AN ABSTRACT OF THE THESIS OF

Maria Angela Artania Widyasari for the degree of Master of Science in Food Science and Technology presented on June 14, 2002

Title: Apple Polyphenolics and Their Antioxidant Properties: Influence of Cutivars, Post-harvest Storage and 1- MCP Treatment

Abstract approved: _____

Ronald E. Wrolstad

The distribution of total phenolics, antioxidant capacity, monomeric anthocyanin, and ascorbic acid contents in the peel and flesh of Red Delicious, Granny Smith, and Fuji apples during a six- month storage period were determined. In addition, the effect of 1-MCP (1- Methyl cyclopropene) on these parameters of the edible portion of apples during storage was also investigated. Two different assays were employed to determine antioxidant activities: the spectrofluorometric-based Oxygen Radical absorbing Capacity (ORAC) and the spectrophotometric-based Ferric Reducing Antioxidant Power (FRAP). Total phenolics were measured using the Folin- Ciocalteau method. A determination of monomeric anthocyanin content was also performed using the pH- differential method for Red Delicious and Fuji apples.

Antioxidant activity was predominantly observed in the aqueous fraction and was attributed to polyphenolics. Antioxidant activities were highest in the peel, with Red Delicious peel having the highest values, presumably due to anthocyanin pigments. At zero time month storage, the ORAC, FRAP, total phenolics, and total monomeric anthocyanin contents of Red Delicious peel were: 37.7μmol T.E./g, 62.7 μmol T.E./g, 6.63 mg/g GAE, and 26.4 mg/100g, respectively. The three tested cultivars were significantly different with respect to ORAC, FRAP, total phenolics, and monomeric anthocyanin contents. 1- MCP treatment did not have a

significant influence on antioxidant activities and monomeric anthocyanin contents of the three cultivars during storage. Storage did not have significant influence on ORAC values of the edible portion of apple, however it had significant influence on the FRAP values. The contribution of ascorbic acid to the total antioxidant capacity was small.

Apple Polyphenolics and Their Antioxidant Properties: Influence of Cultivars, Post- Harvest Storage, and 1- MCP Treatment

By

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APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

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

Maria Angela Artania Widyasari, Author

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APPLE POLYPHENOLICS AND THEIR ANTIOXIDANT PROPERTIES: INFLUENCE OF CULTIVAR, POST- HARVEST STORAGE, AND 1-MCP TREATMENT

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The United States is one of the largest apple producers in the world, second only to China (Anonymous 2001a). The leading apple-producing state in the US is Washington, which produces more than 40% of the nation's apples for fresh and processed use. New York is usually second in apple production, followed by California, Michigan, Pennsylvania, and Virginia (Anonymous 2001b). In the year 2000, Washington's apple crop consisted of 48.5% Red Delicious, 17% Golden Delicious, 12% Fuji, 8% Granny Smith, 8.5% Gala, 2.4% Braeburn, and 3.6% other varieties, including Jonagold, Cameo, Pink Lady, and Rome (Anonymous 2001b). There are five grades of Washington apples, the highest being Washington Extra Fancy, followed by US Extra Fancy, Washington Extra Fancy, US Fancy, US no.1, and US No 1. Hail (Anonymous 2001b).

On average, the current per capita apple consumption in the US is about 8,100 grams per year (Anonymous 1998). Studies by Vinson (2001) indicated that apples compare favorably to other fruits in terms of their contribution to each American's average dietary intake, particularly when considering per capita

consumption. In 1998, Vinson reported that apples contributed 50.6 mg phenol/day. Vinson subsequent reported (2001) that apples contribute 57.1 mg phenol/ day.

Apples (*Malus domestica* cv. Borkh), available year- round, are an inexpensive source of fiber. In addition, apples contain phenolic compounds, some of which have demonstrated antioxidant activity. Phenolics, which have been reported in many varieties of apples (Spanos and others 1990; Andrade and others 1998; Guyot and others 1998; Burda and others 1990; Sanoner and others 1999; Hammerstone and others 2000; Kähkönen and others 2001, Schieber and others 2001), include cinnamic acid derivatives and flavanols which represent about 90% of the total phenolic content of apple cortices (Amiot and others 1992); wide variations occur among cultivars (Robards and Prenzler 1999).

While many apples available to the consumer have been stored for up to 6 months, there has been little information on how storage effects antioxidant capacity, total phenolic and monomeric anthocyanin contents of apples. These results should be of value to those studying antioxidants and to researchers investigating the effects of postharvest conditions and seeking to preserve fruit quality. The objectives of this study are 1) to measure antioxidant capacity, total phenolics, and monomeric anthocyanin contents of Red Delicious, Fuji, and Granny Smith apple extracts as well as their distribution in the peel and flesh

during a six- month storage period; and 2) to determine the effect of 1-MCP treatment and postharvest storage on antioxidant capacity, total phenolics, and anthocyanin contents.

LITERATURE REVIEW

Apple Polyphenolics

Apples are among the most ancient crops known to man. Apple trees (*Malus domestica* Borkh) are grown throughout the world; they belong to the Rosaceae family which includes more than 100 genera and more than 2000 species. Apples belong to the subfamily Pomoidae which contains 18 genera and the genus Malus (Rehder 1940). The Rosaceae family includes many other fruit- producing plants such as pear, cherry, blackberry, apricot, and plum as well as the ornamental plant the rose (Harborne 1967).

Apple is a pome fruit developed from an inferior ovary, and is derived from the ovary wall and the floral tube (Teskey and Shoemaker 1982). The floral tube is fused with the ovary wall, becomes fleshy and ripens with it. The fleshy mesocarp constitutes the main edible portion.

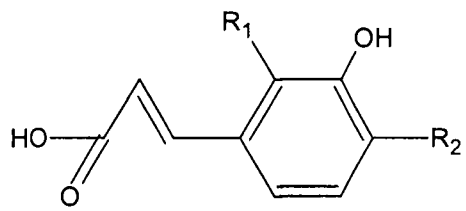
Apples contain phenolic compounds which are secondary plant metabolites derived from the shikimate pathway and phenylpropanoid metabolism. Generally,

phenolic compounds possess an aromatic ring bearing one or more hydroxy groups. Phenolic compounds in plants protect against invading pathogens and damaging UV irradiation, serve as attractants for seed dispersal and pollination, and function as substrates for polyphenoloxidase; phenolic compounds also play a possible role in plant growth and fruit ripening (Macheix and others 1990).

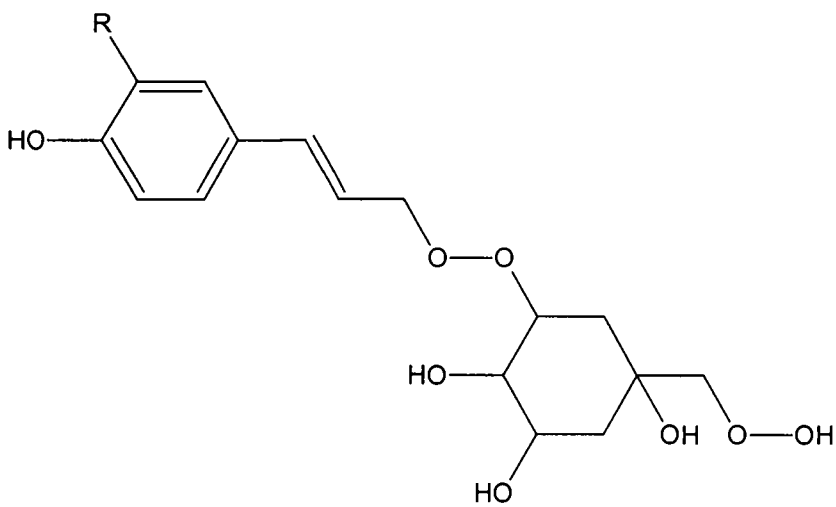
There are a number of recent investigations concerning identification and characterization of apple phenolics (Andrade and others 1998; Guyot and others 1998; Burda and others 1999; Sanoner and others 1999; Hammerstone and others 2000; Schieber and others 2001). Earlier, Spanos and others (1990) analyzed apple phenolics composition and the changes they undergo during processing and storage. The phenolic constituents in apples can be divided into two groups: (a) phenolic acids and related compounds, which include cinnamic acid and benzoic acid, and (b) flavonoids such as flavonol, flavan-3-ols (catechins), phloridzin and anthocyanin (Spanos and Wrolstad 1990; Golding and others 2001). Figure 1.1 shows the structures of phenolic compounds which have been identified in apple. Among the cinnamic acid derivatives, the main compounds are chlorogenic acid and 4-coumaroylquinic acid (Robards and Prenzler 1999). Flavonols are mainly present as quercetin glycosides. The catechins are represented predominantly by (-)-epicatechin. Unlike flavonols and anthocyanins, catechins are not glycosylated. Catechins are present in monomeric form as well as in oligomeric form

(procyanidins) (Van Der Sluis and others 2001). Phloridzin; a dihydrochalcone glucoside, is not widely distributed in fruit, it is restricted to the subfamily Pomoidae and the genus *Malus* (Durkee and Poapst 1965). Phloridzin is present in substantial quantities in apples, it has been used as a marker compound for apple juice authenticity. Phloridzin has been reported to have a regulatory effect in apple seedlings (Jones 1976). The most common anthocyanin in apples is cyanidin-3-galactoside (Robards and Prenzler 1999).

At the subcellular level, phenolic compounds accumulate at two major sites. The first site is the cell wall where lignin and simpler molecules such as flavonoids and esterified ferulic acid are deposited. The second site is the vacuole where various soluble phenolic compounds and derivatives accumulate (Macheix and others 1990). In apple cells, 97% of the phenolic compounds accumulate in vacuoles, where the concentration is in the order of 108 mM (Macheix and others 1990). At the tissue level, the amount of soluble phenolic compounds is higher in the external tissues of fleshy fruits (epidermal and subepidermal layers) than in the internal tissues (mesocarp, pulp).

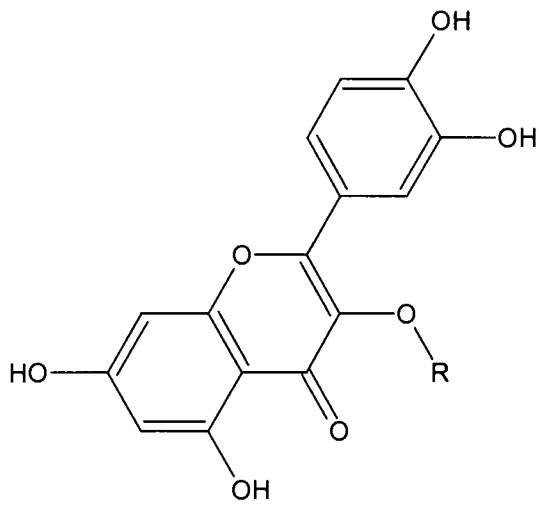


hydroycinnamic acids:
R1=R2=H p-coumaric acid

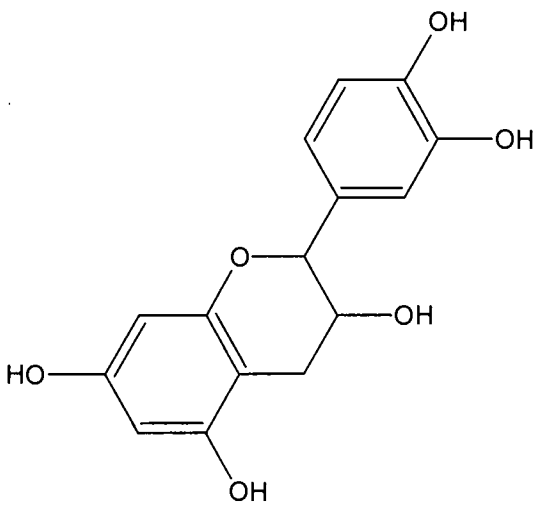


chlorogenic acid

Figure 1.1. Structures of Phenolic Compounds Identified from Apple

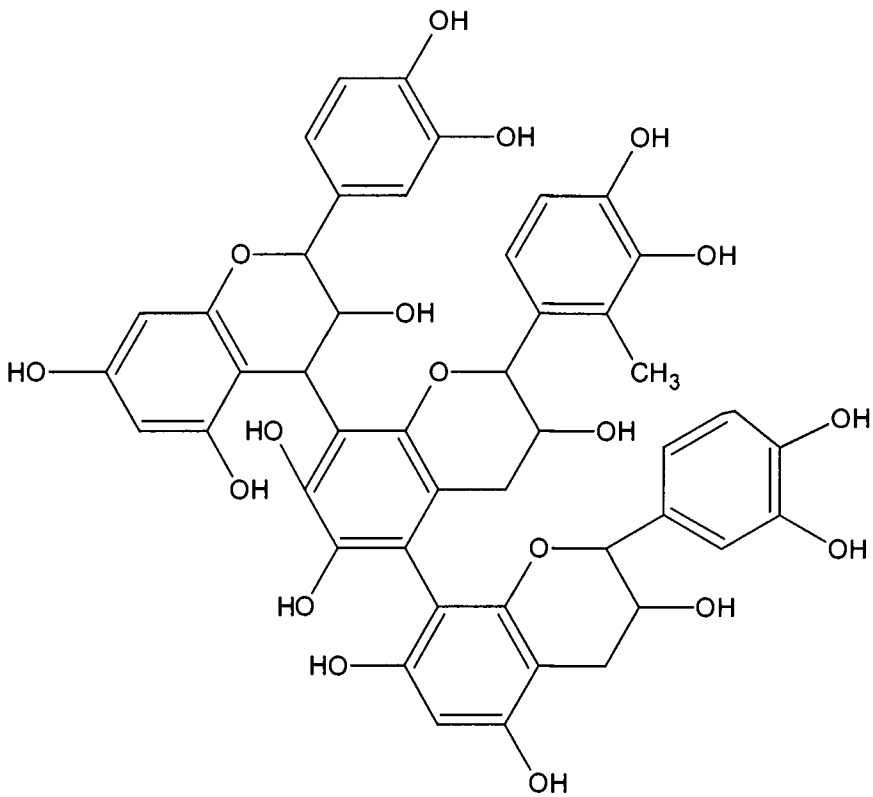


flavonols:
R= glycosyl quercetin- glycosides



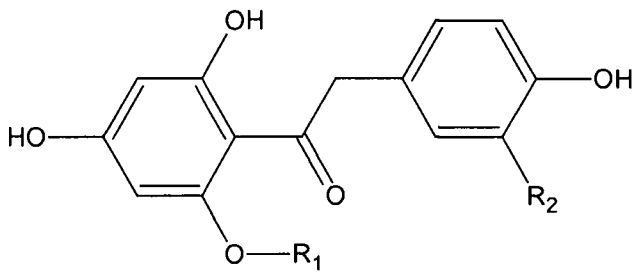
flavanols: epicatechin

Figure 1.1 (Continued)

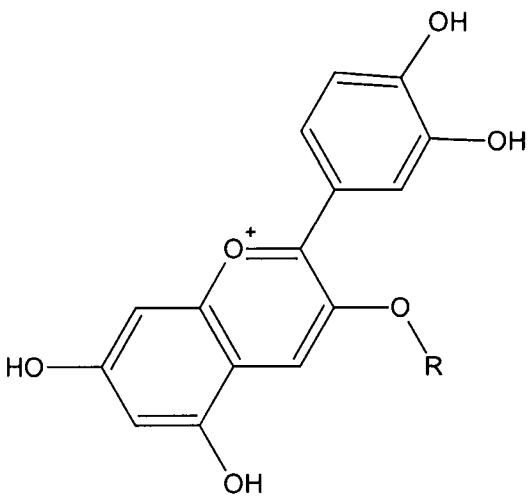


Procyanidin

Figure 1.1 (Continued)



dihydrochalcones:
R1= glycosyl, R2= 3- hydroxyphloridzin



anthocyanidins:
R= glycosyl cyanidin-glycosides

Figure 1.1 (Continued)

The total amount of phenols was 10 times greater in the peel of apples than in the flesh (Macheix and others 1990). Flavan-3-ols, catechin, epicatechin were present in the skin at three times the concentration of the flesh (Sal'kova and Bekbulatova 1965). The contents of ferulic, *p*- coumaric, gallic, and protocatechuic acids, and epicatechin in apple peels were higher than in the whole fruits and pulps (Gorinstein and others 2001).

Apple skin and apple flesh differ in their phenolic glycoside compositions. Olezek and others (1988) examined the Rhode Island Greening apples and isolated five quercetin glycosides: quercetin 3-*O* -galactoside (hyperin), quercetin 3-*O* glucoside (isoquercetin), quercetin 3-*O*- xyloside (reynoutrin), quercetin 3-*O*-arabinose (avicularin), and quercetin 3-*O*-rhamnoside (quercetin) and two phloretin glycosides: phloretin glucoside (phloridzin) and phloretin xyloglucoside from apple skin. They also found the glycoside of phloretin in apple flesh. Apple skin possesses both dihydrochalcones, phloretin glucoside (phloridzin) and phloretin xyloglucoside,and five quercetin glycosides. In contrast, apple flesh contains mainly two dihydrochalcone glycosides (Oleszek and others 1988). Burda and others (1990) examined three apple cultivars: Golden Delicious, Empire, and Rhode Island Greening apples and reported that the phenolics in apple flesh consisted of five main compounds: epicatechin, procyanidin B_2, chlorogenic acid, phloretin xylogalactoside and phloretin glucoside; the skins contained an additional

five quercetin glycosides (glucoside, galactoside, xyloside, arabinoside, rhamnoside). In addition, Burda and others (1990) noticed a large variation in phenolics concentration with respect to cultivar. Rhode Island Greening apples contained a higher concentration of epicatechin, procyanidin B2, and phloretin xylogalactoside in the skins and flesh whereas the Empire cultivar contained the lowest. Table 1.1 shows the concentration of phenolic compounds in the flesh and skins of apples during maturation and storage as reported by Burda and others (1990).

Lister (1994) reported that flavonoid compounds were higher in the skin when compared to the flesh, with the exception of phloridzin. Phloridzin, a dihydrochalcone glucoside, is a major phenolic constituent of the leaves, bark, roots, and seeds of apple. It has been stated that phloridzin is absent from the apple fruit (Macheix and others 1990). However, studies by a number of researchers disproved this (Dick and others 1987; Oleszek and others 1988; Burda and others 1990; Lister 1994)

Studies by Walker (1963) had shown that chlorogenic acid was the major phenolic compound in apples. However, Burda and others (1990) reported that epicatechin and procyanidins were the major phenolics in apples.

Table 1.1 Concentration of Phenolic Compounds in Flesh and Skins of Apples During Maturation and Storage (adapted from: Burda and others 1990); GD: Golden Delicious; E: Empire; RIG: Rhode Island Greening; nd: not detected

Phenolic (μg/g fresh wt.) ave± SD	GD flesh	GS skin	E flesh	E skin	R.I.G. flesh	R.I.G. skin
Epicatechin	40± 9	210± 130	10± 11	130± 107	140± 22	670± 214
Procyanidin B2	60± 19	200± 132	40± 29	120± 87	150± 31	600± 210
Phloretin Xylogalactoside	20± 4	130± 51	10± 3	60± 44	30± 6	230± 59
Phloretin Glucoside	10± 3	150±40	10± 2	120± 36	10± 5	100± 29
Chlorogenic Acid	10± 11	40± 29	50± 31	30± 21	60± 19	60± 44
Quercetin Galactoside	nd	290± 84	nd	220± 86	nd	370± 96
Quercetin Glucoside	nd	70± 31	nd	110± 31	nd	130± 77
Quercetin Xyloside	nd	100± 32	nd	110± 32	nd	150± 57
Quercetin Arabinoside	nd	130± 28	nd	140±36	nd	200± 54
Quercetin Rhamnoside	nd	230± 70	nd	200± 68	nd	220± 37

Anthocyanin in Apples

Anthocyanins occur ubiquitously in the plant kingdom; they are responsible for the blue, purple, violet, magenta, red and orange coloration in plants. The biosynthesis of the anthocyanin can be considered as the formation of a C_6-C_3-C_6 skeleton. The two aromatic rings of the generalized structure of anthocyanin are derived from two different precursors: acetate units for ring A and phenylpropanoid precursor for ring B; which are joined by a condensation reaction (Gross 1987) .

Anthocyanin stability is clearly influenced by environmental factors and processing conditions, such as pH, temperature, O_2, enzymes, and condensation reactions. Anthocyanins differ from other natural flavonoids by strongly absorbing Visible light. The range of colors associated with the anthocyanins results from their ability to form resonance structures from distinct and varied substitutions of the parent C_3-C_6-C_3 nucleus and is also affected by various environmental factors. The anthocyanins are glycosides of eighteen different naturally occurring anthocyanidins (Jackman and Smith 1996). Glycosidic substitution increases stability and water solubility. Anthocyanins are reactive compounds. Cyanidin, petunidin, and delphinidin contain *ortho* phenolic groups, which are more susceptible to oxidation and which will complex with metal ions (Wrolstad 2000). The skin color of apples, particularly for red cultivars, is an important factor in consumer acceptance. The pigment responsible for the red coloration of apple peel

is mainly anthocyanin, although colorless phenolic compounds such as flavonols, flavan-3-ols, dihydrochalcones, phenolic acids, and tannins also contribute to color intensification through a process known as the copigmentation reaction. The main anthocyanin of apples is idaein (cyanidin-3- galactoside). Cyanidin 3- arabinose , cyanidin 3-glucoside, and cyanidin-3-xyloside are the minor anthocyanins of apples (Mazza and Miniati 1993). Table 1.2 shows anthocyanin contents of the peel from five different apple cultivars. The synthesis of anthocyanin occurs during fruit growth, while color changes during ripening depends mostly on the simultaneous disappearance of chlorophyll a and b (Knee 1993). Several factors such as genetics, light, temperature, fertilization, storage and processing affect the formation of anthocyanins in apples.

Table 1.2 Anthocyanins in Peel from Different Apple Cultivars (*Malus pumila* L.) (adapted from: Mazza and Miniatti 1993); t: trace amounts)
Cultivar and Relative Concentration (%)

Anthocyanins	Red Delicious (Starkrimson)	Stoke Red	Jonathan	Tremletts	Cox's Orange Pippin	Ingrid Marie
Cyanidin 3-galactoside	85	94	94	90	92	90
Cyanidin 3-arabinose	10	5	4	8	6	6
Cyanidin 3-glucoside	5	1	3	2	2	4
Cyanidin 3-xyloside	t	t	t	t	t	t
Their acylated derivatives	t	t	t	t	t	t

Total Polyphenolics

Total phenolics by Folin- Ciocalteau (FC) were reported by several studies for various cultivars (Cilliers and others 1990; Spanos and others 1990; Sanoner and others 1999; Kähkönen and others 2001; Liu and others 2001). Table 1.3 shows the total phenolic content of various apples cultivars. Cilliers and others (1990) examined apple juices and ciders. They reported that the total polyphenolics content varied considerably between the different cultivars. Sanoner and others (1999) studied the polyphenolic composition of the cortex of 14 French cider apple varieties, one English apple, and one desert apple. They also reported that the total polyphenolic concentration varied depending on the variety. However, Kähkönen and others (2001) found no significant difference in the total polyphenolic content of the two cultivars they examined (*Malus pumila* cv. Punakaneli and *Malus pumila* cv. Valkea Kuulas). Kähkönen and others (2001) found considerable difference in total phenolics depending on extraction solvent (Table1.3). Kähkönen and others (2001) reported that extraction with acetone/ water gave more active extracts than methanol/ water or pure water extraction. Liu and others (2001) analyzed 10 apple cultivars and reported that the total polyphenolics content was higher in all varieties for apples with skin when compared to apples without skin, with the exception of NY 674.

Table 1.3 Total Phenolic Content by Folin Ciocalteau (FC) of Various Apple Cultivars

Cultivar	Total Polyphenolics by FC	References
Fuji with skin	230.49±4.4 mg GAE./100g	Liu and others (2001)
Fuji without skin	131.39±1.0 mg GAE/100g	Liu and others (2001)
Red Delicious with skin	204.49±2.1 mg GAE/100g	Liu and others (2001)
Red Delicious without skin	167.82± 1.7 mg GAE/100g	Liu and others (2001)
Liberty with skin	196.75±0.32 mg GAE/ 100g	Liu and others (2001)
Liberty without skin	127.95±3.2 mg GAE/100g	Liu and others (2001)
Northern Spy with skin	191.50±0.84 mg GAE/100g	Liu and others (2001)
Northern Spy without skin	142.34±0.83 mg GAE/100g	Liu and others (2001)
Golden Delicious with skin	179.19±5.9 mg GAE/ 100g	Liu and others (2001)
Golden Delicious without skin	124.14±3.6 mg GAE/ 100g	Liu and others (2001)
Fortune with skin	152.04±1.5 mg GAE/100g	Liu and others (2001)
Fortune without skin	137.60±1.8 mg GAE/ 100g	Liu and others (2001)
Jonagold with skin	126.49±0.88 mg GAE/100g	Liu and others (2001)
Jonagold without skin	126.49±0.88 mg GAE/100g	Liu and others (2001)
Empire with skin	115.07±1.1 mg GAE/100g	Liu and others (2001)
Empire without skin	71.61± 1.4 mg GAE/100g	Liu and others (2001)
NY 647 with skin	110.68±1.5 mg GAE/100g	Liu and others (2001)
NY 647 without skin	117.42±3.8 mg GAE/100g	Liu and others (2001)
Gala with skin	200.39±2.6mg GAE/100g	Liu and others (2001)
Gala without skin	133.76±1.7 mg GAE/100g	Liu and others (2001)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Apple Punakaneli 60% methanol as extraction solvent	984±5 mg GAE/100g	Kähkönen and others (2001)
Apple Punakaneli 70% acetone as extraction solvent	1148±20 mg GAE/100g	Kähkönen and others (2001)
Apple Punakaneli H_2O as extraction solvent	391±6 mg GAE/100g	Kähkönen and others (2001)
Apple Punakaneli using refluxing as extraction method	857±10 mg GAE/100g	Kähkönen and others (2001)
Apple Punakaneli Hexane as extraction solvent	Not Detected	Kähkönen and others (2001)
Foxwhelp fermented	6448 mg Chlorogenic Acid/L	Cilliers and others (1990)
Foxwhelp Juice	6396 mg Chlorogenic Acid/L	Cilliers and others (1990)
Medaille d'Or fermented	12725 mg Chlorogenic Acid/L	Cilliers and others (1990)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Medaille d'Or juice	15203 mg Chlorogenic Acid/L	Cilliers and others (1990)
Jonathan fermented	1310 mg Chlorogenic Acid/L	Cilliers and others (1990)
Jonathan juice	1269 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou fermented	1523 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou juice	1198 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou juice	672 mg Chlorogenic Acid/L	Cilliers and others (1990)
Old Gold fermented	254 mg Chlorogenic Acid/L	Cilliers and others (1990)
Medaille d'Or juice	15203 mg Chlorogenic Acid/L	Cilliers and others (1990)
Jonathan fermented	1310 mg Chlorogenic Acid/L	Cilliers and others (1990)
Jonathan juice	1269 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou fermented	1523 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou juice	1198 mg Chlorogenic Acid/L	Cilliers and others (1990)
King/Nehou juice	672 mg Chlorogenic Acid/L	Cilliers and others (1990)
Old Gold fermented	254 mg Chlorogenic Acid/L	Cilliers and others (1990)
Blacktwig fermented	817 mg Chlorogenic Acid/L	Cilliers and others (1990)
Blacktwig fermented	903 mg Chlorogenic Acid/L	Cilliers and others (1990)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Golden Delicious fermented	2512 mg Chlorogenic Acid/L	Cilliers and others (1990)
Yarlington Mill juice	7363 mg Chlorogenic Acid/L	Cilliers and others (1990)
Kingston Black	1639 mg Chlorogenic Acid/L	Cilliers and others (1990)
Granny Smith, short stored, press	188 mg GAE/L	Spanos and others (1990)
Granny Smith, short stored, HTST	251 mg GAE/L	Spanos and others (1990)
Granny Smith, short stored, enzyme clarify.	252 mg GAE/L	Spanos and others (1990)
Granny Smith, Short stored, filtered, fined	216 mg GAE/L	Spanos and others (1990)
Granny Smith, Short stored, bottle, fined	207 mg GAE/L	Spanos and others (1990)
Granny Smith, Short stored, concentrate, fined	224 mg GAE/L	Spanos and others (1990)
Granny Smith, Short Stored, concentrate, stored	310 mg GAE/L	Spanos and others (1990)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Granny Smith, Short Stored, Filtered, not fined	223 mg GAE/L	Spanos and others (1990)
Granny Smith, Short Stored, Bottled, not fined	231 mg GAE/L	Spanos and others (1990)
Granny Smith, Short Stored,Concentrated, not fined	229 mg GAE/L	Spanos and others (1990)
Granny Smith, Short Stored, Concentrate, stored	316 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, press	142 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, HTST	228 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, enzyme clarif	217 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, Bottled, fined	178 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, Concentrate, fined	211 mg GAE/L	Spanos and others (1990)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Granny Smith, Long Stored, Bottled, not fined	219 mg GAE/L	Spanos and others (1990)
Granny Smith, Long Stored, Concentrate, not fined	232 mg GAE/L	Spanos and others (1990)
Red Delicious press	374 mg GAE/L	Spanos and others (1990)
Red Delicious, diffusion at 55 C	401 mg GAE/L	Spanos and others (1990)
Red Delicious diffusion at 63 C	579 mg GAE/L	Spanos and others (1990)
Red Delicious Diffusion at 67 C	624 mg GAE/L	Spanos and others (1990)
Red Delicious, Diffusion at 73 C	780 mg GAE/L	Spanos and others (1990)
McIntosh, press	160 mg GAE/L	Spanos and others (1990)
McIntosh, diffusion at 55 C	483 mg GAE/L	Spanos and others (1990)
McIntosh, Diffusion at 67 C	580 mg GAE/L	Spanos and others (1990)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Spartan, press	176 mg GAE/L	Spanos and others (1990)
Spartan, diffusion at 55 C	272 mg GAE/L	Spanos and others (1990)
Spartan diffusion at 63 C	502 mg GAE/L	Spanos and others (1990)
Spartan diffusion at 73 C	567 mg GAE/L	Spanos and others (1990)
Commercial Concentrate A	204 mg GAE/L	Spanos and others (1990)
Commercial Concentrate B	224 mg GAE/L	Spanos and others (1990)
Commercial Concentrate C	113 mg GAE/L	Spanos and others (1990)
Commercial Concentrate D	49 mg GAE/L	Spanos and others (1990)
Guillevic	1.74 ± 0.18 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)

Table 1.3 (continued)

Cultivar	Total Polyphenolics by FC	References
Petit Jaune	2.22 ± 0.31 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Binet Rouge	2.22 ± 0.31 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Juliana	2.25 ± 0.26 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Clozette	2.40 ± 0.98 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Old Gold juice	318 mg Chlorogenic Acid/L	Cilliers and others (1990)
Old Gold juice	229 mg Chlorogenic Acid/L	Cilliers and others (1990)
Spitzenberg fermented	1310 mg Chlorogenic Acid/L	Cilliers and others (1990)
Spitzenberg fermented	395 mg Chlorogenic Acid/L	Cilliers and others (1990)

Table 1.3. (continued)

Cultivar	Total Polyphenolics by FC	References
Golden Delicious	1.28± 0.13 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Judor	1.10± 0.17 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Kermerrien	3.57 ± 0.15 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Jeanne Renard	6.00 ± 0.26 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Avrolles	2.56 ± 0.31 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Dous Moen	3.13± 0.51 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Antoinette	3.35 ± 0.18 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Bedan	3.21 ± 0.31 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Dabinett	3.33 ± 0.28 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Douce Coët Ligné	3.38 ± 0.25 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)
Chevalier	3.83 ± 0.04 g /kg fresh weight (-)epicatechin as standard	Sanoner and others (1999)

HPLC is a specific method for quantification of individual phenolic compounds, while FC colorimetric assay is a general assessment of the levels of phenolics. Several studies showed a correlation between HPLC and FC methods (Cilliers and others 1990; Spanos and others 1990; Sanoner and others 1999). Cilliers and others (1990) found that the total chlorogenic acid content (which included caffeic acid) and the sum of all peaks observed at 280nm by HPLC correlated very well with the total polyphenol content by FC methods for all samples. Spanos and others (1990) studied the effect of processing, concentration, and storage on the phenolics composition of Granny Smith apple juice pressed from fruit held at 1° C for 3 and 9 months. In addition, Spanos and others (1990) studied the effect of diffusion extraction at 55, 63, 67, and 73° C on the phenolics composition of Red Delicious, McIntosh, and Spartan apple juices. Four commercial apple juice concentrates of European origin were also included in the tested samples. Spanos and others (1990) found that HPLC and FC assays correlated highly in the group of commercial samples, poorly in the group of diffusion- extracted samples and not at all in the group of samples that consisted of different processing and storage stages of Granny Smith apple juice.

Phenolic Compounds as Antioxidants

Free radicals are molecules with unpaired electrons. They are thought to contribute to many human diseases such as arteriosclerosis, cancer, and chronic diseases associated with aging. Human cells produce free radicals as part of their normal functioning. Some people are also exposed to external free radicals, such as environmental pollutants and cigarette smoke (Langseth 2000). Antioxidants are compounds that, when present at low concentration compared with those of an oxidizable substrate, significantly delay or prevent oxidation of that substrate (Halliwell and Gutteridge 1999). Many phenolic compounds have free radical-scavenging properties and inhibit autooxidation reactions.

There are two mechanisms for oxidation in which antioxidant can play a preventive role, the first is H- atom transfer, demonstrated below for the important case of lipid peroxidation: (1)RH $\rightarrow$ R•, the initiation process in which free radicals remove a hydrogen from a polyunsaturated fatty acid to form a lipid radical; followed by (2) R•+$O_2$$\rightarrow$$RO_2$•, the propagation process in which the lipid radical and the molecular oxygen forms lipid peroxyl radical Next is (3) RO_2•+ RH $\rightarrow$ ROOH+ R• the termination process in which the new radicals react together or with antioxidants to eliminate radicals. Once a free radical R• has been generated, then reactions 2 and 3 form a chain reaction. As the chain cycles through

(2) and (3), many lipid molecules (R-H) are converted into lipid hydroperoxide (ROOH), resulting in oxidation and rancidity of fats (Langseth 2000). For the phenolic antioxidant, the generic term ArOH is used since, by definition, it contains at least one hydroxyl group attached to a benzene ring. The role of the antioxidant ArOH is to interrupt the chain according to $RO_2\bullet$+ ArOH $\rightarrow$ ROOH+ ArO$\bullet$. To be effective, ArO$\bullet$ must be a relatively stable free radical, thus reacting slowly with the substrate RH but rapidly with $RO_2\bullet$ (hence the term"chain- breaking antioxidant") (Wright and others 2001). The second mechanism by which an antioxidant can deactivate a free radical is electron transfer, in which the radical cation is first formed followed by rapid reversible deprotonation in solution : (1) $RO_2\bullet$ + ArOH$\rightarrow$ RO_2^-+ $ArOH^+$ (electron transfer) followed by (2) $ArOH^+$ + $H_2O\leftrightarrow$ ArO$\bullet$ + H_3O^+ (deprotonation equilibrium) and next is RO_2^-+ $H_3O^+ \leftrightarrow$ ROOH+H_2O (hydroperoxide formation). The net results from above are $RO_2\bullet$ +ArOH $\rightarrow$ROOH + ArO$\bullet$. Potential antioxidant actions of flavonoids should be considered in multiple terms because the free radical- scavenging effects of flavonoids are not necessarily single biochemical reactions (DiSilvestro 2001) and because there are at least six different possible antioxidant mechanisms of flavonoids (direct radical scavenging, down regulation of radical production, elimination of radical precursors, metal chelation, inhibition of xanthine oxidase,

and elevation of endogeneous antioxidants). Down regulation of radical production, elimination of radical precursors, metal chelation, inhibition of xanthine oxidase, and elevation of endogenous antioxidants can involve, at least in part, in the prevention of the formation of free radicals. These mechanisms might be termed as indirect antioxidant actions.

Measurement of Antioxidant Activity

A number of methods have been developed to measure the efficiency of dietary antioxidants in food extracts or of pure compounds, as well as to determine the antioxidant activity of plasma. These methods concentrate on different mechanisms of the antioxidant defense system such as chelation of metal ions, inhibition of lipid peroxidation, reduction of lipid peroxyl radicals, and scavenging of oxygen and hydroxyl radicals. TEAC (Trolox Equivalent Antioxidant Capacity), ORAC (Oxygen Radical Absorbance Capacity) and TRAP (Total Reducing Ability of Plasma) assays measure the ability of antioxidants to scavenge free radicals generated in the reaction medium. Some methods, such as the DPPH• (2,2-diphenyl-1-picrylhydrazyl) or DMPD•, measure the scavenging of stable radical species by antioxidants. The xanthine/ xanthine oxidase assay measures the efficiency of antioxidants to quench singlet oxygen. Other methods evaluate the inhibition of lipid peroxidation by antioxidants, quantifying products such as

conjugated dienes, lipid peroxides or hydroperoxides, as well as products resulting from the decomposition of lipid peroxides, including malondialdehyde determined by TBARS (thiobarbituric acid reactive substances) assays. On the basis of redox reactions, Benzie and Strain (1996) developed a methodology to determine the reduction ability of plasma as a measure of its antioxidant power; this method is called FRAP (Ferric Reducing Antioxidant Power) (Pulido and Bravo 2000).

Dietary Implication

It has been suggested that flavonoids and other phenolics play a preventive role in the development of cancer (Block and others 1992; Ames and others 1993). and heart disease (Rimm and others 1996). Apples are considered to be one of the most significant sources of flavonoids in the human diet. It has been estimated that apples provide 186 mg/ 100g total phenols, which equals 256 mg total phenols/ serving size (Vinson and others 2001). Liu and others (2001) examined 10 apple cultivars and found that all tested cultivars exhibited a high antioxidant activity. According to Liu and others (2001), apples with skin, Northern Spy and Red Delicious, had the highest TOSC values at 83.34 and 83.3 mmol vitamin C equivalent/g, followed by Fuji, Gala, Liberty, NY 674, Fortune, Jonagold, and Empire. For apples without skin, Northern Spy had the highest antioxidant activity at a value of 48.54 mmol vitamin C equivalent/g, followed by Fuji, Red Delicious,

Golden Delicious, Liberty, Gala, NY674, Fortune, Jonagold, and Empire. Earlier, Eberhardt and others (2000) reported that the major contribution to the antioxidant activity of apples is not from vitamin C but from other phytochemicals in apples; the combination of different phytochemicals in apples may work additively or synergistically to be responsible for this powerful antioxidant activity. Table 1.4 shows antioxidant capacities of several apple cultivars. Van Der Sluis and others (2001) studied the antioxidant capacities of four apple cultivars: Jonagold, Golden Delicious, Cox' Orange, and Elstar using IC_{50} method. Their studies revealed that Jonagold possessed the highest antioxidant activity, followed by Elstar, Cox'Orange and Golden Delicious. Without a uniform method for extraction and quantification of antioxidant it is very difficult to compare the results from different reports.

Table 1.4 Antioxidant Capacity of Various Apple Cultivars
a: mmol vitamin C Equivalents/g; b:μM; c: g of fw/L; d: μmoles of Trolox Equivalents/g

Author	Cultivar	Method	Antioxidant Capacity
Liu and others (2001)	Northern Spy With skin	TOSC	83.34[a]
Liu and others (2001)	Red Delicious With skin	TOSC	83.3[a]
Liu and others (2001)	Northern Spy Without skin	TOSC	48.54[a]
Liu and others (2001)	Empire Without skin	TOSC	19.66[a]
Vinson and others (2001)	Not specified	IC_{50}	0.31[b]
Van der Sluis (2001)	Jonagold	IC_{50}	5.8[c]
Van der Sluis (2001)	Golden Delicious	IC_{50}	7.6[c]
Van der Sluis (2001)	Cox's Orange	IC_{50}	6.7[c]
Van der Sluis (2001)	Elstar	IC_{50}	6.6[c]
Mazza and Miniati (1993)	Not specified	ORAC	2.18[d]

Apples compare favorably with other fruits in regards to their contribution to the average American's consumption of dietary antioxidants particularly when considering per capita consumption. Table 1.5 presents antioxidant capacities of fruits as determined by the ORAC Assay. Wang and others (1996) analyzed the antioxidant activity of 12 fruits and 5 commercial fruit juices using the ORAC assay. Wang and others (1996) reported that strawberries had the highest antioxidant capacity followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon. Studies by Kalt and others (1999) revealed that lowbush blueberry showed a higher antioxidant capacity than strawberry. When considering per capita consumption, apples provide higher antioxidant capacity than blueberries. For instance, in 1998, the amount of US per capita consumption of apples was 8635.5 grams; for blueberries, it was 180 grams. Hence, in 1998, apples supplied 18,825 micromoles of Trolox equivalents (based on Wang and others (1996)), and blueberries supplied 11,592 micromoles of Trolox equivalent of antioxidant capacity (based on Kalt and others data (1999)) in an average American's diet.

Table 1.5 Antioxidant Capacities of Fruits as Determined by ORAC assay

Authors	Fruit	Antioxidant Capacity μmoles of T.E./g of fruit	Per capita consumption of Antioxidant Capacity (μmoles of T.E.)
Wang and others (1996)	apples	2.18	18,825
Kalt and others (1999)	strawberry	20.6	38,007
Kalt and others (1999)	highbush blueberry	60.1	10,818
Kalt and others (1999)	lowbush blueberry	64.4	11,592
Wang and others (1996)	strawberry	15.36	28,339
Wang and others (1996)	plum	9.49	4,783
Wang and others (1996)	orange	7.50	47,925
Wang and others (1996)	red grape	7.39	21,616
Wang and others (1996)	kiwi fruit	6.02	1,355
Wang and others (1996)	pink grapefruit	4.83	12,606
Wang and others (1996)	white grape	4.46	13,046
Wang and others (1996)	banana	2.21	27,856

Table 1. 5 (continued)

Authors	Fruit	Antioxidant Capacity μmoles of T.E./g of fruit	Per capita consumption of Antioxidant Capacity (μmoles of T.E.)
Wang and others (1996)	tomato	1.89	12,673
Wang and others (1996)	pear	1.34	1,869
Wang and others (1996)	melon	0.97	11,917

Postharvest Physiology of Apples

Apples are climacteric fruits. Carbon dioxide production and oxygen uptake increase by 50 to 100 % during ripening. Ethylene production increases about 1000-fold with concurring respiratory rise (Knee 1993). Ethylene plays a role in the postharvest quality of apples and can often be harmful by accelerating the ripening process and reducing shelf life. On the other hand, ethylene can sometimes have a positive influence on the quality of the product by promoting faster, more uniform ripening before retail distribution (Reid 1992).

Ethylene Biosynthesis

Ethylene is a plant hormone, regulating many aspects of growth, development, senescence and abscission of plants. Ethylene (C_2H_4) is a natural product of plant metabolism and is produced by all tissues of higher plants and by some microorganisms. The biochemical pathway of ethylene biosynthesis in plants starts with the conversion of amino acid methionine to S- Adenosylmethionine (SAM). SAM is the precursor of 1 –aminocyclopropane-1- carboxylic acid (ACC), the immediate precursor of ethylene. The enzyme ACC synthase, which converts SAM to ACC, is the main site of control of ethylene biosynthesis. ACC synthase is activated by a common enzyme co- factor pyridoxal phosphate. Inhibitors of enzymes that require pyridoxal phosphate, such as AVG (amino- ethoxyvinyl

glycine) and AOA (aminooxyacetic acid) can be used to inhibit ethylene production. The conversion of ACC into ethylene is mediated by an enzyme called EFE (Ethylene Forming Enzyme) or ACC oxidase. This enzyme, known to be very labile, is presumably membrane-bound (Kader 1992; Reid 1992).

Many phases of plant growth and development such as fruit ripening and abscission are affected by ethylene. The favored model of the way ethylene induces these effects is by binding to a protein, called a binding site, thus stimulating the release of a so-called "second messenger ", which instructs the DNA to form m RNA molecules specific to one of the effects mentioned above. These molecules are translated into proteins by polyribosomes, and the proteins formed in this manner are the enzymes that cause the actual ethylene response (Kader 1992; Reid 1992).

1-MCP Treatment

Superficial scald is an important postharvest disorder in apples. The susceptibility of apples to scald decreases with advanced fruit maturity (Fan and others 1999). Ethylene is produced as apples mature and ripen. An increase in ethylene production during storage is accompanied by the accumulation of α-farnesene, a compound considered to be vital to scald development (Fan and others 1999). Current methods for scald control rely on the use of antioxidants such as

DPA (Diphenylamine) and ethoxyquin, CA (Controlled Atmosphere) storage, or a combination of CA and antioxidant treatments. A recently developed gaseous compound, 1- MCP (1- Methyl Cyclopropene), shows great promise for maintaining quality and extending storage life of fruits. The advantageous effects of 1- MCP come from its ability to block the action of the gaseous plant hormone ethylene, by competing with ethylene for the plant receptor (Watkins and others 2000). The results of Fan and others (1999) showed that inhibiting ethylene action, using MCP, reduces ethylene production as well as scald, which only develops on fruits producing ethylene.

1-MCP Action

Burg and Burg (1967) postulated the presence of a metal in the ethylene receptor based on a correlation between the relative ethylene- like activity of several compounds and their known order of binding to silver ions. In 1973, Sisler and Pian reported that 2,5-norbornadiene counteracted ethylene in a competitive manner. Since then, a number of studies have shown that other compounds such as *trans*-cyclooctene counteract ethylene by interacting with the receptor. Similar to 2,5-norbornadiene, *trans*- cyclooctene required a high concentration, continuous exposure, and had a strong odor.

Recently, organic molecules such as DACP, CP, 1-MCP, and 3,3-DMCP that block the ethylene receptor for extended periods of time have been discovered. DACP is a weak ethylene inhibitor. However, upon radiation with visible light, it gives rise to one or several much more active components that block ethylene responses for many days. DACP is explosive at high concentration which limits its commercial usefulness. CP, 1-MCP, and 3,3-DMCP have been found to be effective antagonists of the ethylene receptor (Sisler and others 1996 a, b). Most of the studies have been conducted with 1-MCP since 1-MCP is more stable than CP and more active than 3,3-DMCP. 1-MCP presumably binds to the metal in the ethylene receptor (Sisler and Serek 1997). 1-MCP would compete with ethylene for the receptor, preventing ethylene from binding in the tissues. While 1-MCP is bound, ethylene cannot bind (Sisler and Serek 1997).

Ethylene may act by withdrawing electrons from a metal receptor, causing a ligand substitution process that induces an action response (Sisler 1977; Sisler 1991; Sisler and Goren 1981). 1-MCP should be capable of inducing such a response since theoretically, it also would withdraw electrons from a metal. Since 1-MCP is highly strained, its effect would be stronger than that of ethylene. As it binds to the receptor strongly, the formation of an active complex is not completed, thus effectively blocking the receptor. Sisler and Serek (1997) hypothesized that ethylene can leave the receptor, and that departure is necessary

for the formation of an active complex. Ethylene would not be a part of the active complex, but the initiator of its formation. A model involving ligand substitution was proposed by Sisler and Serek (1997). Parts of this model have been presented before by Sisler (1977); Sisler (1991) , and Sisler and Goren (1981). Steps in the proposed model by Sisler and Serek (1997) are: (1) ethylene approaches the metal and electrons are withdrawn; (2) another ligand in a *trans* position to it moves away from the metal; (3) another ligand moves toward the metal, and as it does, ethylene is lost and an active complex is formed; (4) 1- MCP acts in a similar way to ethylene, however it is not lost from the complex and therefore, an active complex is not formed.

The Effect of Storage and 1-MCP Treatment on Antioxidant Capacity and Phenolic Compounds in Apples

Van Der Sluis and others (2001) reported that long- term storage, both at refrigeration temperature and under controlled atmosphere, did not influence flavonoid concentration or antioxidant capacity in Jonagold, Golden Delicious, Cox' Orange, and Elstar apples. He also reported no seasonal effect on antioxidant capacity in the four apple cultivars he examined.

The Granny Smith variety of apples behaves differently than other apple cultivars; it needs an abnormally long time to ripen at room temperature. Its

maturation was accelerated by low- temperature stress (Pèrez- Ilzarbe and others 1997). Pèrez- Ilzarbe and others (1997) reported that in the pulp of Granny Smith apples, phenolic compounds decreased during the development period and during cold treatment. In contrast, in the peel of Granny Smith apples, the quantity of phenolic compounds increased with time after the cold treatment.

Maclean and others (2001) examined the effect of 1- MCP treatment on three apple cultivars; namely Delicious, McIntosh, and Empire. He found that the two scald- susceptible cultivars: McIntosh and Delicious, had a significant enhancement of antioxidant capacities following treatment with 1- MCP, whereas Empire did not demonstrate a significant 1-MCP treatment effect.

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CHAPTER 2

APPLE POLYPHENOLICS AND THEIR ANTIOXIDANT PROPERTIES: INFLUENCE OF CULTIVARS, POST- HARVEST STORAGE AND 1-MCP TREATMENT

ABSTRACT

The distribution of total phenolics, antioxidant capacity, monomeric anthocyanin, and ascorbic acid contents in the peel and flesh of Red Delicious, Granny Smith, and Fuji apples during a six- month storage period were determined. In addition, the effect of 1-MCP (1- Methyl cyclopropene) on these parameters of the edible portion of apples during storage was also investigated. Two different assays were employed to determine antioxidant activities: the spectrofluorometric-based Oxygen Radical absorbing Capacity (ORAC) and the spectrophotometric-based Ferric Reducing Antioxidant Power (FRAP). Total phenolics were measured using the Folin- Ciocalteau method. A determination of monomeric anthocyanin content was also performed using the pH- differential method for Red Delicious and Fuji apples.

Antioxidant activity was predominantly observed in the aqueous fraction and was attributed to polyphenolics. Antioxidant activities were highest in the peel, with Red Delicious peel having the highest values, presumably due to anthocyanin pigments. At zero time storage, the ORAC, FRAP, total phenolics, and total

monomeric anthocyanin contents of Red Delicious peel were: 37.7μmol T.E./g, 62.7 μmol T.E./g, 6.63 mg/g GAE, and 26.4 mg/100g, respectively. The three tested cultivars were significantly different with respect to ORAC, FRAP, total phenolics, and monomeric anthocyanin contents. 1- MCP treatment did not have a significant influence on antioxidant activities and monomeric anthocyanin contents of the three cultivars during storage. Storage did not have a significant influence on ORAC values of the edible portion of apple, however it had significant influence on the FRAP values. The contribution of ascorbic acid to the total antioxidant capacity was small.

INTRODUCTION

Epidemiological studies have indicated that consumption of fruit and vegetables is associated with a lowered risk of cancer (Block and others 1992, Ames and others 1993), heart disease (Rimm and others 1996), and stroke (Gillman and others 1995; Joshipura and others 1999). It is widely believed that the presence of polyphenolic antioxidants in these fruits provides protection against these diseases (Prior and others 1998; Hertog and others 1993; Schramm and German 1998; Miller and Rice- Evans 1997).

In many western countries, apples are the most commonly consumed fruit. On average, the current per capita apple consumption in the US is about 8,100 grams per year (Anonymous 2001a). Among the fruits, apples make the largest contribution of phenols to the U.S. diet, second only to bananas (Vinson and others 2001). Washington is the leading apple- producing state in the United States. Since 1989, Washington fresh apple sales have totaled between 70 million and 100 million boxes each year and accounted for nearly three- fourths of all the apples grown in state. Washington's percentage of fresh apple sales is the highest in the United States (Anonymous 2001b). In the year 2000, Washington's apple crop consisted of 48.5% Red Delicious, 17% Golden Delicious, 12% Fuji, 8% Granny Smith, 8.5% Gala, 2.4% Braeburn, and 3.6% other varieties, including Jonagold, Cameo, Pink Lady, and Rome (Anonymous 2001b). The significance of apples in

the diet may be explained by a number of factors such as availability throughout the year and the fact that they can be consumed in various forms such as fresh fruit, juice, dried, and canned. Apple extracts exhibited high antioxidant activities, presumably because apple extracts contain phenolic compounds. The phenolic constituents in apples are derived from phenylalanine via shikimate and phenylpropanoid pathways and can be divided into two groups: (a) phenolic acids and related compounds and (b) flavonoids (Spanos and Wrolstad 1992).

Apples are harvested commercially before they become ripe for eating. Storing apples for marketing later in the year increases the risk of fruit losses from surface rot and superficial scald. A common factor in susceptibility to these disorders is fruit maturity at harvest, determined in part by ethylene production. Current methods for scald control rely on the use of antioxidants such as Diphenylamine (DPA) and ethoxyquin, Controlled Atmosphere storage (CA), or a combination of CA and antioxidant treatment. Many apple varieties such as Red Delicious and Granny Smith develop non-pathogenic lesions on the skin during storage. A recently- developed gaseous compound, 1- Methylcycloropene (1-MCP), shows great promise for maintaining quality and extending storage life of fruits. The advantageous effects of 1- MCP come from its ability to block action of the gaseous plant hormone ethylene, which generally hastens the rate of deterioration of plant tissues after harvest (Watkins and others 2000).

Because of the increased interest in dietary phenolics from apples, it is important to obtain data on the distribution, concentration and fate of these compounds in various cultivars during postharvest storage. Liu and others (2001) analyzed 10 apples cultivars and reported that the total polyphenolics content was higher in all varieties for apples with skin when compared to apples without skin, with the exception of NY 674. They also analyzed the apples for their antioxidant capacities using the Total Oxyradical Scavenging Capacity (TOSC) assay and found that for apples with skin, Northern Spy and Red Delicious had the highest TOSC values followed by Fuji, Gala, Liberty, NY 674, Golden Delicious, Fortune, Jonagold, and Empire. Eberhardt and others (2000) reported that the major contribution to the antioxidant activity of apple is not from vitamin C but from other phytochemicals in apples; the combination of different phytochemicals in apples may work additively or synergistically to be responsible for this powerful antioxidant activity. The objectives of this study were to measure the antioxidant capacity, total phenolics, and monomeric anthocyanin contents of Red Delicious, Fuji, and Granny Smith apple extracts and to measure their distribution in the peel and flesh during a six- month storage period. The effect of 1-MCP treatment on these parameters of the edible portion of apples during storage was also investigated.

MATERIALS AND METHODS

Samples

A total of 12 boxes, consisting of Washington Extra Fancy Red Delicious, Granny Smith, and Fuji apples (*Malus* X *domestica* Borkh.) was provided by Stemilt Growers, Wenatchee, WA. Red Delicious was selected because it represents an anthocyanin- rich cultivar, Granny Smith was selected because it represents a non- anthocyanin cultivar, and Fuji was selected because it is a new cultivar. The apples had been stored in a cold room (0 °C, 88%RH) after harvest. One box of each variety was treated with 1- MCP. The apples were fumigated with 1ppm of 1- MCP for 18 hours at 20° C in an 800 L steel chamber at the USDA Tree Fruit Research Laboratory, Wenatchee, WA. The fruits were treated two weeks after harvest. The apples were returned to the cold room (0 °C, 88%RH) after 1- MCP treatment.

One third of each 1- MCP treated box was combined into one box and sent along with one box of each variety to the OSU Food Science and Technology Department at each of the three storage intervals of 0, 3, and 6 months. Fruits were stored at 0° C upon arrival.

Reagents

The Folin- Ciocalteau reagent was obtained from Sigma Chemical Co. (St. Louis, MO 63178). Gallic acid standard was obtained from Sigma Chemical Co. All solvents used in this study were high- performance liquid chromatography (HPLC) grade.

Sample Preparation

Four apples of each cultivar were chosen at random (one apple from each layer of the box) and peeled with a mechanical apple peeler. The flesh was further cut into small pieces, approximately 1½ cm height X 1½ cm length X 1½ cm width, with a stainless steel knife. The weight of the whole apple and each tissue fraction, peel and flesh, were measured to determine their proportions in fresh apple. The peel and flesh were separated into two containers, frozen with liquid nitrogen and stored at –70 °C until extraction.

The control apples and those treated with 1-MCP were cored and the edible portions were cut with a stainless steel knife into small pieces, approximately 1½ cm height X 1½ cm length X 1½ cm, , frozen with liquid Nitrogen and stored at – 70 °C until extraction. Figure 2.1 summarizes the sample preparation.

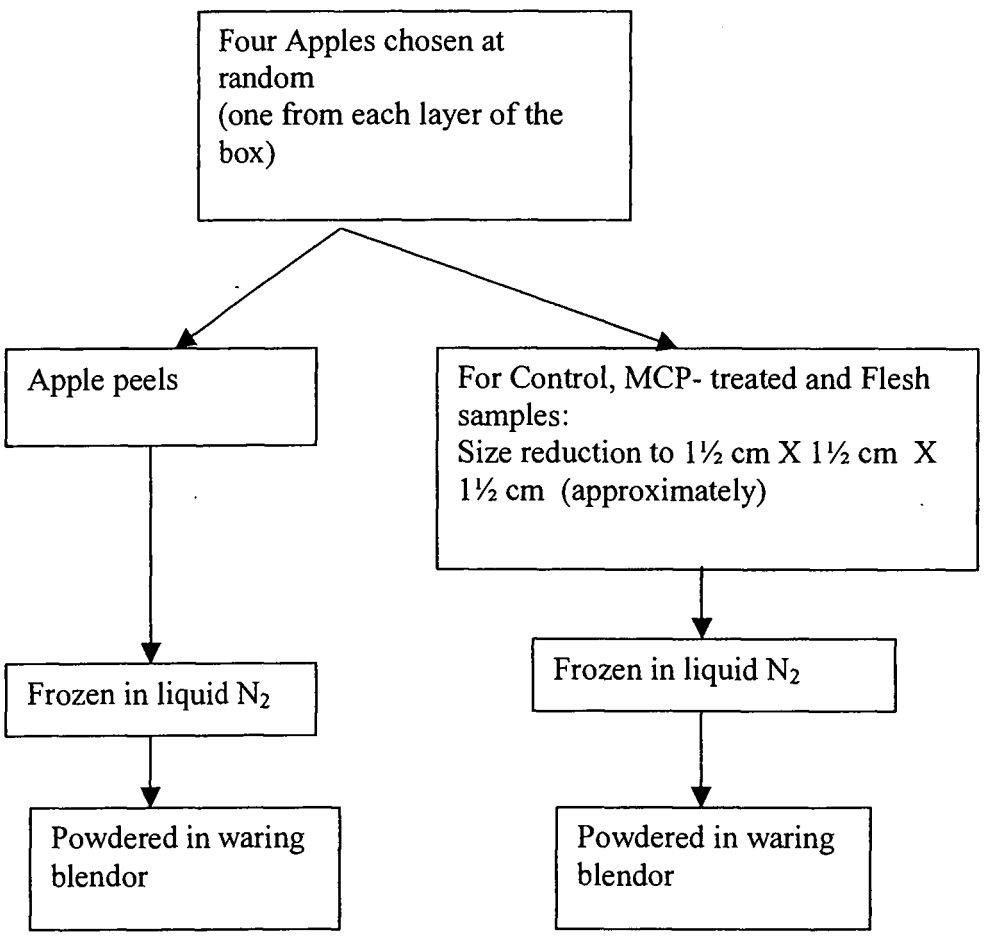

Figure 2.1 Flow chart for the sample preparation

Extraction Procedures

Extracts were prepared according to Rodriguez-Saona and Wrolstad (2001). The tissue samples were frozen with liquid nitrogen and pulverized, using a stainless steel Waring blendor. Ten grams of powdered fruit samples were then blended with 20 mL of acetone and filtered through a Büchner funnel with Whatman filter paper no.4. The filter cake was reextracted with an aqueous solution of acetone: water (70/30 v/v) three times. The filtrates were combined and placed in centrifuge bottles, shaken with 2 volumes of chloroform and centrifuged for 45 min at 750 rpm. The aqueous phase was collected and put onto a Büchi rotary evaporator at 40° C to remove residual acetone. The aqueous extract was diluted to 50 mL with deionized water and stored at –70° C until analysis. The organic phase was also collected and analyzed as a peel sample. The organic phase was evaporated until dry, then redissolved with acetone to 10 mL volume and stored at –70° C until analysis. Figure 2.2.illustrates the extraction process.

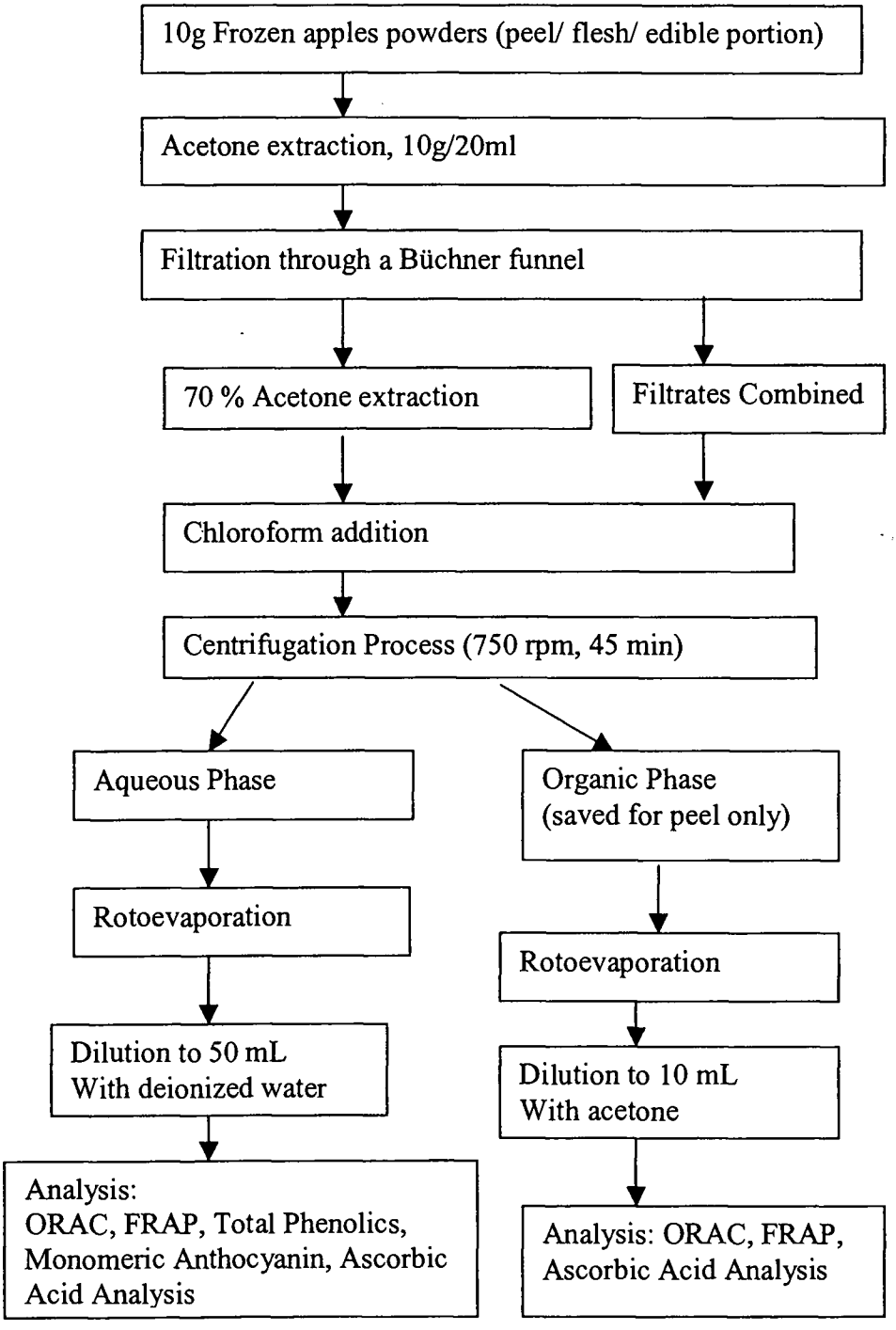

Figure 2.2. Flowchart for preparation of apple extract

Determination of Total Phenolics

The total phenolic contents were determined according to the procedure described by Singleton and Rossi (1976) with some modifications. The aqueous samples were diluted 10 fold in test tubes with distilled water, followed by the addition of 15 mL water and 1 mL Folin- Ciocalteau reagent. The test tubes were shaken, allowed to stand for 10 min at room temperature, and 3 mL of a 20g/100 mL solution of Na_2CO_3 were added. The contents of the test tubes were mixed again, placed in a water bath at 40° C for 20 min and cooled in ice water. Absorbance was measured at 755nm with a Shimadzu 300 UV- Visible spectrophotometer. The results were expressed as Gallic Acid equivalents (GAE) mg / g fresh frozen weight.

Anthocyanins Determination

Monomeric anthocyanin content was determined by the pH differential method of Giusti and Wrolstad (2001). The sample absorbances were measured with a Shimadzu 300 UV- Visible spectrophotometer at 510 and 700 nm. The data were calculated as cyanidin-3-glucoside, using the molar extinction (ε) of 26,900 and a molecular weight of 449,2.

Antioxidant Capacity Determination

Antioxidant capacity was measured by ORAC (Oxygen Radical Absorbing Capacity) and FRAP (Ferric Reducing Antioxidant Power) assays. The ORAC assays were carried out following procedures previously described by Cao and Allesio (1993) and adapted for use in a 96- well microplate fluorometer (model Cytofluor 4000, Perspective Biosystems, Framingham, MA). In the ORAC assays, the antioxidant activity of the sample is estimated as its capacity to delay a complete loss of natural fluoresecene of β- phycoerythrin upon oxidation by AAPH (a peroxyl radical). The ORAC results were derived from triplicate analyses and expressed as Trolox Equivalent/ g of frozen fruit. Trolox is a water soluble tocopherol analogue used as a reference compound for antioxidant capacity.

In addition to the ORAC Assay, the antioxidant capacity was determined by the FRAP assay. This method is based on the ability of the sample to reduce ferric ion to ferrous ion, followed by the formation of a colored ferrous- tripyridyltriazine complex (Benzie and Strain 1996). The FRAP assay was adapted for use in a 96-well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, CA). The FRAP assay results were obtained from duplicate analyses and were expressed as μm Trolox Equivalent/ g of frozen fruit. Both assays were carried out at The Linus Pauling Institute, Oregon State University.

Ascorbic Acid Content

The ascorbic acid analysis was carried out following procedures described by Martin and Frei (1997) at the Linus Pauling Institute, Oregon State University. This assay was performed using HPLC with electrochemical detection. The results were expressed as μmole ascorbate/ g of material.

Statistical Methods

The experiment was conducted using a factorial treatment design with cultivars, storage, and treatment (edible portion only) as factors. The factorial treatment design is a statistical way to investigate the relationship among several types of treatments. Comparison among treatments can be affected substantially by the condition under which they occur. There were three levels of cultivars: Red Delicious, Granny Smith, and Fuji; three levels of storage time: 0,3, and 6 months, and two levels of treatment: control and 1- MCP treatment. The interaction effects (cultivar*storage, cultivar*treatment, storage*treatment) were measured for the edible portion. Since replicate extraction was performed on the same composite apple sample, the process can be considered only a repeat of extraction and analysis, and not a true sample replicate. For this reason , we could not directly test for three ways interaction in the edible portion and two ways interaction in the peel and the flesh portion of apples. A graphical method was employed to check

for the homogeneity of variance. The means were plotted against each other to provide simple visual evaluation of the equal variances assumption.

For statistical analysis, multifactor analysis of variance (ANOVA) was applied to the means with source of variance being cultivars, storage time, and treatments. Significant difference ($p<0.05$) between means were identified using Fischer Protected Least Significant Different. The analysis were performed with S-Plus 2000 (Matsoft,Inc,Seattle,WA).

The probability that the test statistic, F statististic in this case, would take a value as extreme or more extreme than is actually observed is called the P value. The smaller the P-value, the stronger the evidence against Ho provided by the data (Ho: all means are equal, Ha: at least one means differs from the others). P- value between 0 and .01 indicates there is strong evidence against Ho; P- value between .01 and .05 indicates there is moderate evidence against Ho; P- value between .05 and .10 indicates there is suggestive but inconclusive evidence against Ho, and P-value higher than .10 indicates there is no evidence against Ho.

RESULTS AND DISCUSSION

Extraction Methodology

The choice of extraction method should maximize phenolics recovery with minimum degradation or alteration of the phenolics natural state. The use of liquid nitrogen in this study minimizes phenolics degradation by lowering temperature and providing an oxygen- free nitrogen environment. The fine powders, resulting from cryogenic milling, maximize phenolics recovery due their high surface area and disruption of cellular compartments.

In this study, an acetone- chloroform partition was used for two reasons: first, it has been the experience in our laboratory that the highest recovery of phenolic compounds is achieved with acetone extraction as compared to methanol extraction (Rodriguez- Saona and Wrolstad 2001). Kähkönen and others (2001) have reported that extraction with acetone produces more active extracts than methanol or pure water extraction.

The second reason for using an acetone- chloroform partition is that this extraction procedure permits the analyses of both the water soluble fraction and the non-polar lipid fraction. The addition of chloroform results in phase separation between the aqueous portion, which contains the anthocyanin, phenolics, sugars, organic acids, and other water-soluble compounds and the chloroform phase, which

contains the immiscible organic solvents (acetone/ chloroform mixture) with dissolved lipids, carotenoids, chlorophyll pigments and other non- polar compounds. Tables 2.1 and 2.2 contain the antioxidant capacity of the aqueous and chloroform phases (non- polar fraction) for extracts of the peels of the three cultivars. The antioxidant capacity in the aqueous portion was substantially higher than in the chloroform phase, presumably because of phenolic compounds. The results are also presented in Figure 2.3 and Figure 2.4.

Table 2.1 ORAC (Oxygen Radical Absorbing Capacity) Contents of Aqueous (H_20) and Organic Phase ($CHCl_3$) in The Extraction of Apples Peels; RD: Red Delicious, F: Fuji, GS: Granny Smith

	ORAC μmol T.E./g		ORAC μmol T.E./g		ORAC μmol T.E./g	
	Aqueous (H_20)	Organic ($CHCl_3$)	Aqueous (H_2O)	Organic ($CHCl_3$)	Aqueous (H_2O)	Organic ($CHCl_3$)
Storage (months)	0	0	3	3	6	6
RD Peel	37.7	2.1	31.5	1.08	31.8	2.05
F Peel	26.7	1.6	14.9	0.86	13.7	1.18
GS Peel	24.9	2.49	19.2	1.12	9.84	0.86

Table 2.2 FRAP (Ferric Reducing Antioxidant Power) Contents of Aqueous (H_20) and Organic Phase ($CHCl_3$) in The Extraction of Apples Peels; RD: Red Delicious, F: Fuji, GS: Granny Smith

	FRAP μmol T.E./g		FRAP μmol T.E./g		FRAP μmol T.E./g	
	Aqueous (H_20)	Organic ($CHCl_3$)	Aqueous (H_2O)	Organic ($CHCl_3$)	Aqueous (H_2O)	Organic ($CHCl_3$)
Storage (months)	0	0	3	3	6	6
RD Peel	62.7	1.31	31.2	0.33	39.3	0.95
F Peel	21.1	1.15	13.2	0.36	9.08	0.49
GS Peel	36.9	1.61	21.4	0.27	9.09	0.21

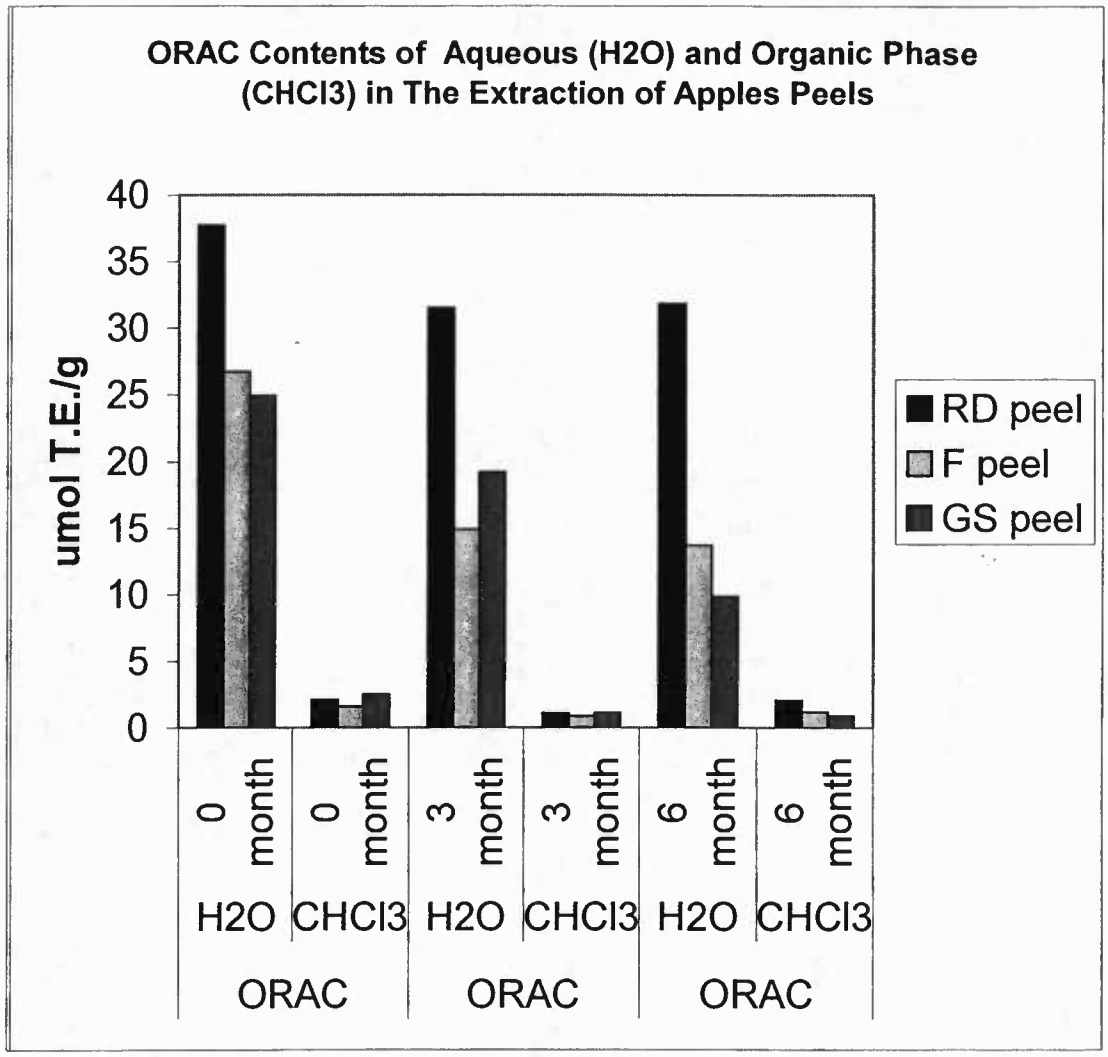

Figure 2.3 ORAC Contents of Aqueous (H2O) and Organic Phase (CHCl3) in The Extraction of Apple Peels

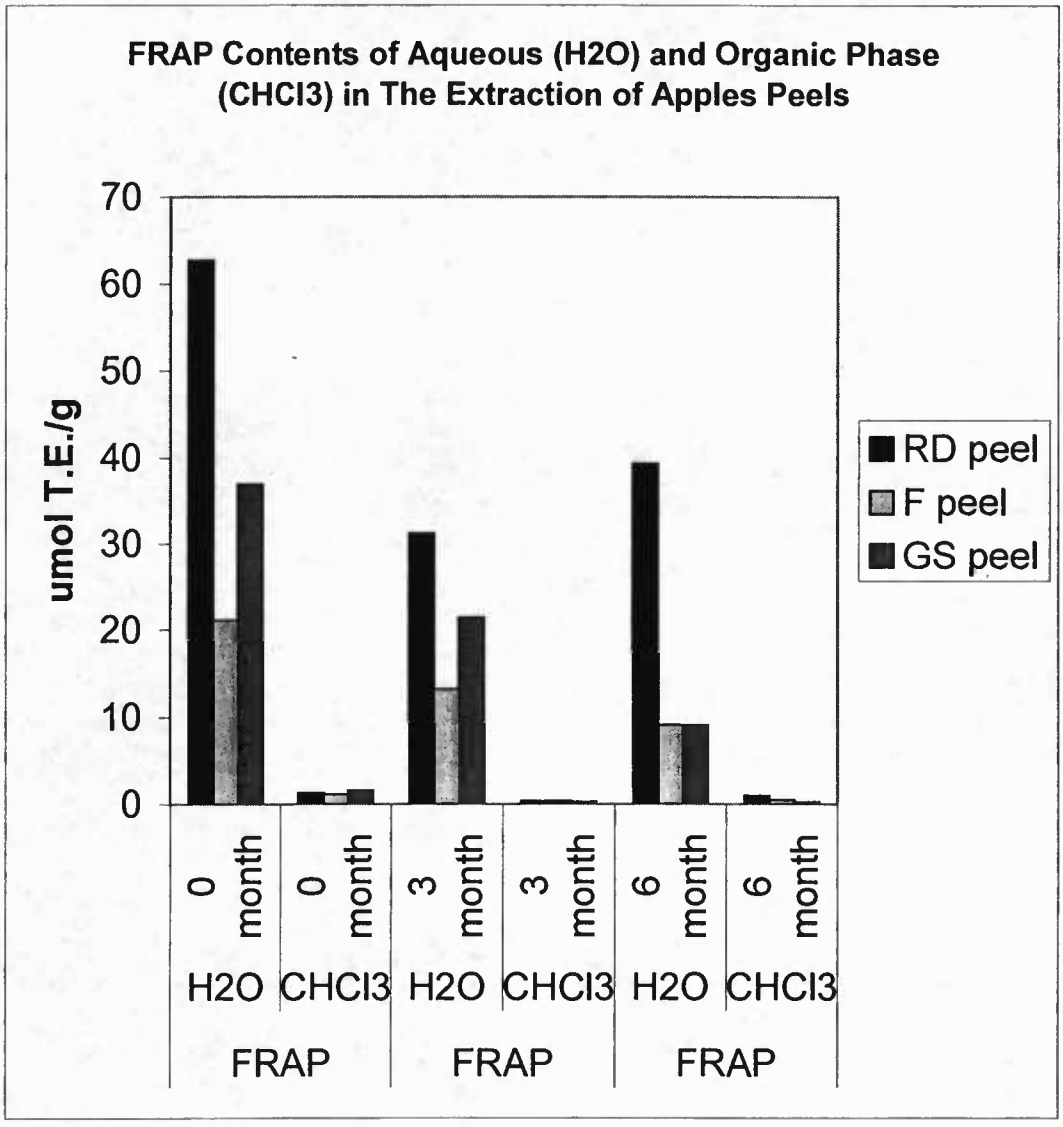

Figure 2.4 FRAP Contents of Aqueous (H2O) and Organic Phase (CHCl3) in The Extraction of Apples Peels

Total Phenolics in The Edible Portion, Peel, and Flesh of Apples

Total phenolic contents of the edible portion (skin and flesh) of apples or control apples showed that Red Delicious had the highest total phenolic content, followed by Granny Smith and Fuji apples (Table 2.3). The three cultivars were significantly different with respect to the total phenolics with p value from ANOVA=0.001. The mean of Red Delicious over storage was significantly different than that of Fuji; the mean of Red Delicious over storage was significantly different than that of Granny Smith, and the mean of Granny Smith over storage was significantly different than that of Fuji (Table 2.4). This result was consistent with the study by Golding and others (2001), which found that phenolic contents can vary greatly depending on the type of cultivars. There were positive correlations between total phenolic contents and ORAC, with r^2= 0.77, Figure 2.5 and FRAP, with r^2= 0.75, Figure 2.6. The total phenolics of apple with skin (the edible portion) and apple without skin (flesh) were compared in this study. Our results were in agreement with Liu and others (2001) that total phenolic contents were higher in all varieties for apples with skin when compared to apples without skin. Liu and others (2001) evaluated total phenolics contents of 10 different apple cultivars, namely Fuji, Red Delicious, Gala, Liberty, Northern Spy, Golden Delicious, Fortune, Jonagold, Empire, and NY 674. They found that Fuji apples with skin had higher total phenolics contents than Red Delicious apples. This

Table 2.3 ORAC, FRAP, Total Phenolics, and Monomeric Anthocyanin Contents of Red Delicious, Fuji, and Granny Smith Apples (1-MCP Treated and Control (Edible portion))

Cultivars	ORAC mean (S.D.)			FRAP mean (S.D.)			Total Phenolic mean (S.D.)			Monomeric Anthocyanin mean (S.D.)		
Storage time (months)	0	3	6	0	3	6	0	3	6	0	3	6
RD Control	13.3 (0.92)	9.09 (0.15)	11.5 (0.28)	20.3 (1.97)	9.15 (0.24)	14.6 (1.2)	2.54 (0.078)	2.3 (0.02)	2.57 (0.02)	4.3 (0.13)	2.1 (0.04)	2.9 (0.001)
RD 1-MCP	12.22 (0.39)	8.78 (0.93)	12.8 (0.67)	15.94 (0.17)	8.87 (0.77)	13.1 (0.28)	2.59 (0.17)	2.27 (0.09)	2.89 (0.002)	3.49 (0.02)	2.08 (0.39)	3.23 (0.02)
F Control	6.65 (0.58)	7.5 (0.23)	7.02 (0.17)	6.84 (0.5)	5.21 (0.17)	5.29 (0.23)	1.42 (0.06)	1.28 (0.06)	1.5 (0.023)	0.49 (0.08)	0.36 (0.004)	0.57 (0.04)

Table 2.3 (continued)

Cultivars	ORAC mean (S.D.)			FRAP mean (S.D.)			Total Phenolic mean (S.D.)			Monomeric Anthocyanin mean (S.D.)		
Storage time (months)	0	3	6	0	3	6	0	3	6	0	3	6
F 1-MCP	5.89 (0.93)	5.94 (0.47)	6.19 (0.46)	6.3 (0.37)	4.87 (0.61)	4.84 (0.29)	1.42 (0.14)	1.31 (0.11)	1.45 (0.065)	0.37 (0.09)	0.364 (0.09)	0.419 (0.07)
GS Control	7.99 (0.44)	7.68 (0.43)	8.99 (0.15)	8.01 (0.87)	7.81 (0.08)	6.17 (0.14)	1.6 (0.04)	1.85 (0.01)	1.64 (0.08)			
GS 1-MCP	7.83 (0.49)	8.72 (0.47)	7.99 (0.38)	11.44 (0.17)	8.14 (0.52)	8.67 (0.65)	1.89 (0.1)	1.86 (0.1)	2.05 (0.035)			

Table 2.3 (continued)

RD: Red Delicious
F: Fuji
GS: Granny Smith
ORAC expressed as μmol T.E./g
FRAP expressed as μmol T.E./g
Total Phenolics expressed as mg/g GAE
Monomeric Anthocyanin expressed as mg/100 g

Table 2.4 Marginal means of Cultivars and Storage Effect of The Edible Portion of Apples

	Cultivar			Storage		
	RD	GS	F	0	3	6
ORAC	11.3	8.21	6.53	8.99	7.95	9.08
FRAP	13.7	7.89	5.56	11.5	6.85	8.78
Phenolics	2.53	1.82	1.38	1.90	1.81	2.02
Anthocyanin	3.01		0.43	2.16	1.23	1.78

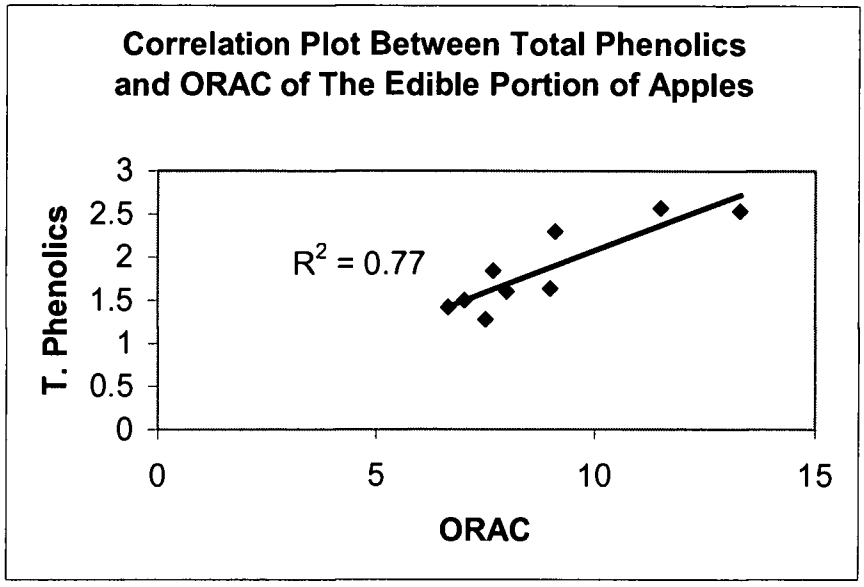

Figure 2.5 Correlation Plot Between Total Phenolics and ORAC of The Edible Portion of Apples

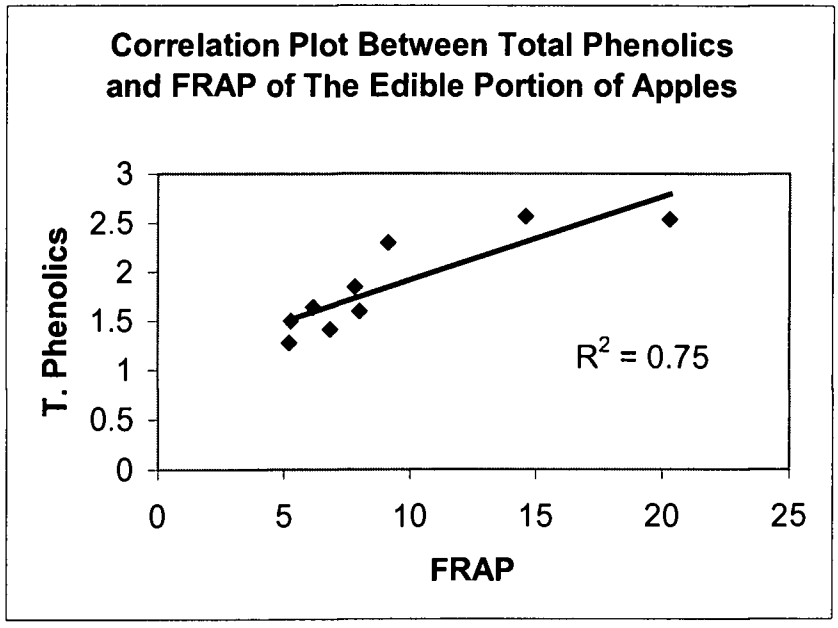

Figure 2.6 Correlation Plot Between Total Phenolics and FRAP of The Edible Portion of Apples

finding differs from ours. Our results showed that for apples with skin at zero time storage, the total phenolic contents of Red Delicious apples were higher than that of Fuji apples. Our total phenolic contents of apples without skin were in agreement with them; Red Delicious apples had higher total phenolics than that of Fuji apples. Our total phenolic contents of Red Delicious with and without skin, 2.54 and 1.89 mg/g GAE, respectively, were higher than the results of Liu and others (2001), which were 2.04 and 1.67 mg/g GAE. However, our total phenolics contents of Fuji with and without skin, 1.42 and 0.95 mg/g GAE, respectively, were lower than that of Liu and others (2001), which were 2.30 and 1.31 mg/g GAE. Vinson and others (2001) estimated that apples provide 186 mg total phenols / 100g apple, which represents 256 mg total phenols/ serving size.

The total phenolic contents of peel and flesh of the three apple cultivars examined are shown in table 2.5. Total phenolics were higher in the peel than in the flesh, with Red Delicious having the highest values, presumably because of the anthocyanin pigments. The relationship between total phenolics and ORAC values are shown in Figure 2.7 for peel and Figure 2.8 for flesh. The total phenolic contents showed a positive correlation with the ORAC values of apples, with r^2=0.85 for peel and r^2= 0.87 for flesh. There was suggestive but inconclusive evidence that cultivars moderately affected the total phenolics in apple flesh with p value of ANOVA = 0.065. The mean of Red Delicious over storage was significantly different than that of Fuji; the mean of Granny Smith over storage

Table 2.5 ORAC, FRAP, Total Phenolics, and Monomeric Anthocyanin Contents of Red Delicious, Fuji, and Granny Smith Apples (peel and flesh)

Cultivars	ORAC mean (S.D.)			FRAP mean (S.D.)			Total Phenolic mean (S.D.)			Monomeric Anthocyanin mean (S.D.)		
Storage time (months)	0	3	6	0	3	6	0	3	6	0	3	6
RD peel	37.7 (2.6)	31.5 (0.35)	31.8 (1.2)	62.7 (2.29)	31.2 (0.49)	32.3 (0.92)	6.63 (0.04)	6.17 (0.06)	6.47 (0.01)	26.4 (0.39)	26.4 (0.1)	30.4 (0.71)
RD flesh	10.1 (0.15)	6.43 (0.2)	9.84 (0.72)	12.5 (1.04)	6.45 (0.59)	6.89 (0.86)	1.89 (0.09)	1.33 (0)	1.83 (0.15)			
F peel	26.7 (2.6)	14.9 (0.1)	13.7 (0.2)	21.1 (2.2)	13.2 (0.42)	9.08 (0.25)	3.65 (0.07)	2.86 (0.08)	2.42 (0.08)	3.9 (0.03)	3.14 (0.01)	1.54 (0.01)

Table 2.5 (continued)

Cultivars	ORAC mean (S.D.)			FRAP mean (S.D.)			Total Phenolic mean (S.D.)			Monomeric Anthocyanin mean (S.D.)		
Storage time (months)	0	3	6	0	3	6	0	3	6	0	3	6
F flesh	4.18 (0.03)	4.93 (0.13)	5.22 (0.12)	4.3 (0.1)	3.32 (0.11)	3.56 (0.03)	0.95 (0.02)	0.92 (0.003)	1.31 (0.25)			
GS peel	24.9 (4.04)	19.2 (0.28)	9.84 (1.01)	36.9 (0.15)	21.4 (1.9)	9.09 (0.37)	3.65 (0.07)	4.24 (0.01)	2.36 (0.017)			
GS flesh	6.8 (0.2)	7.42 (0.25)	6.26 (1.07)	6.03 (0.6)	5.85 (0.09)	5.64 (0.12)	1.49 (0.07)	1.4 (0.04)	1.49 (0.03)			

Table 2.5 (continued)

RD: Red Delicious
F: Fuji
GS: Granny Smith
ORAC expressed as μmol T.E./g
FRAP : expressed as μmol T.E./g
Total Phenolics : expressed as mg/g GAE
Monomeric Anthocyanin : expressed as mg/100g

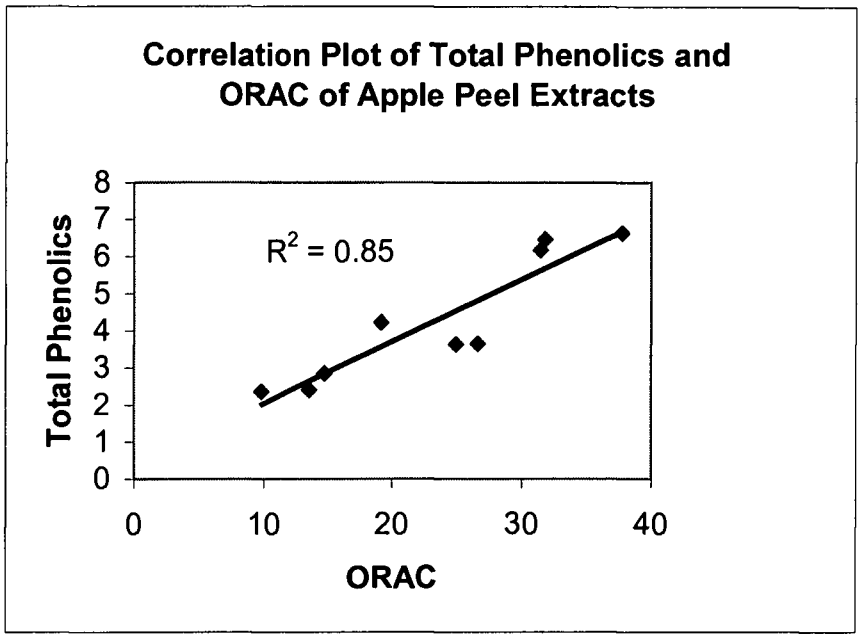

Figure 2.7 Correlation Plot of Total Phenolics and ORAC of Apple Peel Extracts

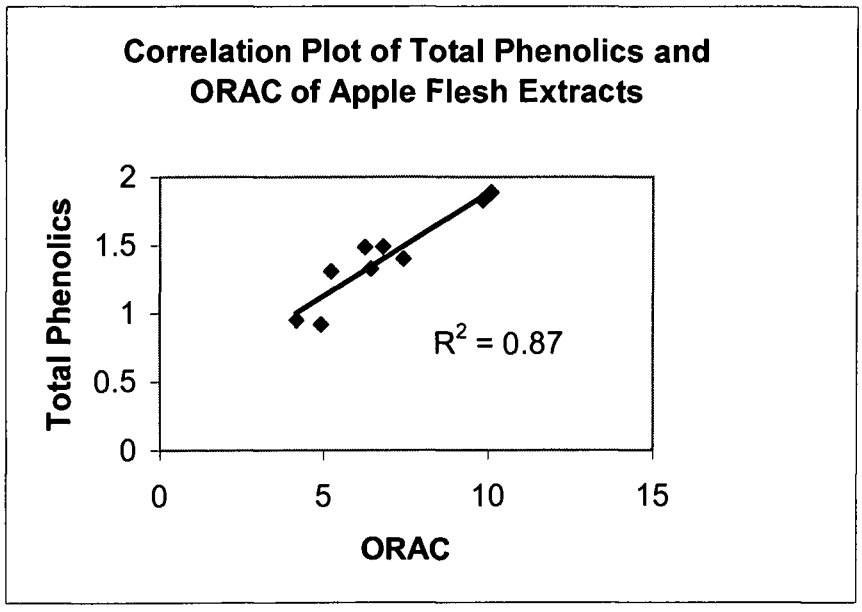

Figure 2.8 Correlation Plot of Total Phenolics and ORAC of Apple Flesh Extracts

was significantly different than that Fuji (Table 2.6). The cultivars type had a significant influence on the total phenolics of apple peels with p value of ANOVA= 0.006. The mean value of Red Delicious over storage was significantly different than that of Fuji; the mean value of Red Delicious over storage was significantly different than that of Granny Smith (Table 2.7).

The relationships between total phenolic contents and FRAP values were also positive, with r^2= 0.71 (Figure 2.9) for peel and r^2= 0.63 for flesh (Figure 2.10). The correlation coefficient of total phenolics and FRAP values was lower than the correlation coefficient of total phenolics and ORAC values.

Monomeric Anthocyanin of Red Delicious and Fuji Apple Cultivars

The skin of Red Delicious apples had a uniform, intensely red color whereas the skin of Fuji apples was paler. Our studies showed that the edible portion of Red Delicious apples had a higher monomeric anthocyanin content than that of Fuji apples. The monomeric anthocyanin contents of Red Delicious and Fuji apples are displayed in table 2.8. At zero month storage, Red Delicious apples contained almost a nine times higher monomeric anthocyanin content than Fuji apples. The correlation between monomeric anthocyanin and ORAC, FRAP, and total phenolic contents of the edible portion of apples were 0.97, 0.96, and 0.87, respectively (Figures 2.11, 2.12, and 2.13). The two varieties were significantly different with respect to their monomeric anthocyanin contents, with p value of

Table 2.6 Marginal means of Cultivars and Storage Effects of Apple Flesh

	Cultivar			Storage		
	RD	GS	F	0	3	6
ORAC	8.78	6.83	4.78	7.02	6.26	7.10
FRAP	8.63	6.50	3.73	8.28	5.20	5.36
Phenolics	1.68	1.50	1.06	1.48	1.22	1.55

Table 2.7 Marginal means of Cultivars and Storage Effects of Apple Peels

	Cultivar			Storage		
	RD	GS	F	0	3	6
ORAC	33.7	18	18.4	29.8	21.8	18.4
FRAP	44.4	22.4	14.4	40.2	21.9	19.2
Phenolics	6.42	3.41	2.97	4.64	4.42	3.75
Anthocyanin	27.7		2.86	15.1	14.8	16

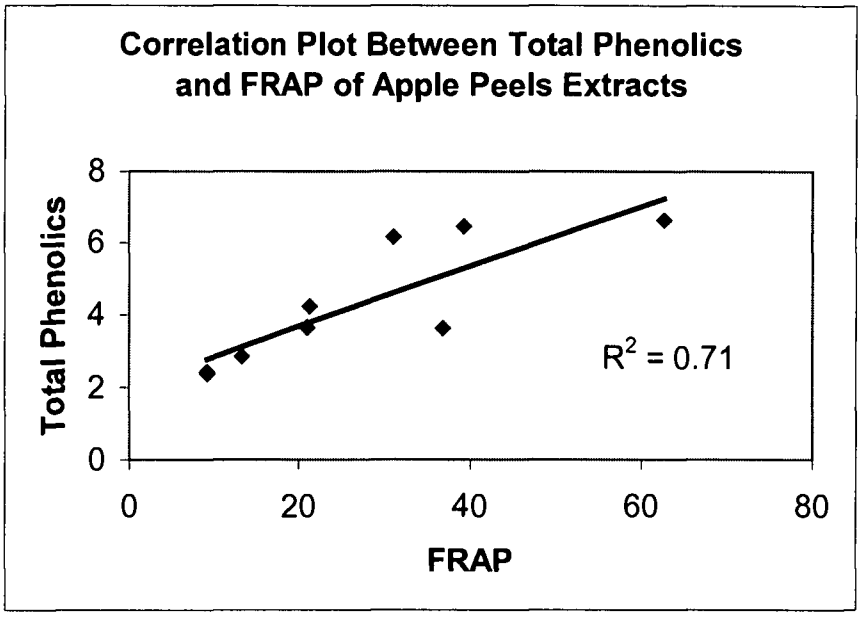

Figure 2.9 Correlation Plot Between Total Phenolics and FRAP of Apple Peels Extracts

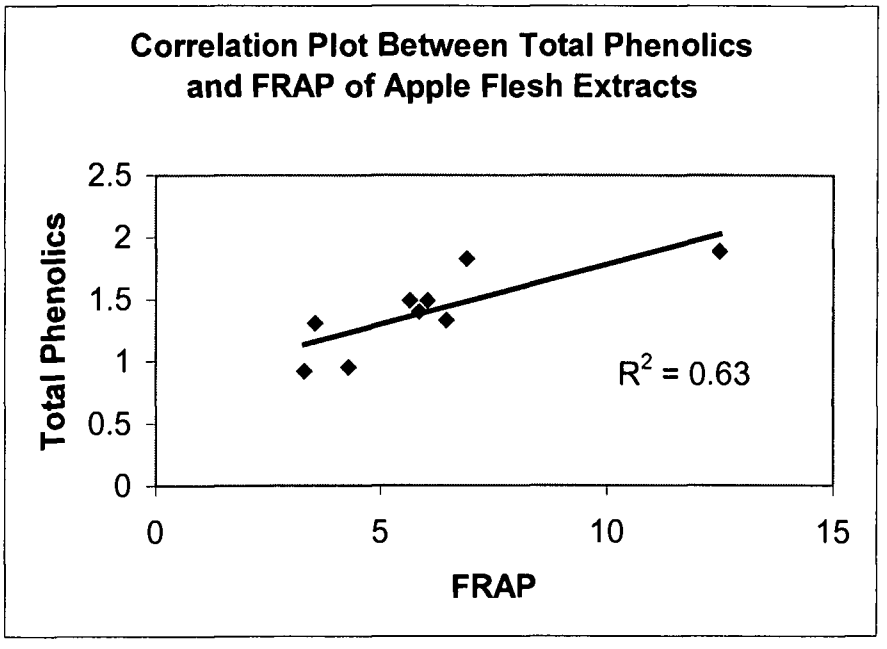

Figure 2.10 Correlation Plot Between Total Phenolics and FRAP of Apple Flesh Extracts

Table 2.8 Monomeric Anthocyanin Content of Red Delicious and Fuji apple peels during a six- month storage period

Cultivar	Monomeric Anthocyanin (mg/100g fw)	Monomeric Anthocyanin (mg/100g fw)	Monomeric Anthocyanin (mg/100g fw)
Storage (months)	0 month	3 month	6 month
Red Delicious peel	26.4 (0.39)	26.4 (0.10)	30.4 (0.71)
Red Delicious Control	4.3 (0.13)	2.1 (0.04)	2.9 (0.0014)
Red Delicious 1- MCP	3.49 (0.021)	2.08 (0.39)	3.23 (0.021)
Fuji peel	3.9 (0.028)	3.14 (0.014)	1.54 (0.014)
Fuji Control	0.49 (0.085)	0.36 (0.0042)	0.57 (0.042)
Fuji 1-MCP	0.37 (0.086)	0.36 (0.086)	0.42 (0.074)

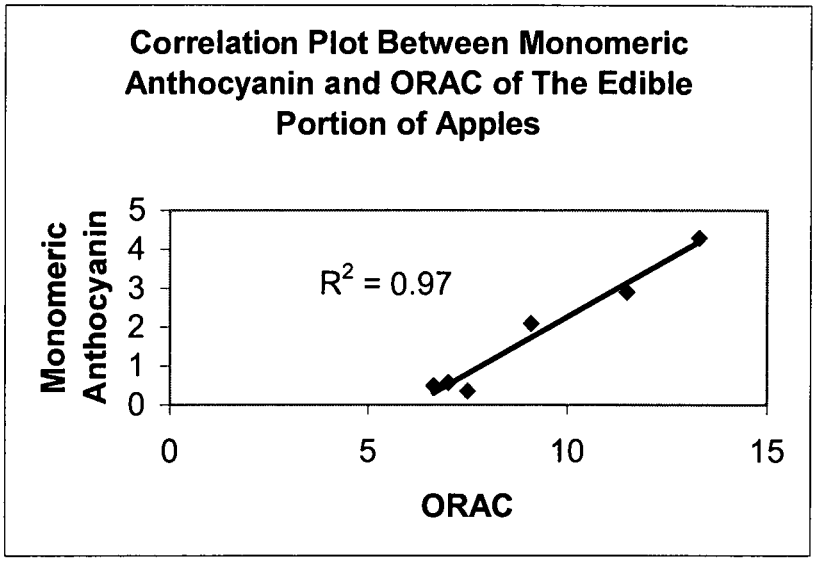

Figure 2.11 Correlation Plot Between Monomeric Anthocyanin and ORAC of The Edible Portion of Apples

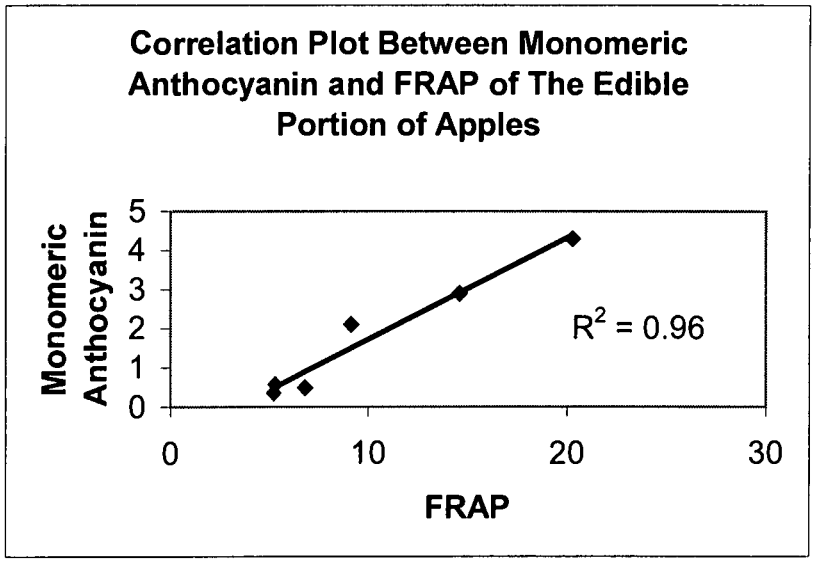

Figure 2.12 Correlation Plot Between Monomeric Anthocyanin and FRAP of The Edible Portion of Apples

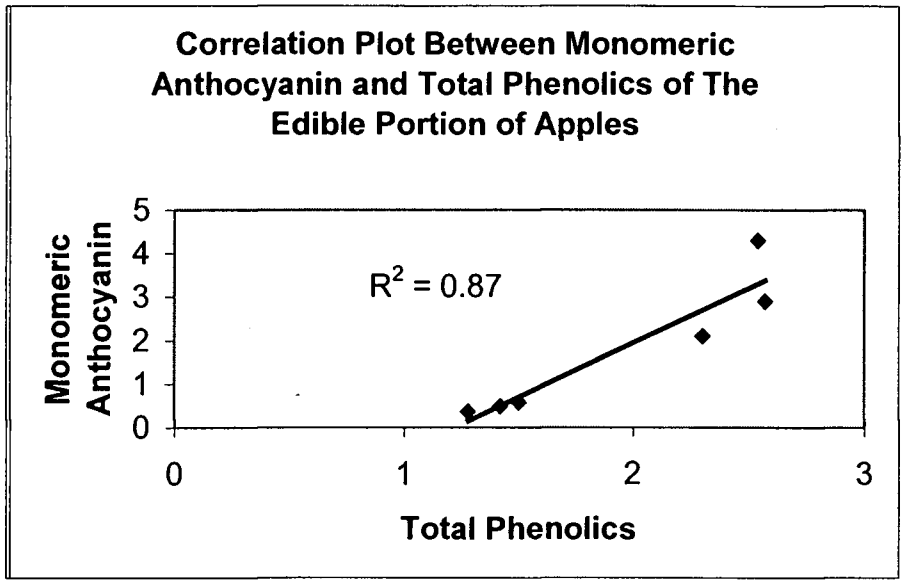

Figure 2.13 Correlation Plot Between Monomeric Anthocyanin and Total Phenolics of The Edible Portion of Apples

ANOVA= 0.0042. Mazza and Miniati (1993) reported that Scugog apple contained 10 mg/ 100g fresh weight of total anthocyanin.

At zero month storage, the monomeric anthocyanin content of Red Delicious peels was almost seven times higher than that of Fuji apples. The two peel cultivars were significantly different with respect to their ORAC content with p value of ANOVA= 0.006. Red Delicious was significantly different than Fuji. The marginal means of cultivars and storage effects of apple peels were presented previously in table 2.7. Our studies also found that the correlation between monomeric anthocyanin contents and ORAC values of apple peels (r^2= 0.74, Figure 2.14) was higher than the correlation between monomeric anthocyanin contents and FRAP values of apple peel (r^2= 0.67, Figure 2.15). Total phenolics of the apple peels correlated strongly with the monomeric anthocyanin contents, with r^2=0.96, Figure 2.16. Anthocyanin contributed significantly to the total phenolic contents of Red Delicious and Fuji apples.

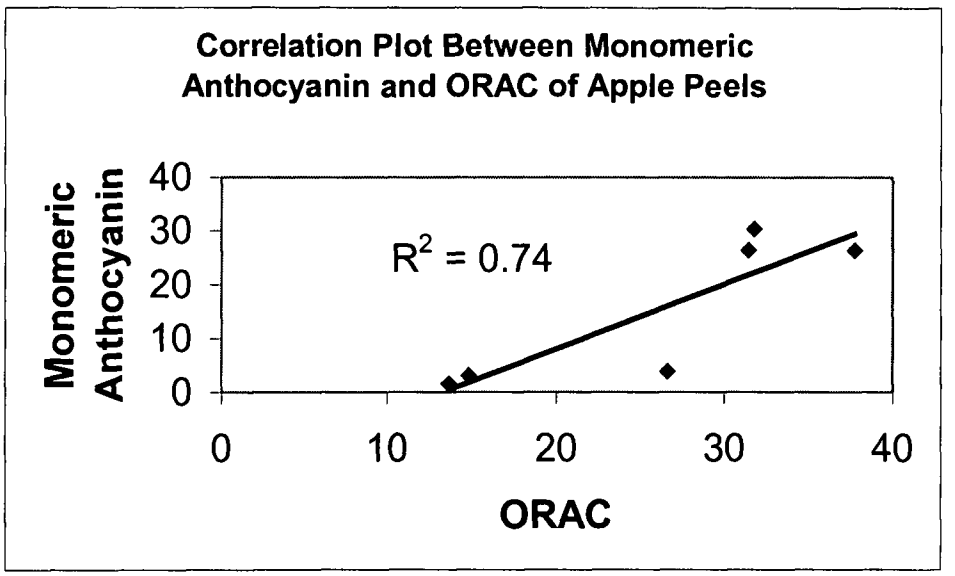

Figure 2.14 Correlation Plot Between Monomeric Anthocyanin and ORAC of Apple Peels

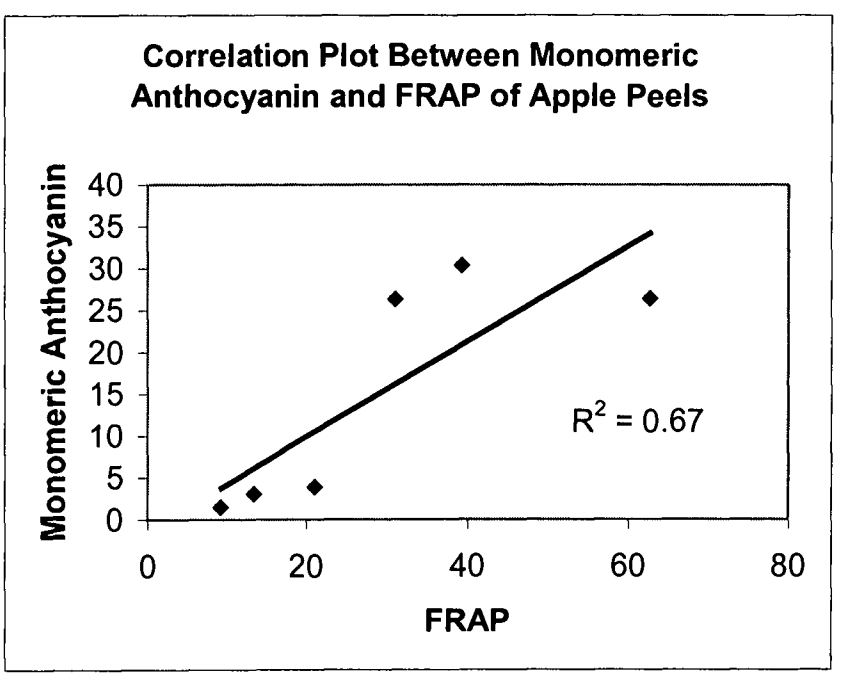

Figure 2.15 Correlation Plot Between Monomeric Anthocyanin and FRAP of Apple Peels

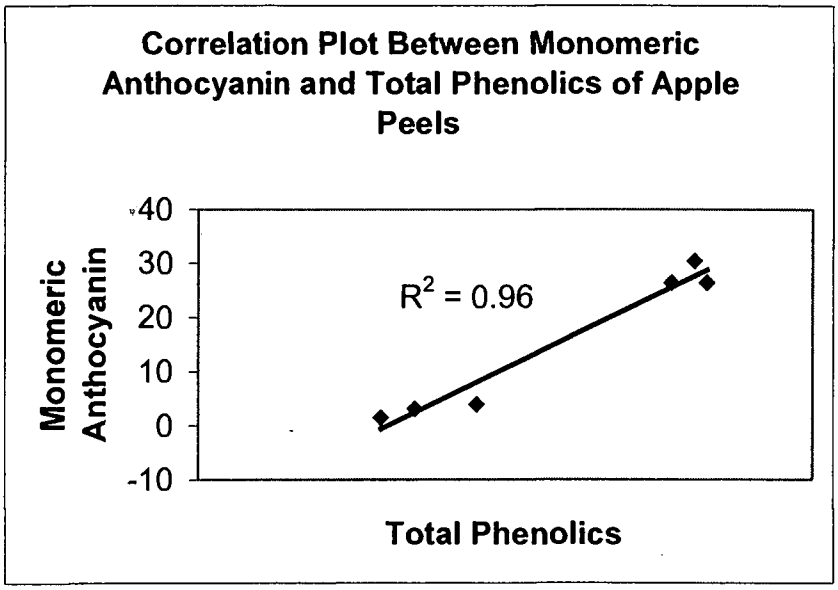

Figure 2.16 Correlation Plot Between Monomeric Anthocyanin and Total Phenolics of Apple Peels

Ascorbic Acid Analysis in Apples

All of the apple extracts were subjected to ascorbic acid analysis. However, only a few extracts contained ascorbic acid. At zero time storage, only Fuji and Granny Smith peels, Fuji control, and 1- MCP treated Granny Smith apple extracts contained ascorbic acid; their values were: 5.64 mgl/100g, 2.29mg/100g, 0.39 mg/100g, and 0.95 mg/100g respectively. At three-month storage periods, only Fuji and Granny Smith peels contained ascorbic acid. Their ascorbic acid contents were 2.11 mg/100g and 2.82mg/100g, respectively. One apple with skin (138 g) contained 7.9 mg of Vitamin C (anonymous 2002). Liu and others (2000) reported that the vitamin C content of raw Red Delicious apples with skin was 5.7 mg/100g. The antioxidant activity of 1 g of Red Delicious apple with skin and without skin was 83.3 and 46.07 TOSC (mmol vitamin C equivalents/g), respectively. The calculated antioxidant activity of vitamin C in 1 g of Red Delicious apple with skin was only 0.32 TOSC (mmol vitamin C equivalents/g). The vitamin C in apple with skin accounts for only 0.4 % of total antioxidant activity. Liu and others (2000) concluded that the main contribution to the antioxidant activity of apple is not due to vitamin C but to a variety of phytochemicals in apples. The combination of different phytochemicals in apples may have additive or synergistic functions, which may be responsible for the potent antioxidant effects (Eberhardt and others 2000).

Antioxidant Capacity of The Edible Portion, Peels, and Flesh of Apples in The Aqueous Phase

Both ORAC and FRAP assay results of the edible portion (skin and flesh) of apples, also called control apples, showed that Red Delicious had the highest antioxidant capacities, followed by Granny Smith and Fuji apples. The three cultivars were significantly different with respect to ORAC with p value of ANOVA= 0.001 and FRAP with p value of =0.00024. For ORAC and FRAP, Red Delicious was significantly different than Granny Smith; Red Delicious was significantly different than Fuji; Fuji was significantly different than Granny Smith. The marginal means were previously presented in table 2.4. The storage trends of ORAC and FRAP of the three apple cultivars were shown on figure 2.17 and 2.18. There was a positive correlation between ORAC and FRAP values for the edible portion of apples (r^2=0.89, figure 2.19). The statistical findings for the edible portion of apples are presented in table 2.9. Wang and others (1996) analyzed 12 fruits: strawberry, orange, apple, pink grapefruit, plum, red grape, white grape, kiwi fruit, banana, tomato, pear, and honeydew melon. They extracted the fruits with acetone. They found that the ORAC value for apple was 2.18± 0.35μmoles of Trolox equivalents per gram of fruits. Liu and others (2001) employed the TOSC (Total Oxyradical Scavenging Capacity) antioxidant assay in analyzing 10 apple cultivars. Liu and others (2001) reported that for apples with skin, Northern Spy and Red Delicious had the highest TOSC values at 83.34 mmol vitamin C

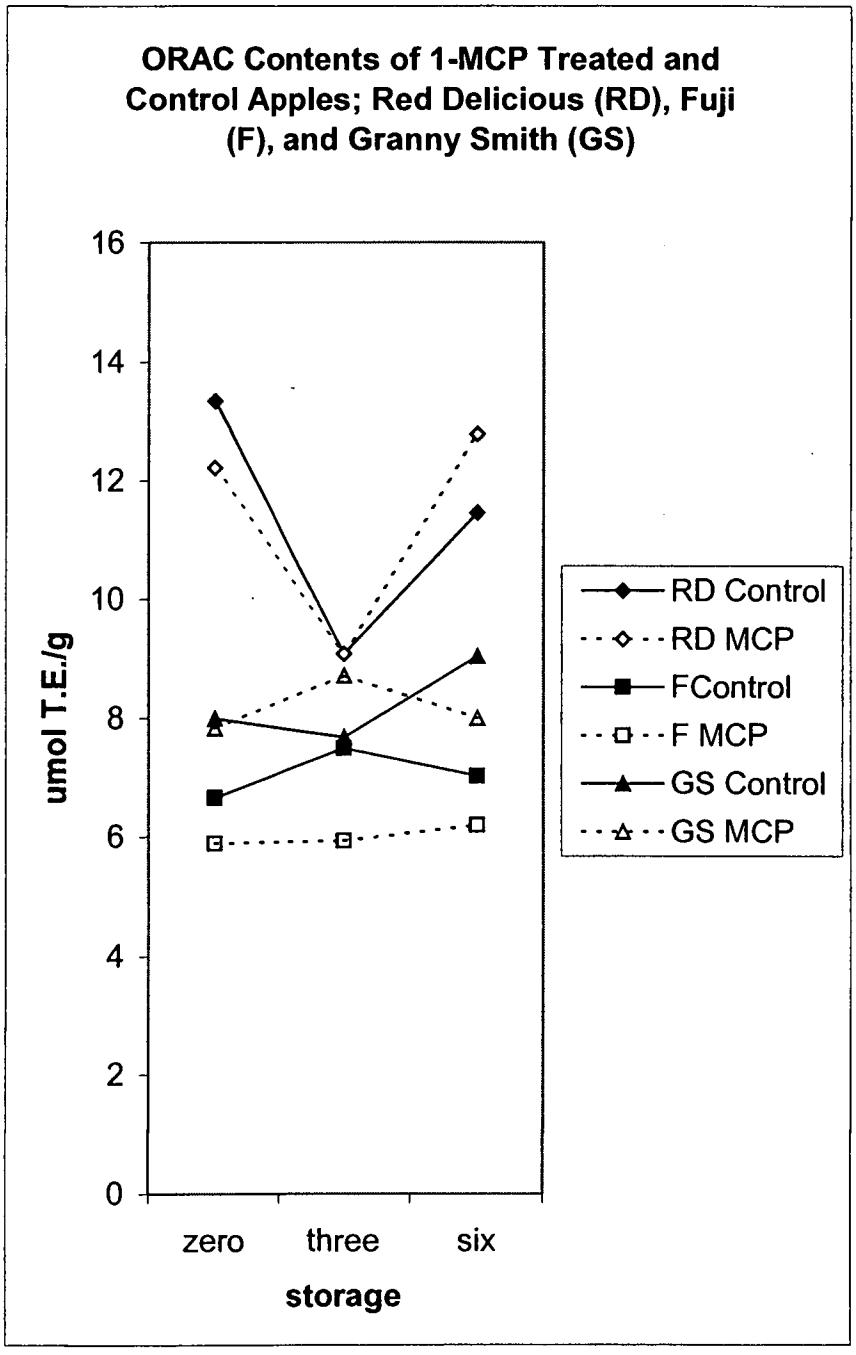

Figure 2.17 ORAC Contents of 1-MCP Treated and Control Apples; Red Delicious (RD), Fuji (F), and Granny Smith (GS)

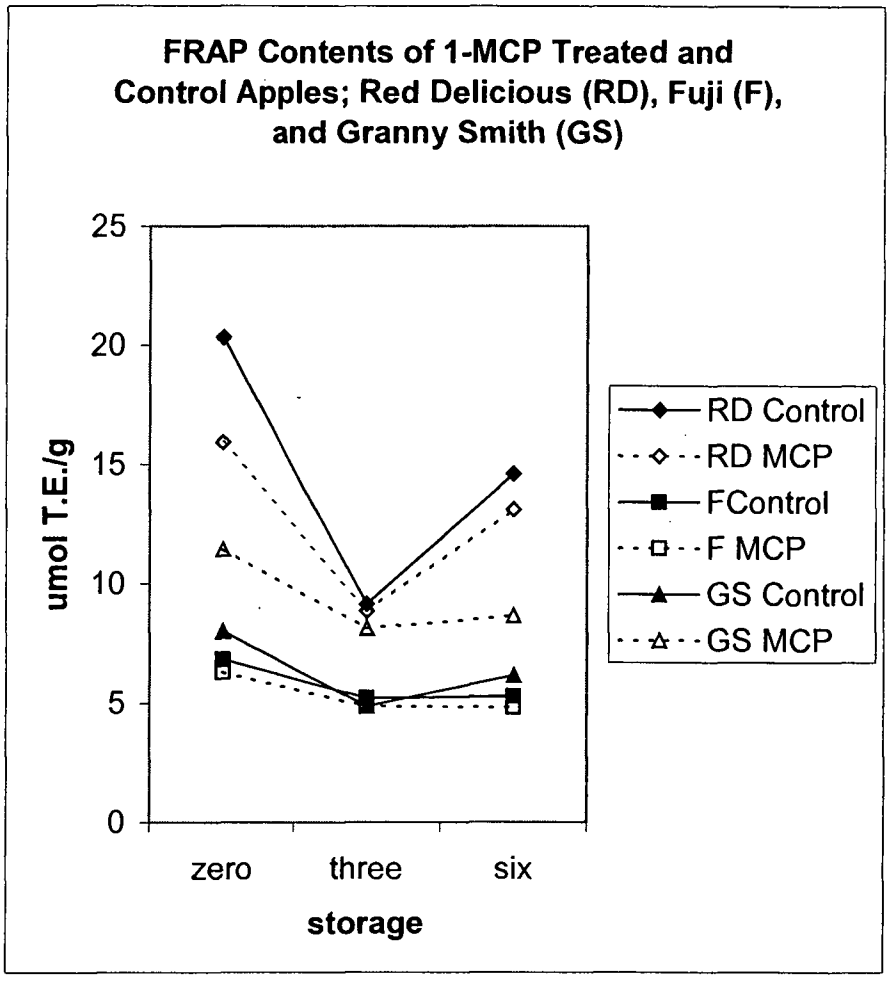

Figure 2.18 FRAP Contents of 1-MCP Treated and Control Apples; Red Delicious (RD), Fuji (F), and Granny Smith (GS)

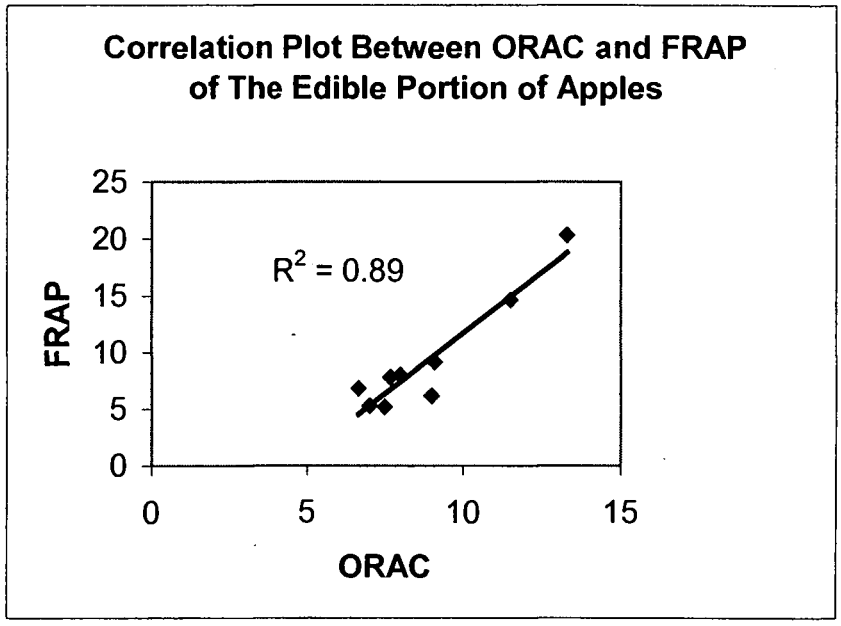

Figure 2.19 Correlation Plot Between ORAC and FRAP of The Edible Portion of Apples

Table 2.9 Statistical findings of ORAC, FRAP, Total Phenolics, and Monomeric Anthocyanin Contents of Control and 1-MCP Treated Apples

Effect	ORAC	FRAP	Total Phenolics	Monomeric Anthocyanin
Cultivar (C)	Significant	Significant	Significant	Significant
MCP treatment (T)	Not Significant	Not Significant	Suggestive but inconclusive	Not Significant
Storage (S)	Not Significant	Significant	Suggestive but inconclusive	Suggestive but inconclusive
C*T	Not significant	Moderate	Not Significant	Not Significant
C*S	Suggestive but inconclusive	Moderate	Not Significant	Not Significant
T*S	Not Significant	Not Significant	Not Significant	Not Significant

equivalents per gram TOSC values and 83.3 mmol vitamin C equivalents per gram TOSC values, followed by Fuji, Gala, Liberty, NY 674, Golden Delicious, Fortune, Jonagold and Empire. Although we had different method in determining antioxidant activity, our result for apples with skin were in agreement with them, Red Delicious had higher antioxidant activity than Fuji apples. In addition, our results were in agreement with Liu and others (2001) who reported that apples with skin had higher antioxidant activities than apples without skin for all varieties tested.

Liu and others (2001) also reported that Northern Spy apples without skin had the highest antioxidant activity (48.54 mmol vitamin C equivalents per gram TOSC values) followed by Fuji, Red Delicious, Golden Delicious, Liberty, Gala, NY 674, Fortune, Jonagold, and Gala. This finding differs from ours. Our results showed that for apples without skin (flesh), Red Delicious had higher antioxidant activity than Fuji apples.

The antioxidant capacities were higher in the peel than in the flesh, with Red Delicious having the highest values, presumably because of the anthocyanin pigments. The antioxidant capacity storage trends in the peel and the flesh of the three apple cultivars are shown in Figures 2.20 , 2.21 ,2.22 , and 2.23.

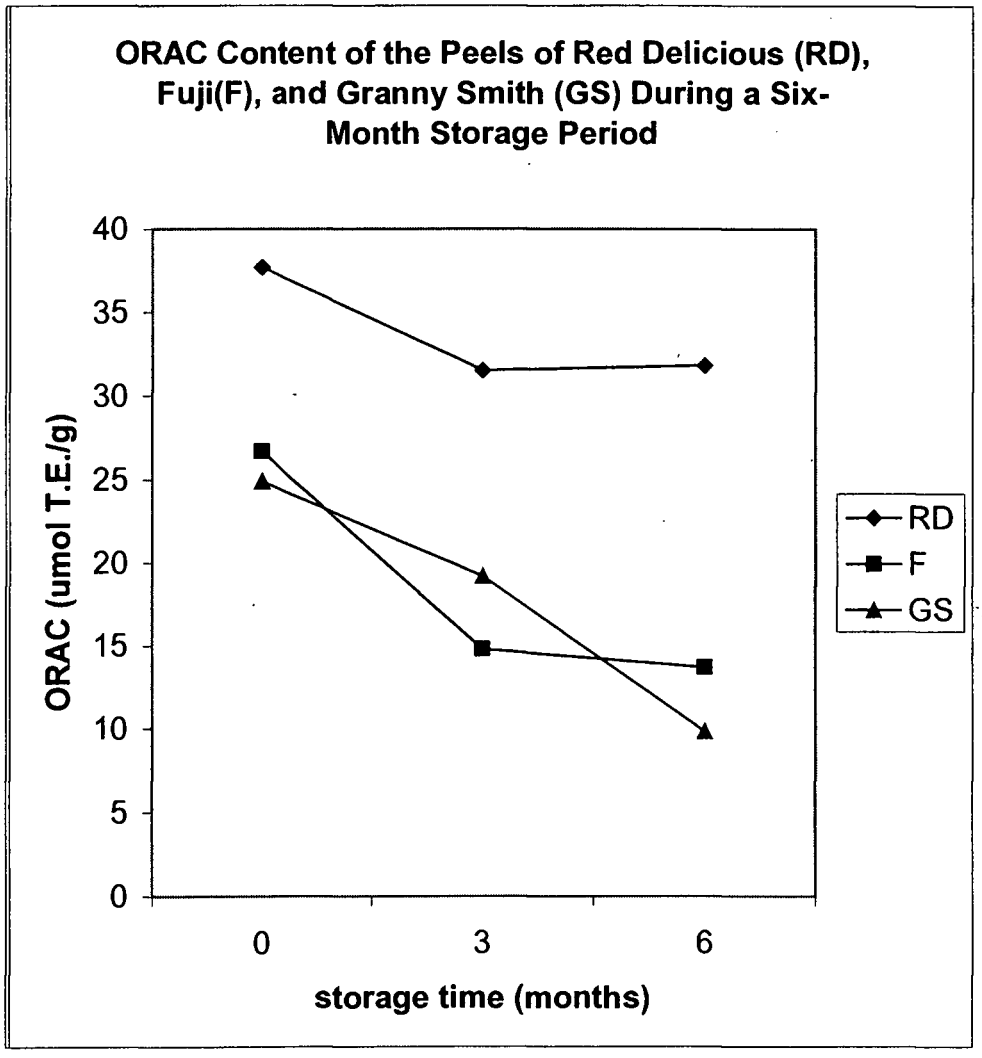

Figure 2.20 ORAC Content of The Peels of Red Delicious (RD), Fuji (F), and Granny Smith (GS) During a Six- Month Storage Period

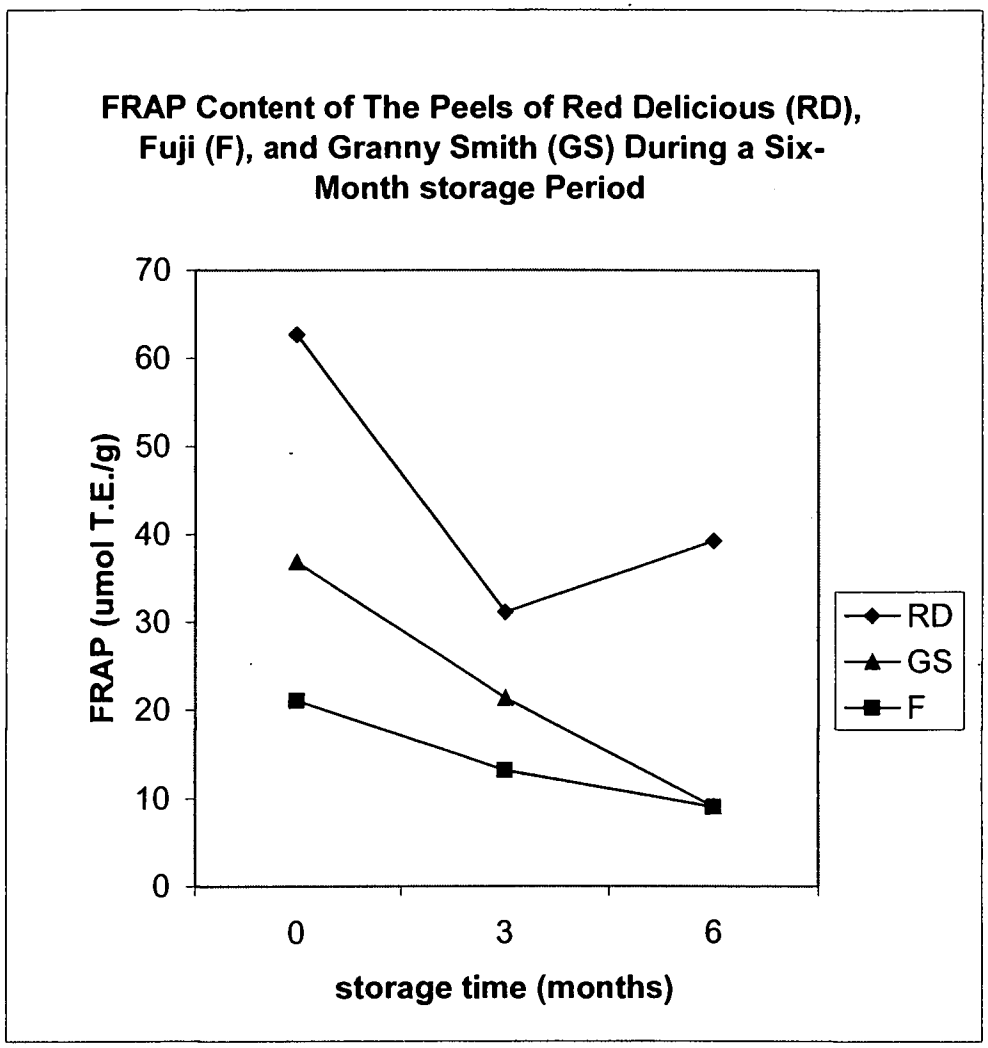

Figure 2.21 FRAP Content of The Peels of Red Delicious (RD), Fuji (F), and Granny Smith (GS) During a Six- Month Storage Period

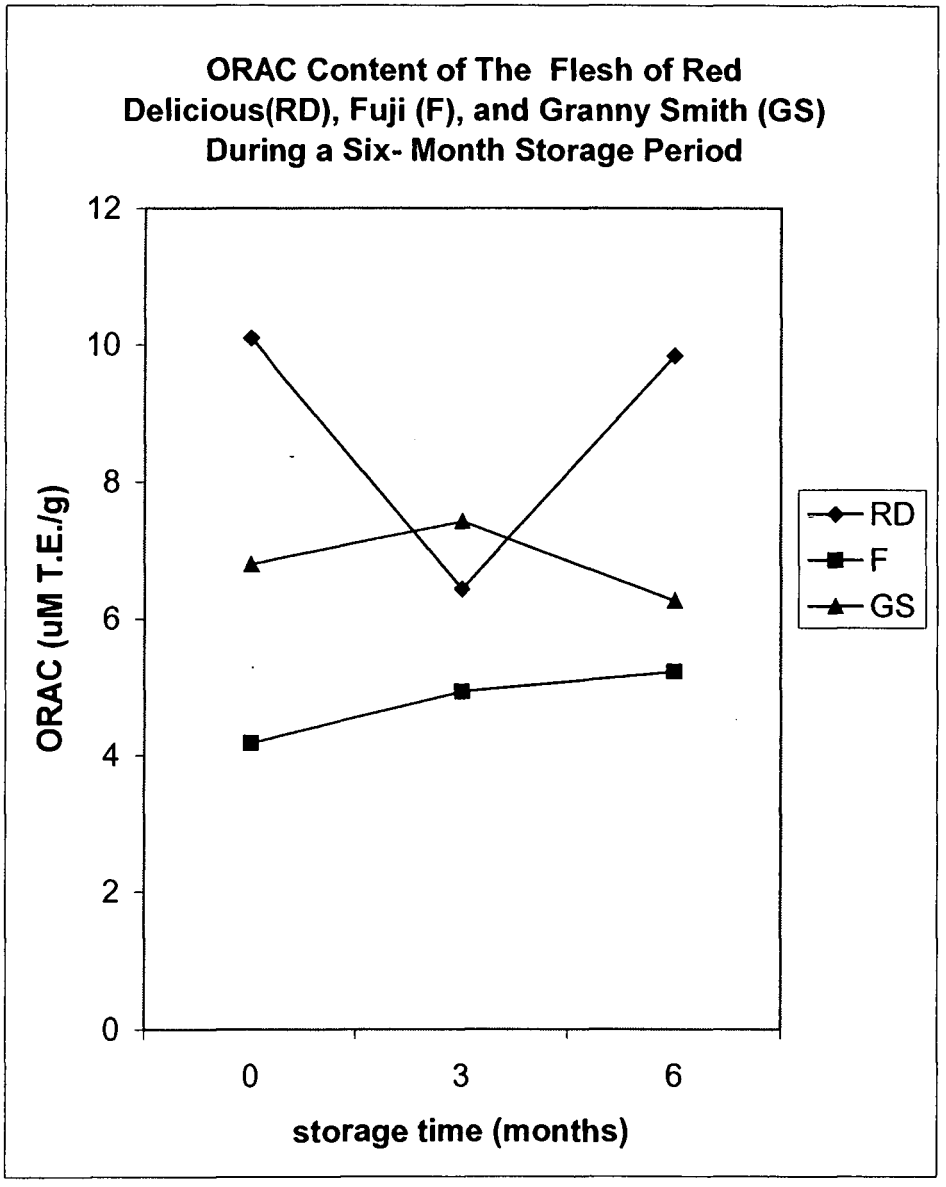

Figure 2.22 ORAC Content of The Flesh Red Delicious (RD), Fuji (F), and Granny Smith (GS) During a Six- Month Storage Period

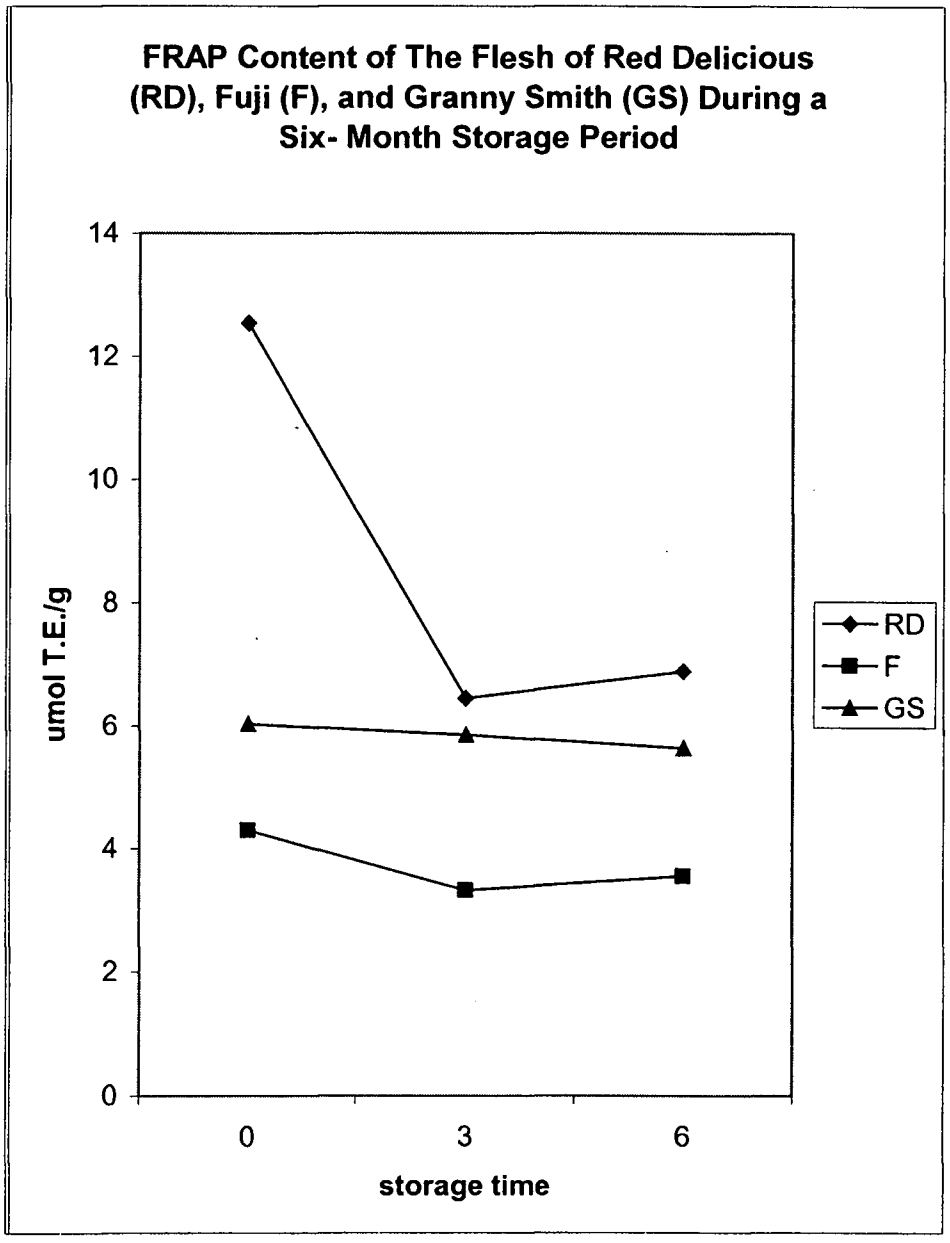

Figure 2.23 FRAP Contents of The Flesh Red Delicious (RD), Fuji (F), and Granny Smith(GS) During a Six- Month Storage Period

The two antioxidant assays, ORAC and FRAP showed different antioxidant activity trends. The differences in the behavior of the apples occurred partly because we employed these two methods. The difference between these two assays is based on the chemistry principles upon which they were built. The ORAC is based on the hydrogen atom transfer between an oxidant and a free radical; the FRAP assay is based on the single electron transfer reaction between an oxidant and a free radical. ORAC values represent the peroxyl radical scavenging capacity of apples. On the contrary, FRAP assays estimates only the Fe (III) reducing capacity and are conducted at the non- physiological condition (with low pH of 3.6). FRAP is not necessarily relevant to antioxidant capacity physiologically and mechanistically (Ou and others 2002). However, FRAP is a simpler, faster, and cheaper method than ORAC. In our studies, the correlation between ORAC and FRAP assays of apple peels (r^2= 0.82, Figure 2.24) was better than the correlation between ORAC and FRAP assays of apple flesh (r^2= 0.70, Figure 2.25). There was suggestive but inconclusive evidence that ORAC values in the flesh of apples were influenced by cultivars with p value of ANOVA= 0.065. The mean of ORAC values of Red Delicious over storage was significantly different than that of Fuji apples. However, the cultivar type had a significant influence on the ORAC values of apple peels with p value of ANOVA= 0.006; the mean of ORAC values of Red Delicious over storage was significantly different than that of Fuji and the mean of

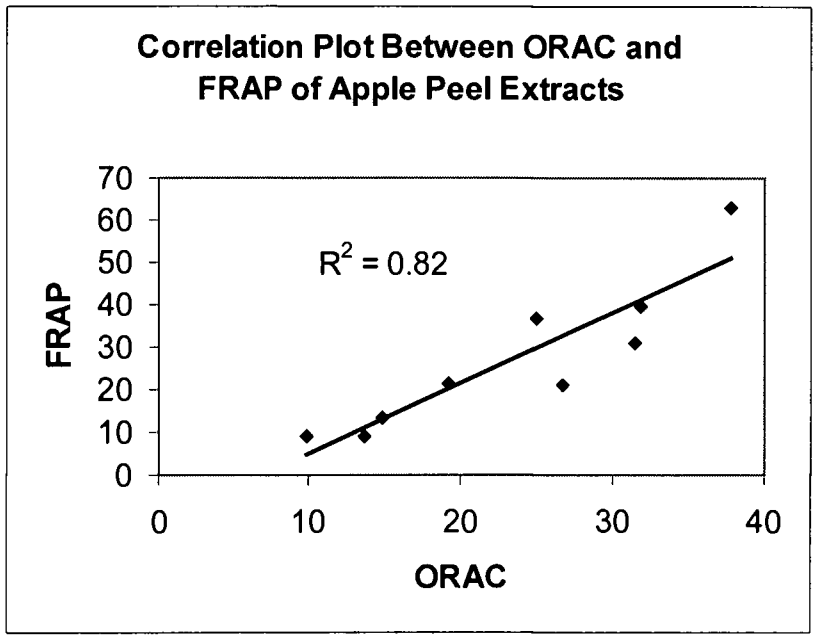

Figure 2.24 Correlation Plot Between ORAC and FRAP of Apple Peel Extracts

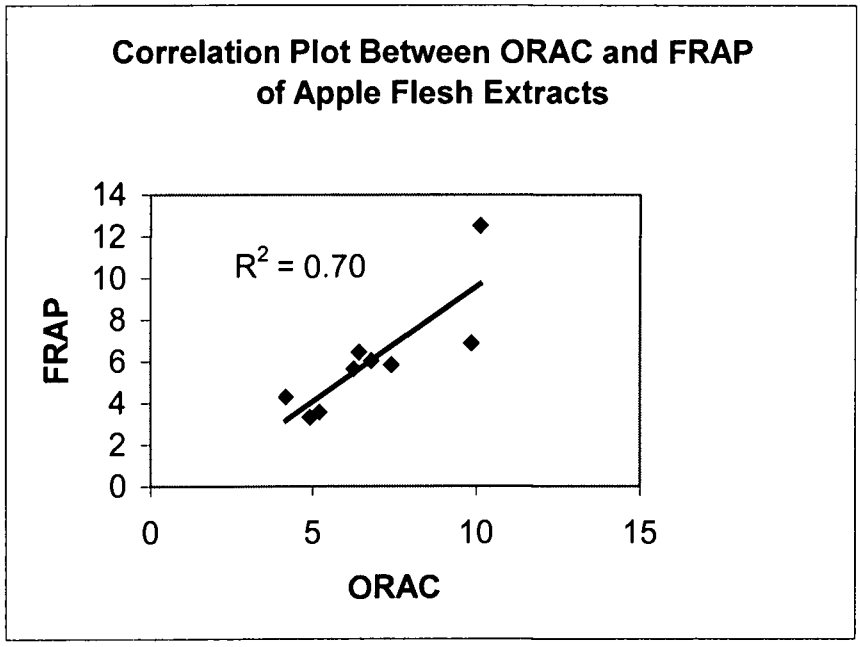

Figure 2.25 Correlation Plot Between ORAC and FRAP of Apple Flesh Extracts

ORAC values of Red Delicious over storage was significantly different than that of Granny Smith. FRAP assay results of the apple peel and flesh extracts were moderately affected by the type of cultivar with $p = 0.016$ and $p = 0.039$ respectively. For apple peels, the mean of FRAP values of Red Delicious over storage was significantly different that that of Fuji and the mean of FRAP values of Red Delicious over storage was significantly different that that of Granny Smith apples. For flesh, the mean of FRAP values of Red Delicious over storage was significantly different that that of Fuji apples.

Antioxidant Capacity in The Non- Polar Phase

At 0 and 3 months storage periods, the chloroform phase of Granny Smith apple peel extract had the highest ORAC values, probably because of the chlorophyll content. During storage, the chloroform phase of Red Delicious and Fuji apple peel extracts behaved in a similar way; their ORAC values decreased during the 0 to 3 month storage period, then increased during the 3 to 6 month period. The ORAC values of the chloroform phase of Granny Smith apple peel extracts decreased during the six- month storage period. There was suggestive but inconclusive evidence that storage influenced the ORAC values of the chloroform phase of three apple cultivars extracts examined ($p= 0.0976$). The type of cultivar did not influence the ORAC values ($p=0.419$). At zero time storage, the chloroform phase of Granny Smith apple peel extracts had the highest FRAP value, followed

by Red Delicious and Fuji apple peel extracts. Similar to ORAC values, FRAP values of the chloroform phase of Red Delicious and Fuji apple peel extracts decreased during the 0 to 3 month storage period, then increased during the 3 to 6 month period. During the six- month storage period, the FRAP values of the chloroform phase of Granny Smith apple peel extracts decreased. Cultivars type did not affect the FRAP values ($p= 0.692$), however storage had a moderate influence on the FRAP values of the chloroform phase of the three apple extracts ($p=0.0237$).

The Effect of Storage on Total Phenolics, Monomeric Anthocyanin and Antioxidant Capacities of The Edible Portion, Peels, and Flesh of Apples

The storage trends of total phenolic contents of the edible portion (control) of Red Delicious, Fuji, and Granny Smith apples are shown on figure 2.26. The total phenolic contents of the edible portion of Red Delicious and Fuji apples decreased from zero and three- month storage and then increased from three- to six-month storage. The total phenolic contents of the edible portion of Granny Smith apples increased from zero to three- months storage and then decreased from three to six- month storage. There was suggestive but inconclusive evidence of a storage effect on the total phenolic contents of the edible portion of the apples with p value of ANOVA= 0.0559. The mean phenolics over cultivars of the three- month storage was significantly different than that of the six- month storage.

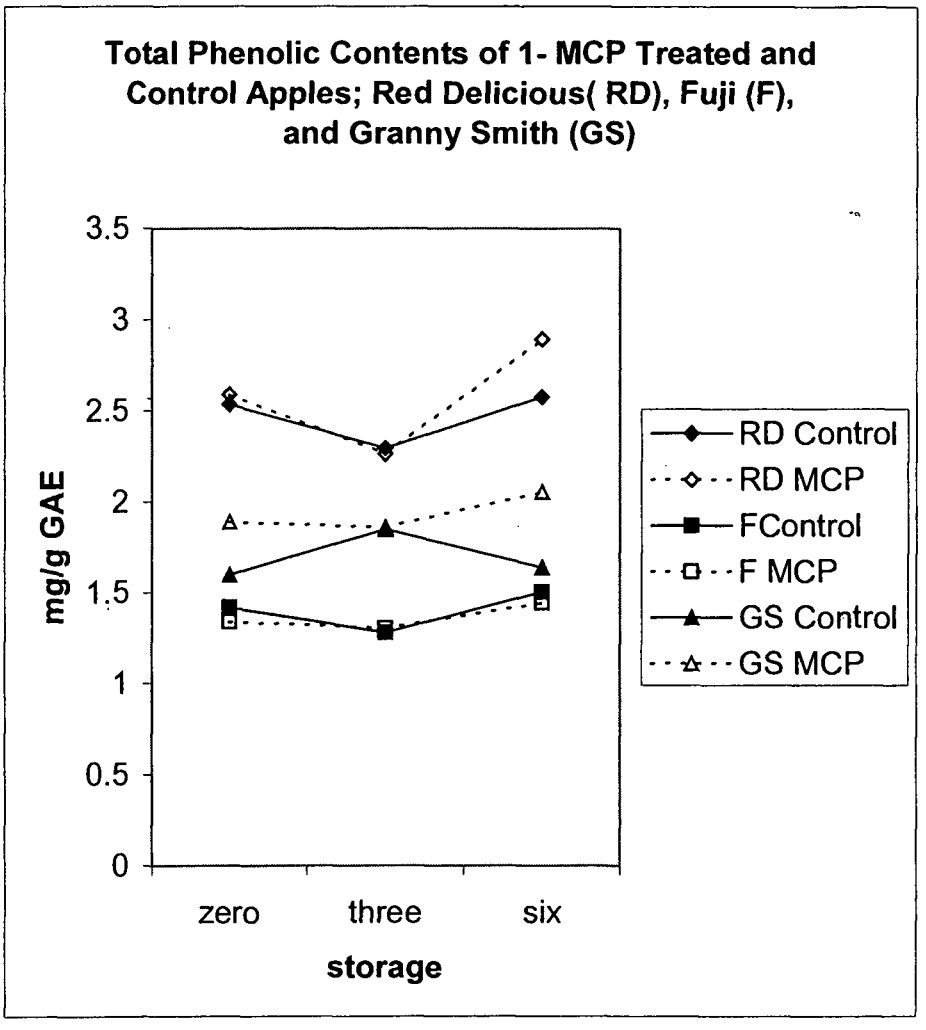

Figure 2.26 Total Phenolic Contents of 1- MCP Treated and Control Apples; Red Delicious (RD), Fuji (F), and Granny Smith (GS)

The storage trends of the total phenolic contents of the peel of the three apples cultivars are shown in figure 2.27. Cold storage did not have a significant effect on the total phenolic contents of apple peel extracts, p value of ANOVA= 0.27. The statistical findings for apple peel are presented in table 2.10.

The total phenolic contents of Red Delicious, Fuji and Granny Smith apple flesh extracts are displayed in figure 2.28. Storage at cold room (0 °C, 88%RH) did not have a significant influence on the total phenolics of apple flesh extracts, with p value of ANOVA= 0.163. The statistical findings for apple flesh are presented in table 2.11.

In 1997, Perez- Ilzarbe and others analyzed Granny Smith apples peels and flesh. After harvest, their apples were stored in controlled chamber at 85% relative humidity and 4° C for 10 days, and then rewarmed at 22° C for 21 days. Samples were taken immediately after leaving the chamber at 4° C and at 3 h, 2 days, 6, 9,14,17, and 21 days after rewarming. They extracted the apples with methanol/ HCl and purified the extracts with ethyl acetate. The extracts were analyzed using RP-HPLC. They reported that in the flesh, apple phenolic compounds decreased during the cold treatment. Our results showed that the total phenolics contents of Granny Smith apple flesh extracts decreased during 0 to 3 month storage. In addition, Perez- Ilzarbe (1997) reported that the quantity of phenolic compounds in the peel increased with time after the cold treatment. They also reported that

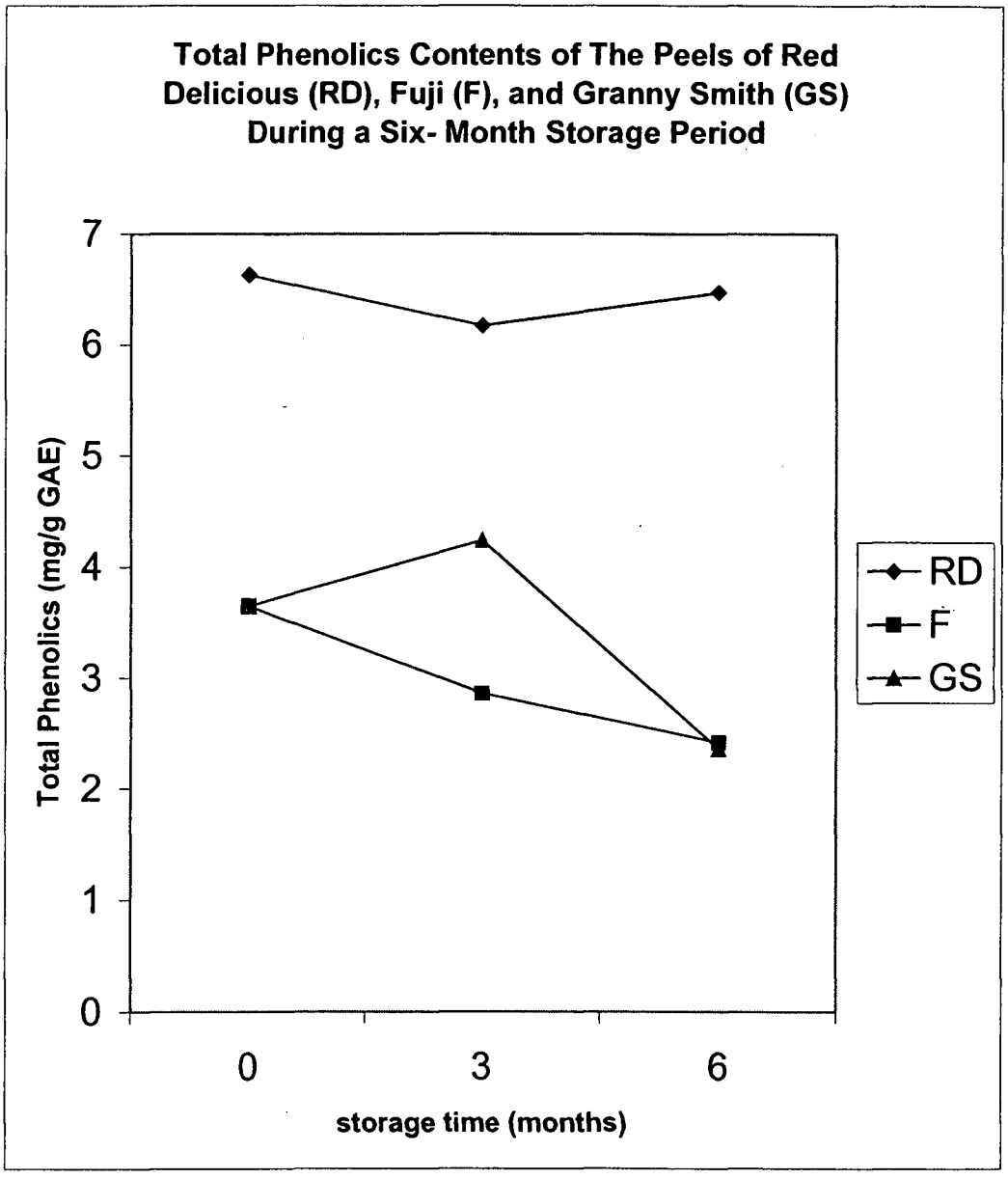

Figure 2.27 Total Phenolic Contents of The Peels of Red Delicious (RD), Fuji (F), and Granny Smith (GS) During a Six- Month Storage Period

Table 2.10 Statistical findings of ORAC, FRAP, Total Phenolics, and Monomeric Anthocyanin Contents of Apples Peels

Effect	ORAC	FRAP	Total Phenolics	Monomeric Anthocyanin
Cultivar	Significant	Moderate	Significant	Significant
Storage	Moderate	Moderate	Not significant	Not significant

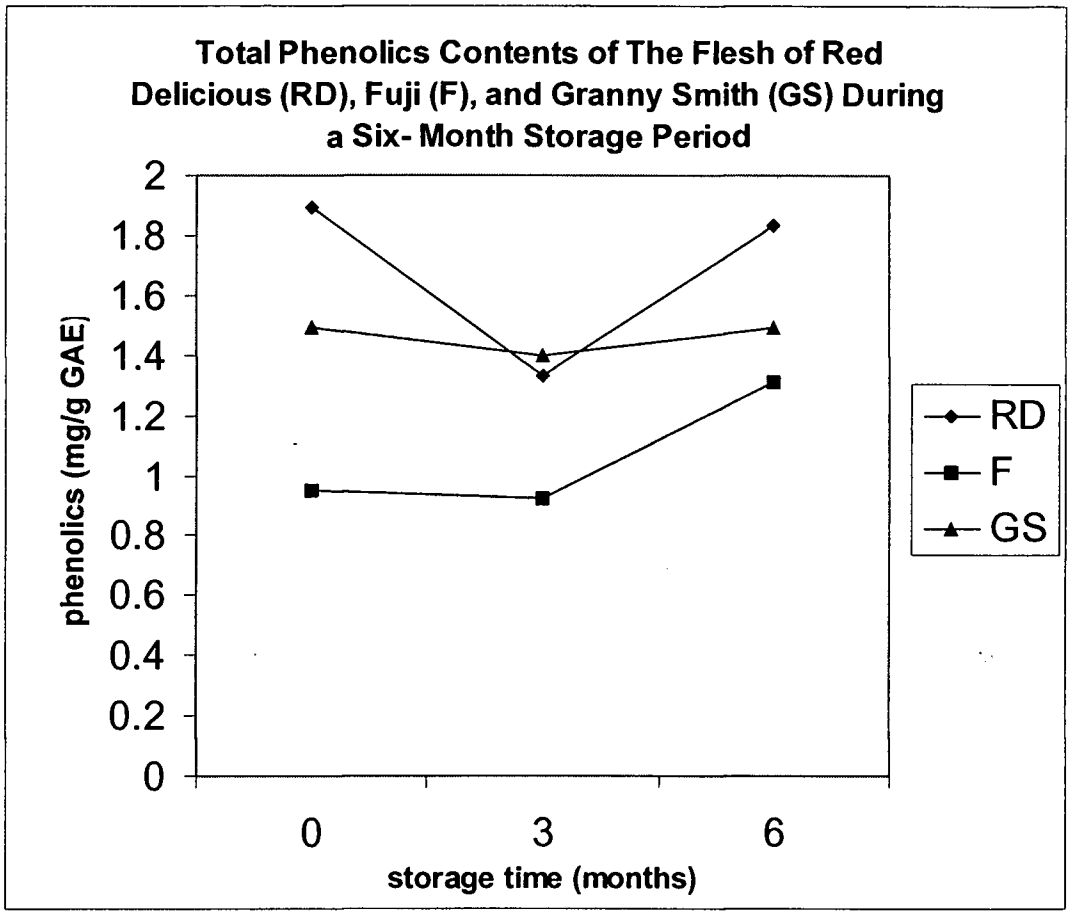

Figure 2.28 Total Phenolic Contents of The Flesh of Red Delicious (RD), Fuji (F), and Granny Smith (GS) During a Six- Month Storage Period

Table 2.11 Statistical findings of ORAC, FRAP, Total Phenolics, and Monomeric Anthocyanin Contents of Apples Flesh

Effect	ORAC	FRAP	Total Phenolics
Cultivar	Suggestive, but inconclusive	Moderate	Moderate
Storage	Not significant	Not significant	Not Significant

cold treatment followed by storage at ambient temperature had no effect on phenolic compounds of apple flesh.

The behavior trends of monomeric anthocyanin contents of the edible portion of Red Delicious and Fuji apples during the six- month storage period are shown in figures 2.29. The monomeric anthocyanin contents of Red Delicious and Fuji apples decreased from zero to three- month storage and then increased from the three to six- month storage period. There was suggestive but inconclusive evidence of a storage effect on the monomeric anthocyanin contents of the edible portion of the apples with p value of ANOVA= 0.0887. The mean of monomeric anthocyanin over cultivars at zero month storage was significantly different than that of the three months storage.

The monomeric anthocyanin contents of Red Delicious and Fuji apple peels during the six- months storage period are presented previously in table 2.7. Cold storage did not have an effect on the monomeric anthocyanin contents of apple peel extracts (p value of ANOVA= 0.8896).

The ORAC and FRAP values of the edible portion of Red Delicious, Fuji, and Granny Smith apples during the six- month storage period are listed previously in table 2.3. Storage did not have a significant influence on ORAC values of the edible portion of apples (p= 0.126). The FRAP values of Red Delicious and Fuji apples decreased from zero to three months storage and then increased. The FRAP

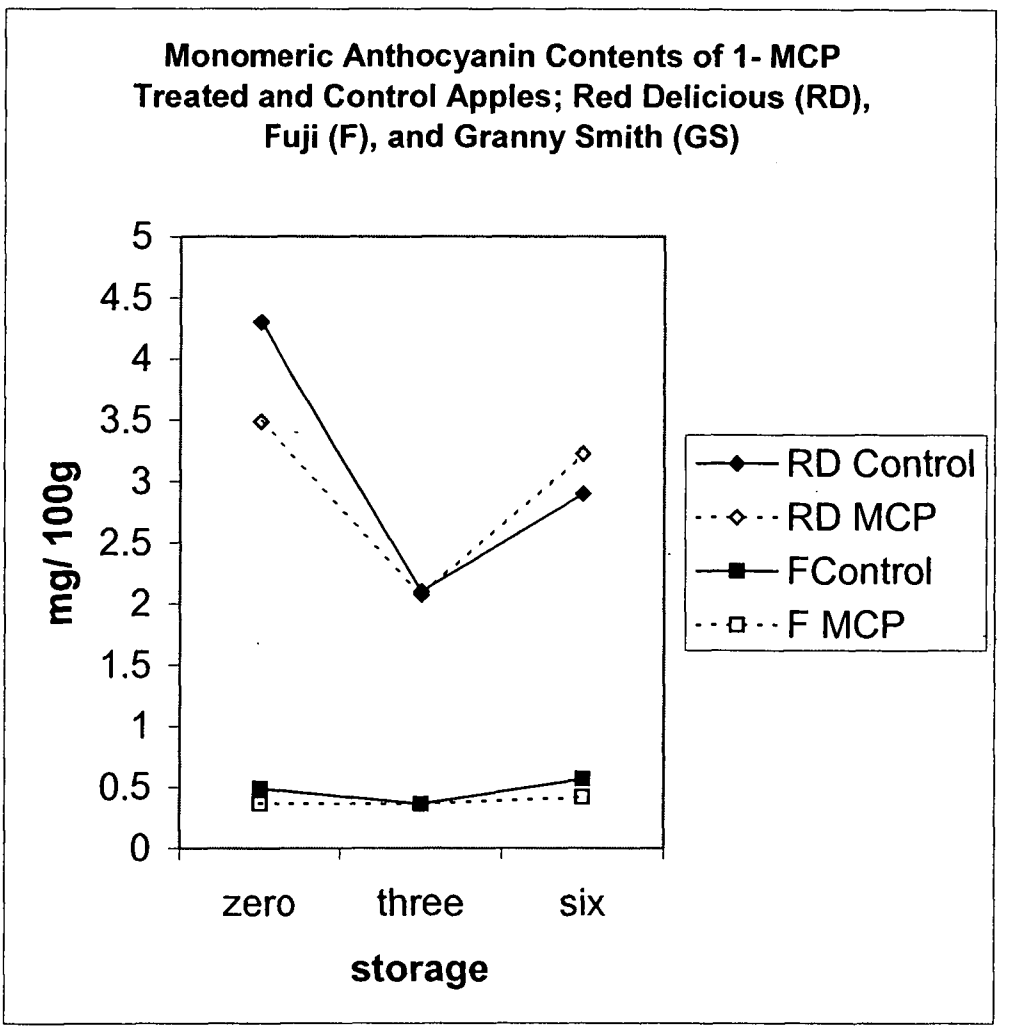

Figure 2.29 Monomeric Anthocyanin Contents of 1- MCP Treated and Control Apples; Red Delicious (RD), Fuji (F), and Granny Smith (GS)

values of Granny Smith apples decreased during the six- month storage period. Storage had a significant influence on the FRAP values of the three apple cultivars with p value of ANOVA= 0.00235. The mean of FRAP values over cultivars at zero month storage was significantly different than that of three month storage; at zero month storage was significantly different than that of six month storage and at three month storage was significantly different than that of six month storage.

The storage trends of the ORAC and FRAP values of the apple peel extracts are shown previously in figure 2.20 and 2.21. The ORAC values of Red Delicious peels decreased from zero to three months storage and then increased. The ORAC values of Fuji and Granny Smith apples decreased during the six- ınonths storage period. Similar to ORAC values, the FRAP values of Red Delicious peels decreased from zero to three months storage and then increased. The FRAP values of Fuji and Granny Smith apples decreased during the six- months storage period. There were moderate storage effects on the ORAC and FRAP values of the apple peel extracts with p value of ANOVA= 0.0283 and 0.045, respectively. The mean of ORAC values over cultivars at zero month storage was significantly different than that of three months storage; at zero month storage was significantly different than that of six month storage. The mean of FRAP values over cultivars at zero month storage was significantly different than that of three month storage; at zero

month storage was significantly different than that of six month storage.

The storage trends of ORAC and FRAP values of Red Delicious, Fuji, and Granny Smith apple flesh extracts are displayed previously in figure 2.22 and 2.23. Over the storage period, ORAC and FRAP values of Granny Smith apple flesh extracts were higher than those of Fuji apple flesh extracts. Storage at cold room (0 °C, 88%RH) did not have a significant influence on ORAC values (p value of ANOVA = 0.111) and FRAP values (p value of ANOVA= 0.74) of apple flesh extracts.

The Effect of 1-MCP Treatment on The Edible Portion of Apples

At the zero and six- months storage period, Red Delicious control apples had a higher total phenolics content than 1- MCP treated apples. At three- month storage, Red Delicious control apples had a lower total phenolics content than 1-MCP treated apples. Fuji control and 1- MCP treated apples had the same total phenolics content at zero storage time. At three- month storage, 1-MCP treated Fuji apples had a higher total phenolics content than control apples. However, at six-month storage, Fuji control apples had a higher total phenolics content than 1-MCP treated apples. During the six- month storage period, 1- MCP Granny Smith apples had a higher total phenolics content than the control apples. There was suggestive but inconclusive evidence of 1- MCP treatment on the total phenolics of the three

apple cultivars with p of ANOVA = 0.0937. At α= 0.05, no treatment effect was detected.

The behavior trends of monomeric contents of Red Delicious and Fuji apples during the six- month storage period are displayed previously in figure 2.29. There were no significant effects of 1- MCP treatment on monomeric anthocyanin contents (p value of ANOVA= 0.5195).

The ORAC contents of control and 1- MCP treated apples of the three apples cultivars are shown previously in figure 2.17. Treatment with 1-MCP had no significant influence on ORAC values of Red Delicious, Fuji, Granny Smith apples (p value of ANOVA= 0.375). The FRAP contents of control and 1- MCP treated apples of Red Delicious, Fuji, and Granny Smith are shown previously in figure 2.18. Treatment with 1-MCP had no significant influence on FRAP values of the three apples cultivars (p value of ANOVA =0.6849). The marginal means for the treatment effect are presented in table 2.12.

In 1-MCP treated apples, there was a positive relationship between ORAC and FRAP values with r^2= 0.81, Figure 2.30. The value of r^2 of the correlation between ORAC and FRAP of 1-MCP apples was lower than the r^2 values of the correlation between ORAC and FRAP of the control apples. ORAC correlated well with total phenolic contents in 1-MCP apples, with r^2=0.93, Figure 2.31.

Table 2.12 Marginal means of Treatment Effects of The Edible Portion of Apples

	Treatment	
	Control	1-MCP
ORAC	8.86	8.48
FRAP	8.94	9.13
Phenolics	1.86	1.96
Anthocyanin	1.79	1.66

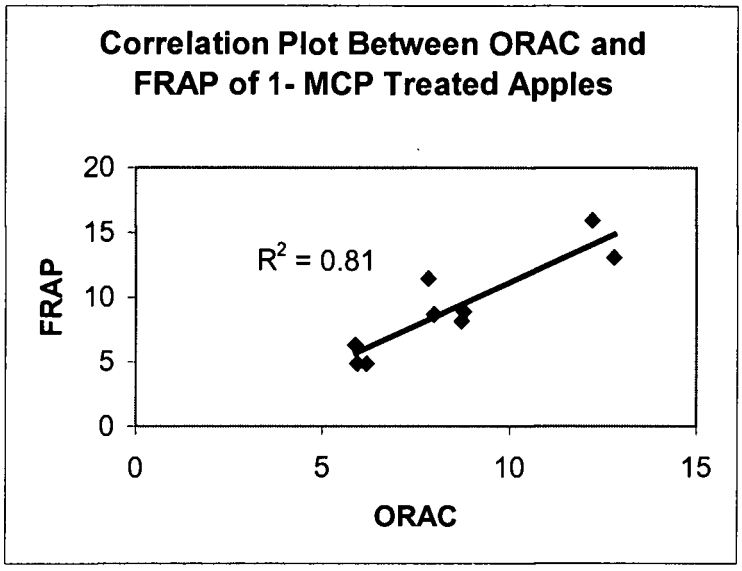

Figure 2.30 Correlation Plot Between ORAC and FRAP of 1- MCP Treated Apples

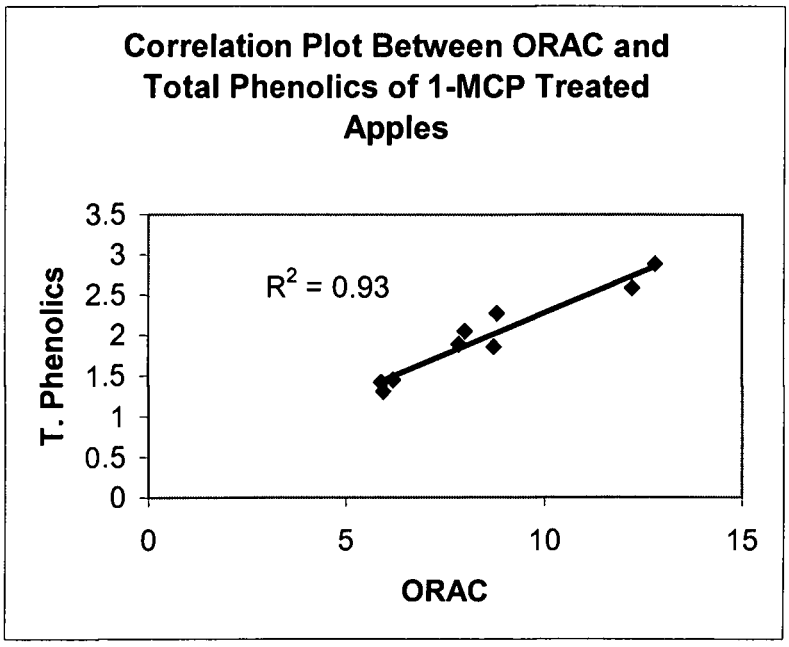

Figure 2.31 Correlation Plot Between ORAC and Total Phenolics of 1- MCP Treated Apples

There was a positive relationship between FRAP values and total phenolic contents, with r^2= 0.76, Figure 2.32. Monomeric anthocyanin contents of 1- MCP treated apples correlated well with ORAC (Figure 2.33), FRAP (Figure 2.34), and total phenolics (Figure 2.35), with r^2= 0.97,0.94, and 0.95, correspondingly.

Maclean and others (2001) studied the effect of 1- MCP treatment in three apple cultivars, namely Delicious, McIntosh, and Empire, on their antioxidant capacities using the TOSC assay. Their apples were treated with 600nL.L $^{-1}$ of 1-MCP for 18 h at 21° C. They extracted the apples with potassium phosphate buffer. In contrast to our studies, Maclean and others (2001) reported that antioxidant capacities of McIntosh and Delicious apples were significantly enhanced as a result of 1- MCP treatment, while Empire did not exhibit a significant 1- MCP treatment effect.

Dietary Implication

Apples compare favorably with other fruits in regards to their contribution to the average American's consumption of dietary antioxidants, particularly when considering per capita consumption. Our research indicated that apples, particularly the Red Delicious variety, could contribute an important amount of antioxidants to the American diet, considering the serving size of 1835.4 μmol T.E./serving (based on 138g/ serving size, USDA) and annual consumption of 114, 852.15 μmol T.E./year.

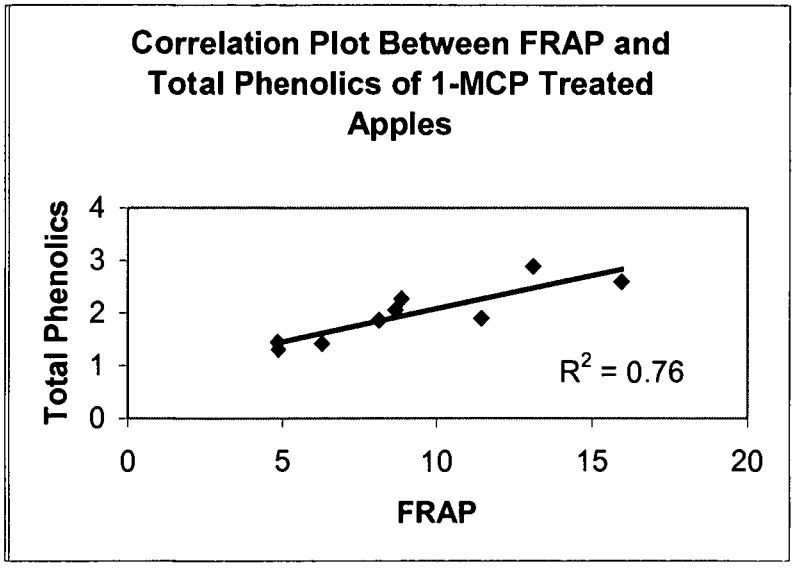

Figure 2.32 Correlation Plot Between FRAP and Total Phenolics of 1- MCP Treated Apples

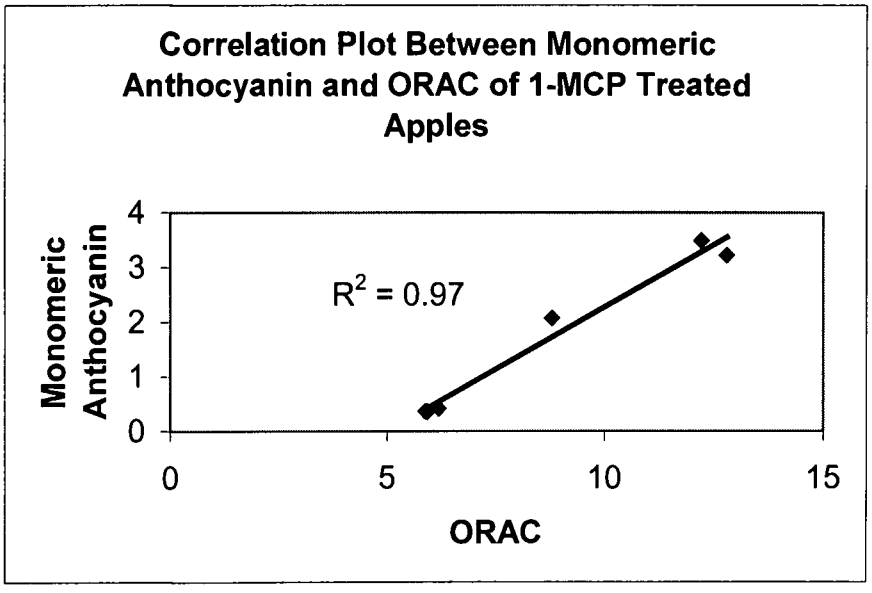

Figure 2.33 Correlation Plot Between Monomeric Anthocyanin and ORAC of 1-MCP Treated Apples

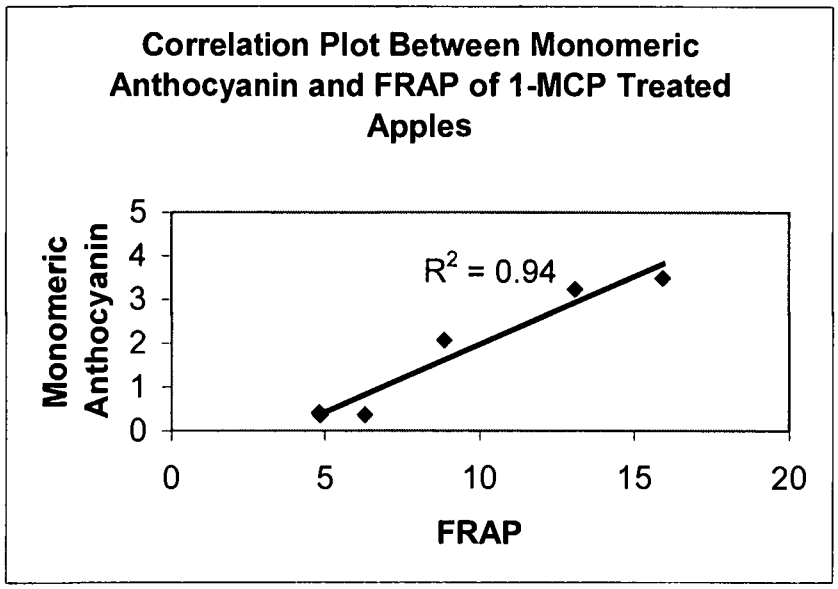

Figure 2.34 Correlation Plot Between Monomeric Anthocyanin and FRAP of 1-MCP Treated Apples

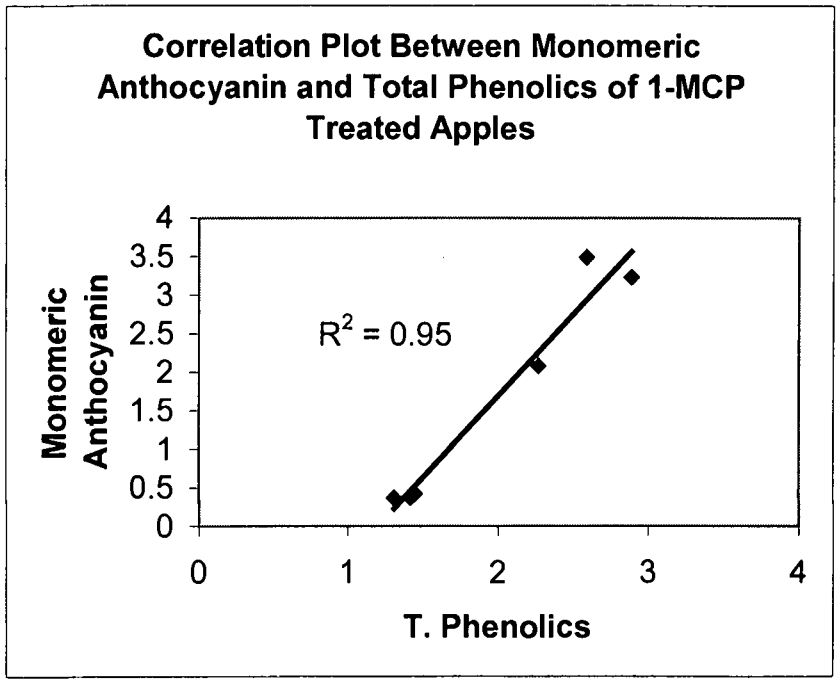

Figure 2.35 Correlation Plot Between Monomeric Anthocyanin and Total Phenolics of 1- MCP Treated Apples

Kalt and others (1999) reported that lowbush blueberry has a high antioxidant capacity, with a value of 64.4 μmoles/g. Lowbush blueberry has higher ORAC values than Red Delicious apples. However, when considering per capita consumption, apples provide a higher antioxidant capacity than blueberries. For instance, in 1998, the amount of US per capita consumption of apples was 8,635.5 grams, and for blueberries, it was 180 grams (Anonymous 2001a).Therefore, in 1998, apples supplied 114,852.15 μmoles of Trolox equivalents, and blueberries supplied 11,592 μmoles of Trolox equivalent of antioxidant capacity in an average American's diet.

CONCLUSION

Apple peel displays higher antioxidant activity than apple flesh. Peel phenolics vary among cultivars, however, they remain relatively stable during storage (0 °C, 88% RH). The total phenolic content was positively related to antioxidant capacity. Ascorbic acid contributed only minimally to the antioxidant capacity. The findings suggest that the combination of phytochemicals in apples is important to the antioxidant activity. These results also suggest that consumption of unpeeled apples provides additional health benefits.

1-MCP treatment did not have a significant influence on ORAC and FRAP values and monomeric anthocyanin content. There was suggestive but inconclusive

evidence that 1-MCP treatment influenced total phenolic contents of the three apples cultivars. This study would be of interest to the apple industry which could greatly benefit on a commercial scale from 1-MCP treatment of a variety of cultivars.

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