis spectrophotometer	DPPH radicals may interact with other radicals, i.e. alkyl, may require long reaction time. Carotenoids, Anthocyanins may interfere, thus pH dependent, not standardized.
FRAP	Simple, inexpensive, robust, fast, measures only the reducing capability based on ferric ion and does not require specialized equipment,	Does not detect compounds that act by radical quenching, i.e. thiols, and proteins oxidizable substrates,
VCEAC	Simple, reliable, correlates with ORAC, Trolox and phenolics, calculated on weight basis familiar with scientists and general, soluble in aqueous and organic phases	

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