

EFFECTS OF 1-METHYLCYCLOPROPENE ON ANTIOXIDANT STATUS AND
EXTERNAL CARBON DIOXIDE INJURY OF 'EMPIRE' APPLES

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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January 2009

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Cornell University, 2009

Extensive studies have suggested that apple consumption is associated with prevention of chronic diseases such as cardiovascular disease, certain cancers, diabetes and asthma as apple is a rich source of phytochemicals with strong antioxidant and antiproliferative activity. 1-Methylcyclopropene (1-MCP), an ethylene inhibitor has been extensively used by the apple industry to maintain quality of various apple cultivars. In New York, use of 1-MCP has been especially high because it maintains firmness throughout the marketing chain. The use of 1-MCP has raised two issues that are addressed in this thesis. The first is the relatively small amount of information about the effects of 1-MCP on nutritional quality of apple fruit. The second is that 1-MCP appears to increase susceptibility of certain cultivars to physiological injuries, and in 'Empire', external CO₂ injury.

The effect of 1-MCP treatment on the phytochemical concentrations and activity and ascorbic acid concentrations of 'Empire' apple as affected by air and controlled atmosphere (CA) storage was investigated. Fruit were stored in air for up to 5 months, and in CA in 2 and 3 kPa O₂ (2% CO₂) at 0.5 and 2.2 °C for 4.5 and 9 months. Ripening was delayed by 1-MCP treatment in both air and CA storage as indicated by internal ethylene concentrations and flesh firmness. Overall, total phenolic, flavonoid and anthocyanin concentrations as well as antioxidant activity are relatively stable during air and CA storage. In CA, interactions among oxygen level, temperature and storage duration were detected but no consistent trends were observed. The only effects of 1-MCP on flavonoid or anthocyanin concentrations of

fruit stored in CA were found in the flesh. Flavonoid concentrations were higher in the flesh of 1-MCP treated than untreated fruit kept in 2 kPa O₂ while anthocyanin concentrations only measured in the peel were not affected. Phenolic concentrations were higher in the peel while lower in the flesh of 1-MCP treated fruit compared to the control fruit stored in air. There were no correlations found between total phenolics and antioxidant activity. Ascorbic acid concentrations declined in both peel and flesh tissues of untreated and 1-MCP treated fruit stored in air. In CA, the change was affected by several storage parameters and there were inconsistent pattern in the decline of ascorbic acid concentrations in CA-stored fruit.

The effects of CO₂ partial pressure, the timing of elevated CO₂ exposure, delays between harvest and exposure to elevated CO₂, DPA concentration, and the timing of DPA treatment after exposure of fruit to 1-MCP on the susceptibility of untreated and 1-MCP-treated 'Empire' apple fruit to external CO₂ injury have also been investigated. 1-MCP-treated fruit were more susceptible to external CO₂ injury than untreated fruit when stored in 5 kPa, but not 1 kPa CO₂ (in 2 kPa O₂). 1-MCP did not increase the period of highest susceptibility to injury during CA storage. The greatest sensitivity to injury occurred 0–3 weeks after harvest. Sensitivity to injury decreased when untreated fruit were kept in air for up to 14 days before exposure to 5 kPa CO₂, but not for 1-MCP-treated fruit. DPA treatment prevented development of CO₂ injury even at a level as low as 250 µL L⁻¹. DPA treatment could be delayed for 4 days after 1-MCP treatment while fruit were exposed to 5 kPa CO₂ in air without injury development.

BIOGRAPHICAL SKETCH

Fanjaniaina Razafimbelo was born on February 3rd, 1974 in Antananarivo, Madagascar. She received her engineering diploma from the “Ecole Supérieure des Sciences Agronomiques” the School of Agronomy in Antananarivo, Madagascar, where she specialized in Food Science and Technology. She won a national academic award and was granted a Fulbright scholarship to pursue her US training.

In 2001, Fanja came to Cornell University to study Agricultural Engineering, but with the insight and guidance of her advisor James Bartsch, decided to pursue a Ph.D. degree in Horticulture which better fit her practical interest. She joined Chris Watkins’s research group in 2003 to study postharvest physiology and technology of preservation of horticultural crops, as well as the health benefits of phytochemicals in fruits and vegetables in chronic disease prevention.

Fanja participated in diverse organizations such as the ISPB at Cornell University to promote cultural diversity on campus. She was an active member of the Bahá’í Club at Cornell and contributed in organizing devotional gatherings and interfaith events. She was the coordinator of social events for the SOHO graduate student association in the Horticulture department in 2004.

She participated in the Annual Swimming across Cayuga Lake, a fundraising event for hospice care of Ithaca. She served the Bahá’í community of the Finger Lakes area in coordinating moral education and empowerment for children and junior youth through study circles and service projects. She participated in activities to raise awareness of racial prejudice and unity in diversity within the Ithaca community.

She will return to Madagascar to fulfill her obligation as a Fulbright grantee, and will work in agribusiness to promote rural development in Vakinankaratra, the central area of Madagascar where fruit and vegetable production is a promising field.

To my cherished parents and parents-in-law,
To my beloved husband,
To the Gicheru family,
To Roberta Harold and Wayne Fawbush,
To the Gotterts, the Dahls, the McHughs, the Meads and the Brinn/Beers
To the Bahá'í Club at Cornell and the Bahá'í Community of Ithaca
To my family and many friends
For their vision and guidance, support, encouragement and patience, and
For their love, prayers and presence in times of tests and in moments of happiness

*“ ... The harder they strive to widen the scope of their knowledge,
the better and more gratifying will be the result.
Let the loved ones of God, whether young or old, whether male or female,
each according to his capabilities, bestir themselves and spare no efforts
to acquire the various current branches of knowledge,
both spiritual and secular and of the arts.”*

‘Abdu’l-Bahá

ACKNOWLEDGMENTS

I would like to thank my special committee chair, Dr. Christopher B. Watkins, for his instructions and advice, guidance, unfailing support, encouragement and trust in my personal potential which propelled my personal growth in the past four years. Your enthusiasm and effectiveness at work have been a great example for me. I could not ask for a better advisor.

My profound gratitude also goes to Dr. James A. Bartsch for your invaluable advice on the direction of my graduate degree at Cornell, and for devoting your time and energy to provide me a special training in refrigeration and CA systems which will constitute a great tool for my immediate career.

My sincere appreciation also goes to Dr. Rui Hai Liu, for conveying in an effective manner the health benefits of fruits, vegetables and whole food consumption. I appreciate how this concept has impacted my food choice and daily habits. I also would like to show appreciation for Dr Liu's lab especially Dr. Kafui K. Adom for the assistance he provided in using the technique of antioxidant assay he developed.

I would like to show my sincere appreciation to Dr. Lailiang Cheng for his assistance and for welcoming me to use his lab with the help of his group to perform experiments related to antioxidant enzymes and physiological injury of apple.

I would like to give special thanks and appreciation to Jackie Nock for her excellent technical support. I could not imagine carrying out so many experiments without your help. You have been a role model for me in both being professional at work and in caring for your family.

I also want to thank my labmates, Monica Ponce Valadez, Beatriz Calderon, Irene Kadzere, Adirek Rugkong, Rujira Deewatanawong, Youngjae Shin, Jin Wook

Lee. I learned a lot from each one of you and I appreciate your sincere friendship. I feel blessed to have had the opportunity to work with such a brilliant and diverse group.

My sincere gratitude also goes to the IIE – Fulbright Program, the Smock Foundation and the Department of Horticulture for their financial support which allowed me to fulfill my academic training and research projects.

And finally, I would like to thank my husband Shahbaz Vikram Lee Fawbush, for joining me in Ithaca and for being a supportive companion in my academic life, for postponing his career to move to Madagascar so that I can fulfill my obligation towards my country as a Fulbright graduate. I feel blessed and I am deeply grateful.

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CHAPTER ONE

INTRODUCTION

1.1. The apple: background

Apples, (*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.) are members of the genus *Malus* Miller, under the Maloideae subfamily and the family *Rosaceae* (1990). It is believed that apple originated in central Asia (Hampson and Kemp, 2003). From the beginning of the 16th centuries, apples were first introduced to the Americas in the eastern USA and Canada, which became the two major apple-producing nations by the early 20th century. Today the states of New York, Michigan and Ohio still constitute the major apple production areas in the Great Lakes region of the USA (O'Rourke, 2003).

1.1.1. Apple morphology and development

The apple fruit develops from its flower, the edible part being from the accessory part of the flower. Typically, the flower has five sepals, petals, stamens and carpels at the center containing the ovary that has two or more ovules. The seeds of an apple fruit are formed after pollination and fertilization of ovules by the sperm of germinated pollen. Seed production is necessary to stimulate the production of the edible part, the fruit setting and contributes to the symmetry in the shape of the fruit (Dennis, 2003). The core, which conventionally is the inedible part of the apple, is formed from the ovary walls, the inner part of the carpel when the fruit matures. The

edible part is formed from the outer layer of cells. The tissue of the fusion of calices, corolla and stamens develops to form the major edible part of the mature fruit.

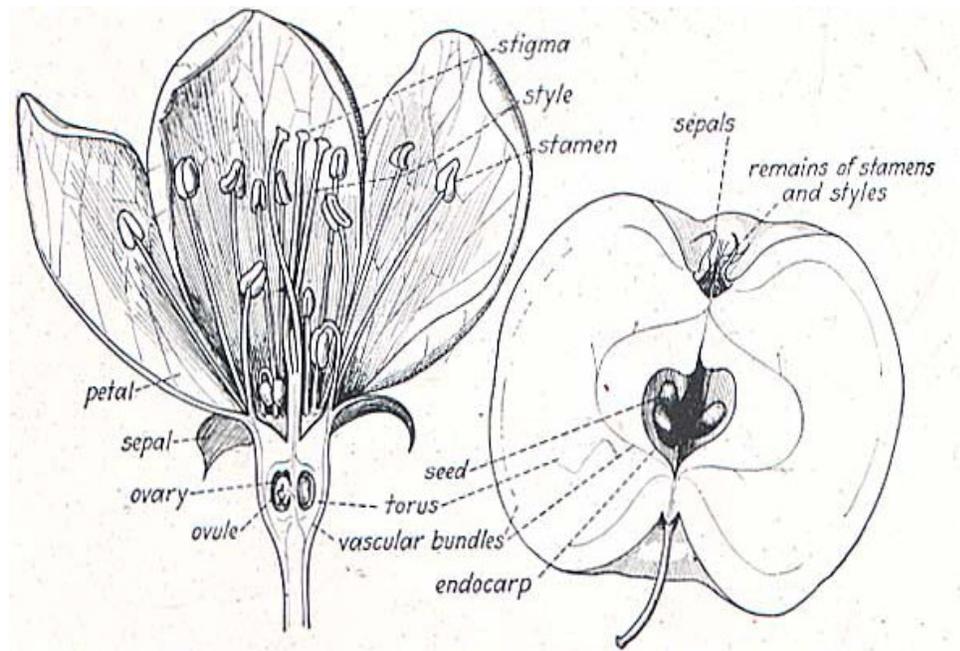


Figure 1.1 Anatomy of apple flower and fruit

1.1.2. Apple ripening and harvest indices

Apple is a climacteric fruit, characterized by a rise in respiration rates during fruit ripening which translates in increased internal concentrations of carbon dioxide and ethylene production (Lelievre et al., 1997; Saltveit, 1999; Watkins, 2002; Watkins, 2003).

Physiological and biochemical changes thus occur during the ripening of apple due to the autocatalytic production of ethylene. From a commercial perspective, these important changes are conducive to the development of quality attributes desired by consumers. In general, these changes include softening, chlorophyll loss with subsequent yellowing of the green part of the apple skin, development of the red color on the peel in some apple cultivars, loss of acidity, starch conversion to sugars and decrease in the bitterness, and the development of flavors through synthesis of aromatic compounds by the apple (Knee et al., 1983). Consequently, postharvest handling and storage technologies are necessary to control and minimize the rates of those changes in order to provide apple of acceptable quality to the consumer yearlong. The timing of harvest is thus a management decision, the dilemma is that when ripening advances the fruit reaches optimum quality, but its storability decreases (Watkins, 2003). Moreover, consumer's preferences in terms of quality attributes vary greatly from one cultivar to another and from one market to another. In Northeast region of the US for example, certain cultivars such as 'McIntosh' require a further aromatic and softening development to meet the consumer's expectations of acceptable quality for that particular cultivar. For 'Empire' apple on the other hand, a certain level of acidity, firm texture and crispiness are some of the most expected attributes.

Many harvest indices are used by the apple industry to determine the appropriate period of harvest depending on several factors including cultivar, production region and the end use of the product. In general, the most important and practical in terms of quality indicators are:

- Internal ethylene concentrations (IEC) (Dilley, 1980; Lau, 1985), which is easily measured using gas chromatography;

- Starch test, which allows estimating the advancement of ripening through starch hydrolysis to sugars by staining starch on sliced apples with iodine solution. This procedure was made easy with the availability of generic or cultivar specific starch charts (Reid et al., 1982; Lau, 1985);
- Flesh firmness, which constitutes an important quality index and indicates internal quality of the apple (Liu and King, 1978). This is not a reliable harvest index as it varies depending on many preharvest factors (Blanpied et al., 1978; Reid et al., 1982);
- Soluble-solids concentration, which increases as fruit mature and ripen generally in relation to starch-to-sugar conversion (Brookfield et al., 1997) and is generally interpreted in association with other indices such as flesh firmness or acidity;
- Titratable acidity (TA), which is relative to cultivars and season (Lau, 1985), but generally decreases as fruit mature and ripen;
- Background color or ground color, assessed using color chart or chromameters. This index is more important for bicolored cultivars such as ‘Gala’, ‘Braeburn’ and ‘Fuji’, than others (Watkins et al., 1993; Plotto et al., 1995);
- Calendar date, days from full bloom and temperature records. This index varies depending on cultivars, temperatures and growing regions, but can be useful for particular cultivars in regions with consistent growing conditions (Blanpied and Silsby, 1992; Beaudry et al., 1993).

1.2. Apple antioxidants and health benefits

The apple is one of the most consumed fruits in the US and worldwide (Wolfe et al., 2003), and is available throughout the year as a result of increased apple production, extensive range of cultivars, progress in postharvest handling and storage technologies, and international trade (O'Rourke, 2003). Apples have numerous nutraceutical benefits and as epidemiological studies indicated apple consumption is

associated with reduced risk of several chronic diseases (Lee and Smith, 2000; Wolfe et al., 2003; Boyer and Liu, 2004). Laboratory studies have shown that apple extracts have potent antioxidants, inhibit cancer cell proliferation, decrease lipid oxidation and lower cholesterol among other their many health benefits (Boyer and Liu, 2004).

Although the health benefits of apples were long believed to be due to their concentrations of vitamins C and E, and β -carotenes, phenolics constitute the major class of phytochemicals which highly contribute to the antioxidant capacity of apples (Rice-Evans et al., 1996; Bravo, 1998; Guo et al., 2003). Figure 1.2 illustrates the classification of dietary phytochemicals, and lists the diverse classes and subclasses of phenolics.

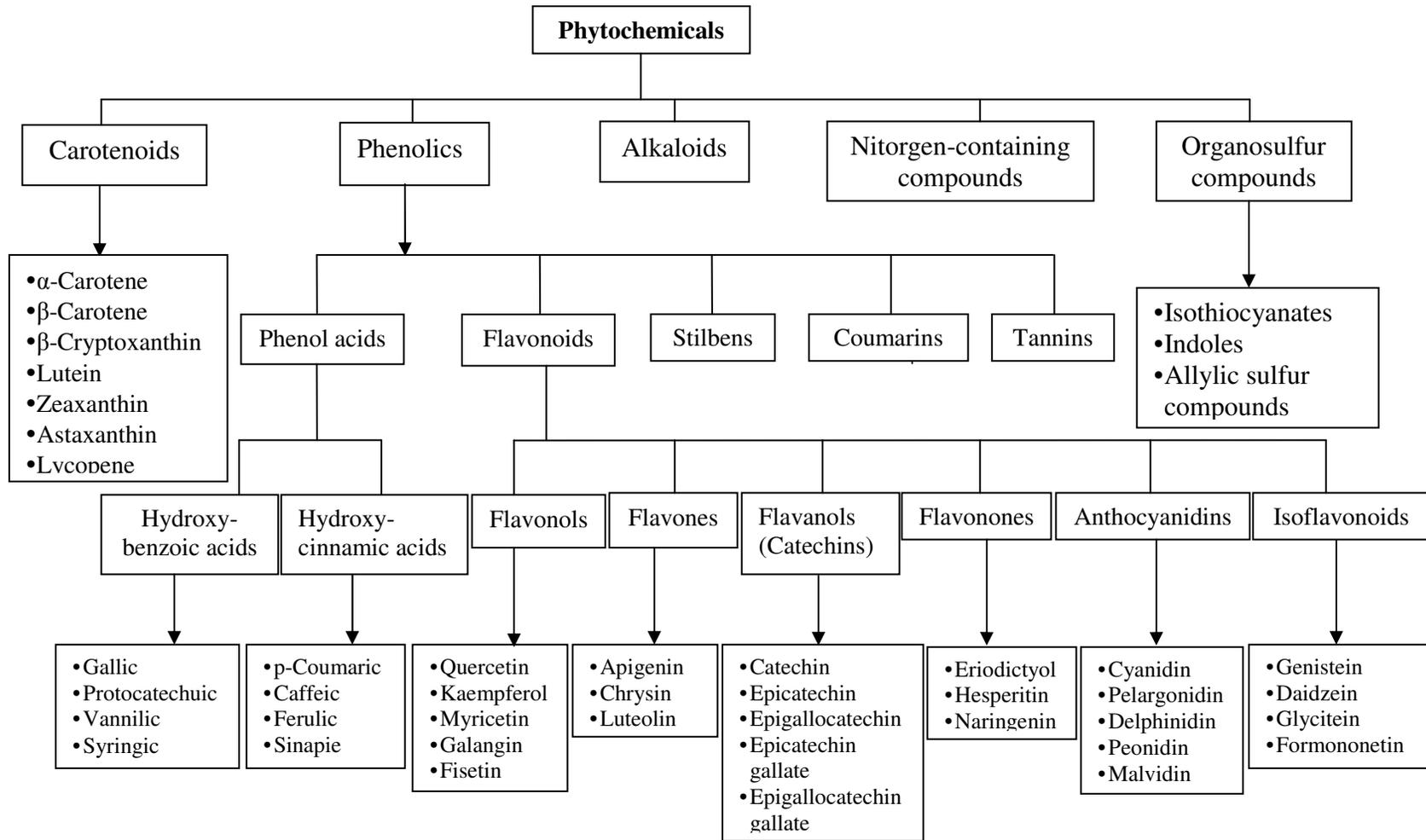


Figure 1.2 Classification of phytochemicals (adapted from Liu, 2004)

1.2.1. Major phytochemicals in apple: phenolics

Apple is the largest source of phenolics in the American diet providing 22% of phenolics from fruit (Vinson et al., 2001). Extractable phenolics assessed in several different varieties of apple were assessed and ranged from 110 to 357 mg/100 g of apple fresh weight (Podsdek et al., 2000; Liu et al., 2001), and that total phenolic concentrations can be twice to six times higher in the peel than in the flesh (Boyer and Liu, 2004). Boyer and Liu (2004) and Sun et al. (2002) suggested that apples have a high proportion of free phenolics that are more readily available for absorption in the bloodstream. Apple phenolics are found to prevent arteriosclerosis development by inhibiting oxidation of low-density lipoprotein (LDL), very low-density lipoproteins (VLDL) and DNA bases by free radicals (Peterson and Dwyer, 1998; Kaur and Kapoor, 2001). Apple phenolics also inhibit the proliferation of human tumor cells in vitro, and reduce the risk of coronary heart disease, cataracts, brain and immune dysfunction, and stroke (Rice-Evans et al., 1997; Vinson et al., 2001; Guo et al., 2003).

The major classes of phenolics in apple comprise phenol acids (hydroxibenzoic acids, hydroxycinnamic acids); flavonoids, stilbens, coumarins and tannins. Phenolics possess ideal chemical structure as antioxidant scavenging free radicals and as metal chelators. The structure of phenolic compounds is constituted of a benzene ring substituted with one or more hydroxyl groups as substitutes, phenolic rings can thus act as hydrogen donor to form hydrogen bonds with specific groups. Phenolics have received particular importance because they have more powerful antioxidant activity compared with that of vitamins C and E (Rice-Evans et al., 1997; Vinson et al., 1998; Wang and Goodman, 1999; Prior and Cao, 2000).

Phenolics are derived from phenylalanine via the shikimate and phenylpropanoid pathways. The first step in phenolic biosynthesis involves the production of trans-cinnamate from phenylalanine (PHE) by phenylalanine ammonia-lyase (PAL). In 'Red Delicious' and 'Golden Delicious' apples increase in PAL activity coincides with ripening (Blankenship and Unrath, 1988). Phenolic compounds also contribute to sensory qualities such as color, astringency, bitterness, and aroma in ripe fruits.

1.2.2. Flavonoids

Flavonoids, a subclass of phenolics are the major secondary metabolites found in fruits and vegetables. Flavonoids in the diet are associated with reduced risk of chronic diseases such as cardiovascular disease and cancer (Hertog and Feskens, 1993; Hertog et al., 1997).

The classes of flavonoids comprise flavonols (quercetin glycosides), flavones, flavanols (catechins), flavanones, anthocyanidins and isoflavonones (Aherne and O'Brien, 2002; Heim et al., 2002; Liu, 2004) (Figure 1.2). The flavonoid structure is made of 2 aromatic rings (A and B) linked by an oxygenated heterocycle ring (ring C) (Figure 1.3). Flavonoids are potent antioxidants because of their chemical structure and can interfere with carcinogenesis in different ways (Bors and Saran, 1987; Wang et al., 1996; Djuric et al., 1998; Hollman, 2001).

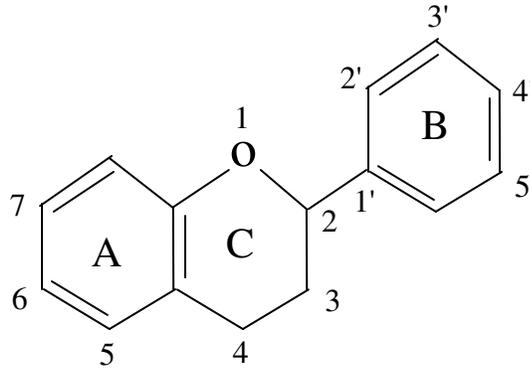


Figure 1.3 Generic structure of Flavonoids

1.2.3. Anthocyanins

Anthocyanins are water-soluble flavonoid pigments found in vacuoles responsible for the color red to blue color of fruits and flowers (Mazza et al., 2002; Garcia-Alonso et al., 2004). Figure 1.4 shows the chemical structure of anthocyanin. Anthocyanins are anthocyanidins with a sugar group, mostly 3-glucosides of a given anthocyanidin. The anthocyanidin could be cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin depending on the type of substitutes R1 to R7 (Figure 1.5), each substitute could be either a hydrogen or hydroxyl or acetyl group. Anthocyanin structure changes in response to the pH and the color this pigment exhibits and varies accordingly from red in acids to blue in bases and colorless at pH 7.0 (Wrolstad, 2001). Anthocyanins are present in red apple peel and its concentrations are mainly related to the presence of cyanidin 3-galactoside (Awad and de Jager, 2000). As a result apple with deep red color such as ‘Idared’ has higher anthocyanin concentrations compared to bicolor peel apple with green patches or pink peel such as ‘Cortland’ or ‘Rome Beauty’ apple, and anthocyanin concentrations in apple such as ‘Golden Delicious’ are insignificant (Wolfe et al., 2003).

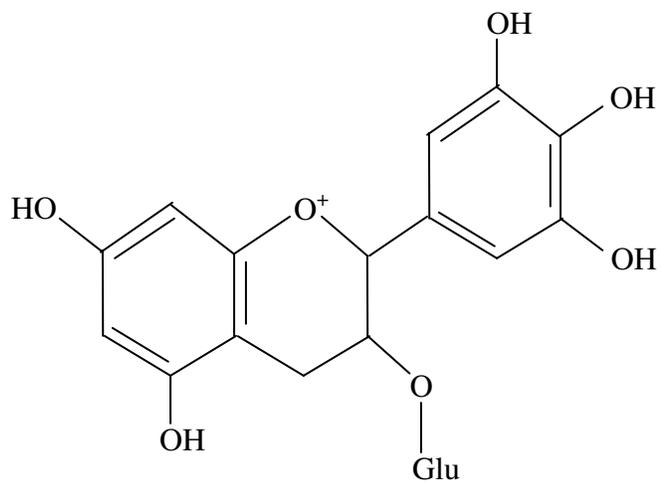


Figure 1.4 Generic structure of Anthocyanins

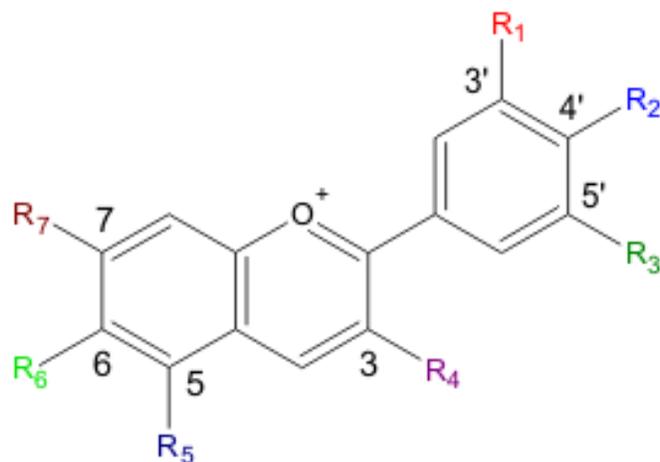


Figure 1.5 Generic structure of Anthocyanidins and derivatives

| Anthocyanidin | Substitutions | | | | | | |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Derivatives | R₁ | R₂ | R₃ | R₄ | R₅ | R₆ | R₇ |
| cyanidin | -OH | -OH | -H | -OH | -OH | -H | -OH |
| delphinidin | -OH | -OH | -OH | -OH | -OH | -H | -OH |
| pelargonidin | -H | -OH | -H | -OH | -H | -H | -OH |
| peonidin | -OCH ₃ | -OH | -H | -OH | -OH | -H | -OH |
| malvidin | -OCH ₃ | -OH | -OCH ₃ | -OH | -OH | -H | -OH |

Anthocyanins also possess antioxidant ability as free radical scavengers (Moller et al., 2004) although its role as antioxidant is less studied. In the recent years however more laboratory studies have been conducted to unravel the biological activities and health benefits of anthocyanins (Kong et al., 2003).

1.2.4. Ascorbic acids (AA)

Although ascorbic acids contribute little to the antioxidant capacity in apple, they still constitute an important vitamin for the human nutrition. Moreover, AA plays an important role in the detoxification mechanism of the plant tissues from accumulation of reactive oxygen species (ROS). Ascorbic acid is used as a substrate in the ascorbate-glutathione cycle which converts ROS such as superoxide into hydrogen peroxide which then needs to be further processed using AA in this detoxification process (Noctor and Foyer, 1998).

AA in plant tissues are mainly reduced ascorbic acids (L-AA), however it is important to consider DHA as it is the first product of L-AA oxidation and could be converted back to L-AA in the human organism (Lee and Kader, 2000; Smirnoff and Wheeler, 2000).

1.2.5. Total antioxidant capacity

In all living organisms, reactive oxygen species (ROS) are formed as a result of aerobic respiration and other mechanisms. Dietary antioxidant can sacrificially scavenge reactive oxygen/nitrogen species (ROS and RNS) thus formed to stop radical chain reactions, or it can inhibit the reactive oxidants from being formed in the first place. In general, dietary antioxidants include radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors, and antioxidant enzyme cofactors.

Traditional antioxidant nutrients such as vitamin C were found to be responsible for only 0.4 % or less of the antioxidant capacity. The rest of the antioxidant capacity is thus attributed to antioxidants such as phenolic compounds (Eberhardt et al., 2000).

As antioxidants have become a subject of increasing interest, many assays have been developed to quantify antioxidant capacity in plants, plant foods and foods, some of the most popular protocols include oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP) (Huang et al., 2005) and more methods convenient and accurate are being developed. The common principle of these antioxidant capacity assays is related to quantifying the concentrations of antioxidants necessary to achieve 50 % inhibition of free radicals under a defined oxidation conditions (Huang et al., 2005).

1.3. Postharvest handling and storage of apple

The main objective of postharvest handling is to maintain quality attributes in order to provide an acceptable product to the consumers at a later period. Those quality attributes pertain to appearance (size, shape, color, freedom of blemishes), and are specific to a given cultivar, internal quality attributes include flavor and texture, which are also specific to cultivar and nowadays there is increased interest as of the preservation of healthful compounds in the apple.

The primary action to reduce the overall metabolism of the apple is refrigeration. For a mid to long term storage of apple controlled atmosphere (CA) is also applied vs. air storage, to further slow down and delay ripening associated process. The principles of CA are to lower O₂ and increase CO₂ to levels that will

decrease fruit respiration and ethylene production and action. Low O₂ concentrations reduce O₂ uptake and CO₂ production proportionally, and therefore the respiratory quotient remains constant. High CO₂ concentrations also reduce CO₂ production more than O₂ uptake, and therefore the fruit respiration declines (Lange and Kader, 1997). Color and flavor losses, as well as physiological and pathological disorders are usually reduced by both temperature and atmosphere low in O₂ and high in CO₂, although both of these factors can exacerbate certain disorders.

However, severe stresses induced by low O₂ and/or high CO₂ outside safe limits at any time-temperature combination can affect metabolic pathways such as glycolysis, fermentation, tricarboxylic acid cycle and mitochondrial respiratory chain, as well as secondary metabolism consequently causing accumulation of acetaldehyde, ethanol, ethyl acetate, as well as succinate in fruit treated with high level of CO₂ (Fernandez-Trujillo et al., 1999). The biochemical changes are often manifested as different types of browning both external and/or internal.

Generally in most fruit and in apple in particular, the factors affecting tolerance to low O₂ and high CO₂ concentrations are:

- Resistance of the fruit skin to gas exchange between the atmospheres and the inside of the fruit.
- Metabolic sensitivity of the fruit to the gas depends on cultivar, and within cultivar factors such as harvest date and growing region (Bramlage et al., 1977; Park et al., 1993; Burmeister and Dilley, 1995).

Apart from its effect in lowering respiration, CA storage can also affect ethylene biosynthesis and action (Watkins, 2002). In the ethylene biosynthesis, methionine (MET) is the starting point. The amino acid is converted into S-adenosyl methionine (SAM) by the addition of adenine. SAM is then converted into 1-amino-cyclopropane carboxylic acid (ACC) by the enzyme ACC synthase. Ethylene

synthesis is controlled by the production of ACC. ACC is oxidized by ACC oxidase to form C₂H₄. This reaction requires the presence of O₂ and ACC oxidase requires a low level of CO₂ for its activation.

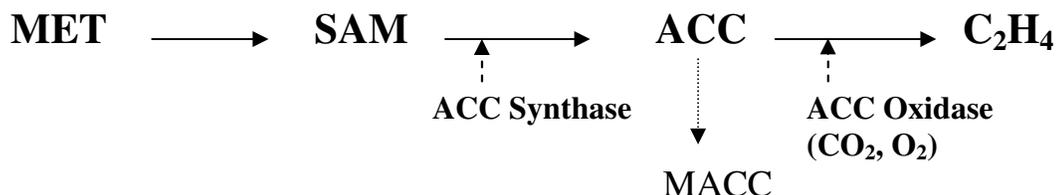


Figure 1.6 Biosynthesis of ethylene in higher vascular plants (Saltveit, 1999)

CA can thus reduce ethylene production through several mechanisms: low O₂ may have a direct effect on ACC oxidase, which plays a critical role in ethylene biosynthesis, requires oxygen for activity (Adams and Yang, 1979; John, 1997). As a result, the low level of O₂ in CA atmosphere (usually 2 to 3% in the case of apple CA storage) can affect the ethylene pathway. It might be possible as well that low O₂ levels in the atmosphere negatively affect ethylene binding. Moreover, increased CO₂ in CA can affect the pH of the cytoplasm which may in turn affect activity of ACC oxidase (Bown, 1985). It has also been proposed that CO₂ may displace ethylene from its receptor sites but may not have any effect on the ethylene perception (de Wild et al., 1999).

1.3.1. Ethylene in postharvest

Ethylene (C₂H₄) is an endogenously produced gas, a plant growth regulator and affects growth, development, ripening and storage potential of fruits, vegetables, and ornamental crops (Saltveit, 1999). Exogenous ethylene can produce the same

effects on exposed crops. Its effects can occur at concentrations of part-per-million to part-per-billion.

Postharvest quality of fruit can be greatly affected by ethylene. Among the beneficial effects are that ethylene promotes color development in fruit, stimulates ripening of climacteric fruit and development of volatiles that usually contributes to an optimal flavor production by the fruit as it ripens. However, undesirable effects of ethylene in postharvest preservation include acceleration of ripening and senescence and enhancement of excessive softening of fruit. As a result, removal or inhibition of ethylene will usually help delay color change, softening, and will increase shelf life of fruit. Non chemical methods used to control and minimize ethylene build up in a postharvest storage environment include use of proper ventilation, temperature control, and use of ethylene scrubbers (Watkins, 2002). However, several technologies have been developed for pre- and post-harvest use to chemically affect ethylene biosynthesis. The first is aminoethoxyvinyl glycine (AVG), an inhibitor of ethylene production, registered for use on apple fruits in preharvest conditions and marketed as ReTain™ (Phan-Thien et al., 2004). AVG acts on the ethylene biosynthesis pathway by inhibiting ACC synthase (Boller et al., 1979) thus inhibits ethylene production (Figure 1.7). The manifestations of AVG effect are delayed and reduced preharvest drop (Bramlage et al., 1980; Williams, 1980; Stover et al., 2003). The second is ethephon (2-chloroethylphosphonic acid), which has been used in order to promote anthocyanin development in apple (Faragher and Brohier, 1984). Thus harvest can occur prior to substantial preharvest drop in environments in which color development is delayed (Stover et al., 2003).

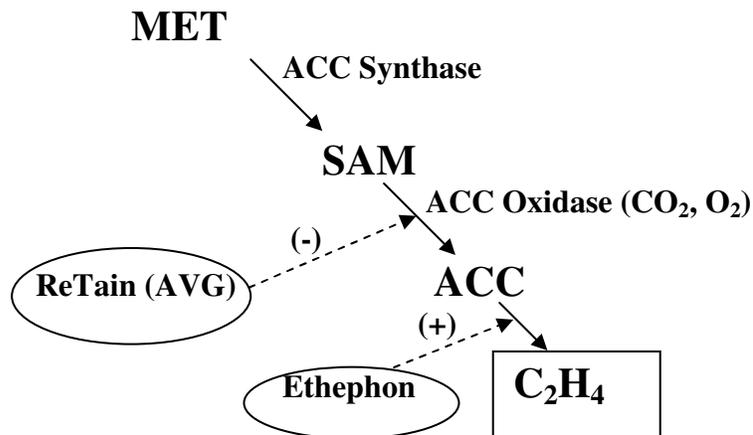


Figure 1.7 Sites of action of ReTainTM and Ethephon on the ethylene biosynthesis pathway

In the recent years, consistent efforts have been directed towards finding compounds that would inhibit or reduce the production of ethylene in postharvest storage conditions (Sisler and Serek, 2003). Several compounds such as 2,5-norbornadiene, a thiosulfate form of silver, diazocyclopentadiene and series of cyclopropenes such as 3,3-dimethylcyclopropene (3,3-DMCP) and 1-alkaline substitute cyclopropene block the ethylene receptors of the cell membrane and prevent ethylene action. However, due to their toxicity, the difficulty of their application and the difficulty in their manufacturing, they could not be commercially used on fruits and vegetables (Sisler and Serek, 1997). However, this effort contributed to the discovery and commercialization of 1-methylcyclopropene (1-MCP), an effective inhibitor of ethylene production which has developed a large momentum in the domain of its application in postharvest preservation of climacteric crops and in the area of fundamental and applied research on ethylene biosynthesis, and ethylene involvement in ripening and senescence processes in plant materials. The responses of

both whole and fresh cut fruits and vegetables as well as ornamental products to 1-MCP has been widely studied in recent years with over 40 papers published in 2004 and 73 papers in 2003, compared with 36 in 2002 (Watkins and Miller, 2006).

The apple was the first to have received registration for 1-MCP application, and the apple industry has rapidly adopted this new technology because of the important benefits 1-MCP provides, even in large scale storage systems: 1-MCP treated apple maintain its firmness and texture after a relatively long storage period in CA and 1-MCP is effective in controlling several physiological disorders among them superficial scald.

Further background on 1-MCP structure and mode of action and its role in postharvest preservation of apple will be reviewed in section 1.4. This fairly new technology has provided opportunities, as well as challenges and limitations in its application that need to be further elucidated. In the frame of this effort my research will be mainly centered on the effects of 1-MCP on the preservation of the antioxidant status of apple, and its effect on the occurrence of CO₂ injury, a physiological disorder in apple.

1.3.2. Changes of phenolic concentrations in apple as affected by storage conditions, time, and postharvest treatment

Limited studies have been conducted that examined the impact of storage conditions on phenolic content and its antioxidant capacity in apple fruit.

Awad and de Jager (2000) measured the levels of major phenolics such as chlorogenic acid and flavonoids in the peel of 'Jonagold' and 'Elstar' apple. They showed that these compounds were relatively stable under air and CA storage conditions. On the other hand, Golding (2001) showed that extractable phenolics changed little during air storage in apple peel of 'Granny Smith', 'Lady Williams', and

'Crofton' apple. The level of phenolics also remained relatively constant in the study by Burda et al. (1990) who used 'Rhode Island Greening', 'Empire' and 'Golden Delicious' apples. Both Awad and de Jager (2000) and Golding et al. (2001) found that phenolic concentrations in apple are cultivar dependent but in general, in most cultivars, total phenolic content increased during the first few months after harvest, then remained constant and eventually declined with storage time.

1.4. 1-methylcyclopropene (1-MCP)

1-MCP has been introduced commercially in postharvest treatment of flowers, fruits and vegetables in order to extend their shelf life, by inhibiting ethylene effects on ripening. At the present time, apple constitute the major crop with which 1-MCP is extensively used both experimentally and commercially, in many countries such as the US, most European countries, Argentina, Australia, Austria, Brazil, Canada, Chile, Costa Rica, Guatemala, and Honduras, Israel, Mexico, the Netherlands, New Zealand, South Africa, UK, and more. Each country has specific crops registered for use with 1-MCP, including: apple, apricot, avocado, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum, squash, tomato, and tulip bulbs. It is expected that registration of 1-MCP use will be granted for other countries and that 1-MCP will be increasingly applied with other varieties of crops.

1.4.1. 1-MCP: structure and mode of action

1-methylcyclopropene (1-MCP) is a simple gas molecule with a double bond in its structure and a methyl substitute on the first carbon of the cyclopropene (Figure 1.8). 1-MCP can thus mimic ethylene and bind to the ethylene receptor sites on the cell wall membranes.

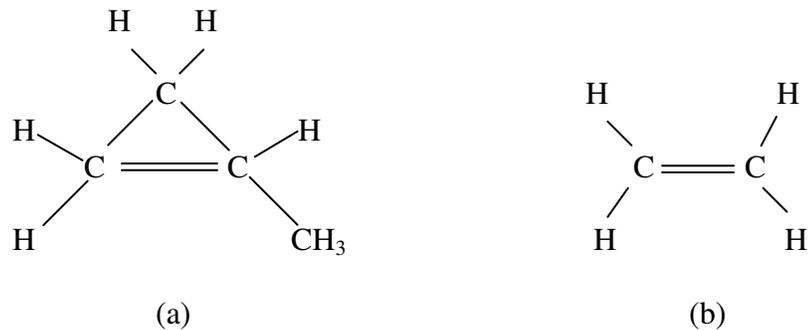


Figure 1.8 Chemical structure of 1-MCP (a) and ethylene (b)

1-MCP is a colorless and odorless gas and has a non-toxic mode of action. 1-MCP is active at very low concentrations and leaves negligible residue in treated commodity. 1-MCP has been classified as a plant growth regulatory by the US Environmental Protecting Agency (US EPA) and its use has been approved (EPA, 2002).

A breakthrough in commercial application of 1-MCP is attributed to formulation of this gas into a stable powder form which provides flexibility and convenience of its use in small and large scale (Watkins and Miller, 2003). The gas is trapped into complex of γ -cyclodextrine, and when the powder is dissolved in water the gas is easily released into the space where the 1-MCP treatment occurs (Feng et al., 2000; Hofman et al., 2000; Blankenship and Dole, 2003). The product is commercially available under the trade name SmartFresh™ AgroFresh, Inc. a subsidiary of Rohm and Haas, for application on edible crops. In a similar way to ethylene, 1-MCP can bind to the ethylene receptor site on the cell wall membrane, blocking the site to ethylene molecules. In contrast to ethylene, which diffuses rapidly from the receptors, and induces the “ethylene responses”, 1-MCP is believed to bind

strongly to the receptor and remains bound to it (Sisler and Serek, 1997). Although 1-MCP binds irreversibly to the receptors, some product can develop responses to ethylene again by formation of new receptors (Sisler and Serek, 1997).

The efficacy of 1-MCP treatment depends on several factors: the concentration and duration of exposure to 1-MCP. Higher concentrations are needed when 1-MCP is used at low temperature (Sisler and Serek, 1997; DeEll et al., 2002; Sisler and Serek, 2003). The type of commodity, cultivar and stage of ripening at the time of treatment as well as the type of storage conditions (CA, or air storage) also constitute factors that determine the concentration of 1-MCP treatment required to inhibit ripening (Watkins et al., 2000).

1.4.2. 1-MCP effect on horticultural crops

Over 150 studies have been undertaken to further understand the action of 1-MCP and its effects, use and applications, with most focus on postharvest preservation of fruits, vegetables and ornamental products (Blankenship and Dole, 2003; Watkins and Nock, 2005). Postharvest responses to 1-MCP vary from crop to crop and within cultivar. Apart from genetic diversity, several factors related to pre and postharvest conditions impact the effect of 1-MCP treatment. A website is available that provides a summary table on the effect of 1-MCP on postharvest physiology of crops (<http://www.hort.cornell.edu/department/faculty/watkins/ethylene/index.htm>).

1.4.3. Effects of 1-MCP on the apple

1-MCP significantly inhibits ripening and respiration rates of treated apple fruit (Fan et al., 1999). 1-MCP prevents and/or delays the increase in ethylene production and internal ethylene concentrations (IEC) associated with the climacteric ripening. The extent of 1-MCP effect is cultivar dependent, and varies depending on type and

length of storage. Softening is also prevented by 1-MCP (Watkins et al., 2000; Watkins et al., 2000; DeLong et al., 2004; Moran and McManus, 2005). Firmness retention and increase of shelf life period have been major commercial attributes that are maintained by 1-MCP treatment even at higher temperature (Mir et al., 2001). The magnitude of IEC changes and softening were directly affected by 1-MCP concentration. During storage, IEC in 1-MCP-treated 'Empire', 'Delicious' increased a little and fruit softened only gradually. 1-MCP concentrations required to delay or prevent ripening are cultivar dependent (Sisler and Serek, 1997). Effects of 1-MCP are greater when combined with controlled atmosphere than used alone meaning in air storage (Watkins et al., 2000). 1-MCP can also inhibit to some extent flavor development and degreening of the apple skin, as both can be affected by ethylene.

1.4.4. 1-MCP and physiological disorders

Brown core and senescent breakdown in apples were prevented in 1-MCP treated fruit. Brown core is predominantly a low temperature-induced type of disorder. Fan et al. (1999) reported reduced incidences of other disorders such as superficial scald, soft scald, core flush, and greasiness in 1-MCP treated fruit compared with control fruit. Moreover, 1-MCP effectively inhibits development of superficial scald in several susceptible cultivars, (Shaham et al., 2003). 1-MCP treated 'Granny Smith', which is a superficial scald susceptible cultivar, had very low levels of alpha-farnesene throughout an experimental storage, and did not develop superficial scald (Shaham et al., 2003).

CO₂ injury has been a concern in CA storage of apple in the past years. Susceptible New York cultivars include 'McIntosh', 'Cortland' and 'Empire'. It has been suggested that 1-MCP exacerbates the occurrence of CO₂ injury. The physiological and biochemical mechanism of the occurrence of this injury is still not

well known, nor the mechanism behind how 1-MCP can aggravate the susceptibility to CO₂ injury. However, observations have shown that, the injury usually appears (but not always) on the greener side of the apple such as in 'Empire'. This fact suggests that greener or less mature tissues are more susceptible to the injury. One hypothesis is that, 1-MCP may increase susceptibility to injury by maintaining the fruit at a younger physiological state during storage.

While an extensive literature base is developing for 1-MCP (Watkins and Miller, 2006) much remains to be learned about its effects. Little is known also about the impact of 1-MCP on the level of phenolics in apples under CA storage. Information regarding the role of ethylene in phenolic biosynthesis may be valuable in enlightening the effect and role of 1-MCP on phenolic content of fruit.

1.4.5. Changes of ascorbic acid and phenolic concentrations in apple as affected by 1-MCP

Although 1-MCP technology has been rapidly adopted by the apple industry, few studies have been conducted to investigate its effects on the nutritional status of apples. MacLean (2003) found that during cold storage, the peel of 'Delicious' and 'Empire' apple treated with 1-MCP had higher water-soluble antioxidant capacity, compared to that of untreated fruit. It has been suggested that PAL activity, the enzyme involved in the biosynthesis of phenolics, has a direct influence on phenolics such as flavonoids and is also influenced by ethylene production during ripening (Murphey and Dilley, 1988; Gomez-Cordoves et al., 1996). It was expected then that 1-MCP treatment would influence flavonoid concentrations and antioxidant capacity. MacLean et al. (2003; 2006) mentioned that 1-MCP could affect the antioxidant composition of apples. However, 1-MCP treatment had various effects on the individual phenolics studied. Total flavonoid concentrations were higher while that of

chlorogenic acid were lower in 1-MCP treated fruit. As of particular flavonoids, anthocyanin concentrations remained constant and flavonols and flavan-3-ols vary little in 1-MCP treated fruit (MacLean et al., 2006).

In some fruits, 1-MCP decreases ascorbic acid loss (Selvarajah et al., 2001; Jiang et al., 2004) while in ‘Golden Smoothie’ apples and ‘Blanquilla’ pears, it lowers ascorbic acid level during cold storage (Larrigaudiere et al., 2001; Vilaplana et al., 2006). Postharvest disorders occurring in storage have been associated with loss of ascorbic acid level in fruit such as pears (Larrigaudiere et al., 2001; Franck et al., 2007), and it would be worthwhile to study the effects of physiological disorders related to 1-MCP treatment on the retention of ascorbic acid in the treated apples.

1.5. Physiological disorders in apple

Understanding the anatomy and histology of the apple fruit constitutes an important tool in describing and localizing where some of the physiological disorders occur in the apple tissues. It will help determine the symptoms and the manifestations of disorders in different tissues of the fruit.

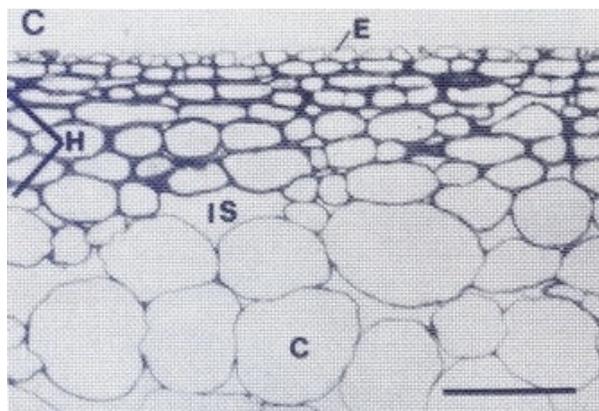


Figure 1.9 Fruit peel in section (Watkins et al., 1997)

E = uniseriate epidermis; H = hypodermis; IS = intercellular space; C = cortex;

Apples have diverse manifestations of physiological disorders. Susceptibility or resistance to a particular injury is variable and is affected greatly by cultivar. Some of the characteristics related to cultivar are skin diffusivity to O₂ and CO₂, cell wall membrane properties, mineral composition, and antioxidant status (Ju and Bramlage, 1999). Factors such as climate, maturity at harvest, nutrition and orchard management methods can also affect these characteristics.

Physiological disorders that have been identified in apple fruit include superficial scald, bitter pit, senescent breakdown, brown core and internal browning, low O₂ injury and CO₂ injury.

1.5.1. Superficial scald

Superficial scald is a physiological disorder that occurs with susceptible cultivars when they are stored for long-term at low-temperature and is probably a chilling injury (Ingle and D'Souza, 1989; Watkins et al., 1995). Cultivar, growing location and harvest date are among the factors influencing its incidence (Watkins et al., 2000). For instance, 'Delicious' and 'Granny Smith' are highly susceptible while 'Gala', 'Empire' and 'Braeburn' are scald resistant.

The development of superficial scald is strongly related to synthesis and oxidation of the sesquiterpene alpha-farnesene (Whitaker, 1998, 2000). In addition a number of studies have indicated that ethylene production is closely associated with that of alpha-farnesene production (Du and Bramlage, 1994; Watkins et al., 1995). Postharvest drenches of the antioxidant diphenylamine (DPA) are commercially used to control superficial scald.

1.5.2. Senescent breakdown

Senescent breakdown generally occurs in fruit stored at higher than optimal temperatures, and/or for too extended a period of time. The apple flesh softens, then becomes mealy and flesh browning develops. In advanced senescent breakdown the skin and flesh tissues may split. ‘McIntosh’, ‘Jonathan’ and ‘Spartan’, ‘Empire’ apples are among the susceptible cultivars (Wilkinson and Fidler, 1973).

1.5.3. Low O₂ injury

The early stages of low-O₂ injury are manifest by loss of flavor of the fruit and skin darkening of the fruit, and then followed by fermentation-related odors. These changes can be reversible if the injury is not advanced and the storage atmosphere is adjusted or fruit is removed to air. Visible symptoms of low O₂ injury can appear externally and internally. External injury shows brownish areas with defined margins on the skin that can cover part of or most of the fruit surface. Internally, it appears as brownish corky sections with occasional cavities that may be underneath the external injury. There are also other forms of low-O₂ injury such as epidermal cracking and ribbon scald (Little and Pegg, 1987; Lau, 1998).

1.5.4. Core browning – low temperature breakdown – internal browning

Low temperature breakdown, brown core and internal browning are physiological disorders that occur due to low temperature conditions and increasing length of storage at that temperature. Generally these disorders increase in incidence and severity with length of storage (Wilkinson and Fidler, 1973).

Brown core, also called core flush, involves browning of core area and later in the cortex, where it becomes difficult to distinguish from low-temperature breakdown.

Low-temperature breakdown is characterized by markedly brown vascular bundles, browning of flesh and a clear halo of unaffected tissue underneath the skin. In contrast to senescent breakdown, the affected tissues are more likely to be firmer, more moist and darker in color. Symptoms and severity of incidence depends on cultivar sensitivity to low temperatures. Climate also affects sensitivity to those disorders, with more browning occurring after colder, wetter growing seasons. (Roelofs and De Jager, 1997; Lentheric et al., 2003).

1.5.5. Internal CO₂ injury

Internal CO₂ is primarily caused by exposure to elevated CO₂ and/or long duration of exposure to the gas in the storage atmosphere. The injury can also occur in air storage conditions where ventilation is poor and CO₂ builds up (Argenta et al., 2002). Internal CO₂ injury manifests as browning spots on the flesh and/or patches with browning depending on the cultivar. Externally, fruit appear normal. However, the disorder becomes apparent on the fruit surface in severe cases. The brown tissue is initially firm and moist but may become dry with cavity formation. Development of brown heart usually ceases when causal conditions are removed.

Several factors affect the disorder: The disorder is dependent on type of fruit. For instance, apple fruit are less sensitive to elevated CO₂ than pear fruit. However within the same type of fruit, cultivar effects are important, supposedly because of anatomical differences such as size of intercellular space and cultivar differences in rates of gas diffusion in the tissues. Internal CO₂ disorder is also associated with late harvest (Lau, 1998). Disorder risk is increased with larger fruit size and with more mature fruit. Susceptibility of fruit to internal CO₂ is also affected by growing regions. It may also occur when cooling is delayed, or with storage at low temperature, and with low O₂ (Johnson et al., 1998). The importance of each factor

can vary greatly with cultivar. Delays between harvest and exposure to elevated CO₂ can reduce susceptibility of fruit to brown heart. In some cultivars, maintenance of low CO₂ during first four to six weeks of CA storage can minimize risk (Watkins et al., 1997). DPA used to control superficial scald also controls carbon dioxide injury (Burmeister and Rowan, 1997).

1.5.6. External CO₂ injury

External CO₂ injury is a physiological disorder that is associated with application of high level of CO₂ in CA storage. The injury has also occurred in air-stored fruit under conditions where CO₂ can accumulate, for example, warm fruit in sealed cartons. CO₂ injury is a skin disorder that occurs usually, but not always on the green or unblushed area of the apple skin. It is manifested initially as a water soaked appearance. The skin is smooth and looks greasy during the first 2 weeks of CA when high level (2.5 kPa and 5 kPa CO₂) was applied from personal observation. The symptoms develop afterwards and affected areas become sunken, wrinkled and pebbly textured, with sharply defined edges and more or less bronze discoloration. The injury is restricted to the skin surface (Watkins et al., 1997). It appears first at the boundary of the innermost hypodermis and outermost cortex then spreads into the outer hypodermis and sometimes into several cell layers of the cortex but the epidermal layer and the overlying cuticle remain intact in even severely CO₂-injured fruit. Several factors have been identified that influence susceptibility of fruit to external CO₂ injury: susceptibility to injury is cultivar dependent; ‘McIntosh’, ‘Cortland’, ‘Empire’ are affected, ‘Fuji’ and ‘Braeburn’ are susceptible (Watkins et al., 1997; Elgar et al., 1998; Volz et al., 1998). Its occurrence can be prevented by DPA treatment of fruit. In the case of ‘Empire’, carbon dioxide injury susceptibility has become more apparent when DPA usage has been stopped. Fruit susceptibility is

aggravated by 1-MCP treatment prior to exposure to CO₂ (DeEll et al., 2003; DeEll et al., 2005).

Susceptibility to injury is the highest during the first four weeks of storage. This is a distinguishing characteristic of external CO₂ injury (Watkins et al., 1997). Early harvested fruit seem to be more susceptible than late harvested fruit (Elgar et al., 1998; 1999). It also appears that susceptibility varies with growing location and for cultivars; fruit injury varies greatly by orchard block (Watkins et al., 1997; Elgar et al., 1999).

Exposure to high level of CO₂ before fruit are cooled as well as the presence of free moisture on the fruit surface can affect disorder incidence (Bramlage et al., 1977). Some level of injury control can be achieved by delaying the exposure of the fruit to high CO₂ level in CA for instance by keeping fruit in cold air storage, and/or initially maintaining low CO₂ concentrations in the storage if this can be done without loss of quality (Wang et al., 2000). The biochemical basis for external CO₂ injury remains unknown. Burmeister (1995) suggested that the mechanism responsible for the CO₂ associated injury in 'Empire' apples is a free-radical-catalyzed oxidation of susceptible amino acid residues in proteins macromolecules, such as membrane lipids containing oxidizable reactive groups.

1.6. Diphenylamine (DPA)

Some cultivars are also treated before storage with DPA in order to control physiological disorders such as superficial scald. It is usually applied using postharvest drenches (Kupferman, 2001), and also requires addition of fungicide to prevent increased decay from wetting the fruit. The maximum DPA concentrations used are regulated for efficiency in controlling superficial scald and minimizing chemical damage and the concentrations used are typically 1000 ppm. Each country

has strict regulations governing maximum DPA residue allowable on fresh fruits. Other concerns related to postharvest application of DPA are related to environmental and waste management issues as well as consumer concerns with chemical treatment of fresh edibles (Zanella, 2003). DPA has also been found to completely control external CO₂ injury in susceptible apple cultivars at the concentrations commercially used. Today, thermofogging of fruit in CA with DPA has been studied as this method which has been adopted by the European apple industry is proven effective and convenient compared to the drenching method.

1.7. Research objectives and experimental approach

The objective of this project was to study the effects of 1-MCP treatment on the preservation of apple fruit quality by investigating its effects on antioxidant composition and capacity, and on the occurrence of physiological disorders mainly that of external CO₂ injury in 'Empire' apple when stored in air and in CA storage system. 'Empire' apple is an important New York variety and has been found to respond well to 1-MCP treatment. When treated with 1-MCP, 'Empire' apple storability increases tremendously and their firmness is remarkably maintained throughout the marketing chains. There is also added benefit in respect to firmness when fruit is only stored in air. Because of increased interest in the contribution of apple in the phytochemicals in the diet and the success of long-term storage of cultivars such as 'Empire' apple, it is justified to carry out experiments looking at the effects of air and different CA factors and importantly of 1-MCP on the fate of phenolic concentrations in apple over the storage duration (Chapter two). Therefore, antioxidant status of 'Empire' apples was assessed. Measurements of total phenolics, flavonoids, anthocyanins and ascorbic acid concentrations as well as the total antioxidant capacity were performed separately on the peel and flesh of apple treated

with 1-MCP and either stored in air or CA. The experiment was carried out for 5 months for air storage and up to 9 months for CA storage. For the CA experiment: in each of the two rooms of 0.5 and 2.2 °C, 2 kPa O₂ and 3 kPa O₂ both in 2 kPa CO₂ which were humidified and balanced with N₂ before delivery was established in two chambers and the effects of these parameters on antioxidant composition and capacity was assessed.

Although quality attributes related to texture and firmness were well maintained in 'Empire' apple, 1-MCP has been found to increase the fruit susceptibility to external CO₂ injury (DeEll et al., 2003) causing important economic loss to the apple industry. Thus, a serie of experiments was conducted in order to investigate how the occurrence of this physiological disorder in 1-MCP treated fruit is affected by the following factors: 1) CO₂ partial pressure in the CA, 2) timing of the exposure of the fruit to high CO₂ partial pressure; 3) delaying the exposure to high CO₂; 4) DPA treatment especially looking at degree effect as well as the timing of the treatment after 1-MCP exposure mainly to study the possible application of DPA thermofogging on highly CO₂ injury susceptible fruits.

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CHAPTER TWO

NUTRITIONAL QUALITY

OF 1-METHYLCYCLOPROPENE (1-MCP)-TREATED 'EMPIRE' APPLES IN AIR AND CONTROLLED ATMOSPHERE (CA) STORAGE

Abstract

The effects of 1-MCP on ripening and concentrations of total phenolics, flavonoids, anthocyanins and total antioxidant activity of 'Empire' apples (*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.) have been studied. Fruit were stored in air for up to 5 months, and in controlled atmosphere (CA) in 2 and 3 kPa O₂ (2 kPa CO₂) at 0.5 and 2.2 °C for 4.5 and 9 months. Ripening was delayed by 1-MCP treatment in both air and CA storage as indicated by internal ethylene concentrations and flesh firmness. Overall, total phenolic, flavonoid and anthocyanin concentrations as well as antioxidant activity are relatively stable during air and CA storage. In CA storage, interactions among oxygen level, temperature and storage duration were detected but no consistent trends were observed. The only effects of 1-MCP on flavonoid or anthocyanin concentrations were found in the flesh and peel respectively of fruit stored in CA. Flavonoid concentrations were higher in the flesh of 1-MCP treated than untreated fruit kept in 2 kPa O₂ while anthocyanin concentrations only measured in the peel was not affected by 1-MCP treatment. Phenolic concentrations were higher in the peel while lower in the flesh of 1-MCP treated fruit compared to the control fruit stored in air. There were no correlations found between total phenolics

and antioxidant activity. Ascorbic acid concentrations declined in both peel and flesh tissues of untreated and 1-MCP treated fruit stored in air, while changes of ascorbic acid concentrations in CA-stored fruit were inconsistent.

Keywords: Apple; Storage; Phenolics; Flavonoids; Anthocyanin; Antioxidant activity; Ascorbic acid

2.1. Introduction

Apples are a widely consumed fruit worldwide, and they are one of the best sources of antioxidant and phenolic compounds, including quercetin, catechin, phlorizdin and chlorogenic acid (Eberhardt et al., 2000; Boyer and Liu, 2004). Cultivars vary greatly in antioxidant components (Imeh and Khokhar, 2002; Wolfe et al., 2003; Boyer et al., 2004; Boyer and Liu, 2004; Napolitano et al., 2004; Lata et al., 2005; Vanzani et al., 2005). Phenolic and flavonoid contents are consistently higher in the skin than in the flesh, and peels have the highest antioxidant activity and anti-proliferation activity (Eberhardt et al., 2000; Eberhardt et al., 2001; Wolfe et al., 2003). In general, the total phenolic concentrations remain relatively stable during storage, but individual components may vary (Burda et al., 1990; Awad and de Jager, 2000; Golding et al., 2001; Awad and de Jager, 2003) and total phenolics and total antioxidant activity may increase in peel of cultivars stored in air or CA conditions (Lattanzio et al., 2001; Leja et al., 2003; Napolitano et al., 2004). Individual phenolic compounds have different antioxidant potential (Lee et al., 2003) but reactions between them may be synergistic. Therefore, total antioxidant activities potentially provide a better estimate of the overall contributions of antioxidant components than

activities of individual components alone (Wang et al., 1996; Vinson et al., 2001; Liu, 2003).

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene perception (Sisler and Blankenship, 1996; Sisler and Serek, 1997, 2003) is now used extensively by apple industries to maintain fruit quality, especially texture (Watkins, 2008). CA can prolong the impact of 1-MCP on both physical and sensory responses of apple and the two technologies generally are most effective when used in combination (Rupasinghe et al., 2000; Watkins et al., 2000; Bai et al., 2005). Nevertheless, 1-MCP can be an excellent replacement for CA storage for short term air storage and for smaller local market operations that do not have CA facilities. Formal data on the extent of 1-MCP use are not available, but it is used in a majority of CA storages of cultivars such as 'Delicious', 'Empire' and 'Gala' in Washington State and New York. The antioxidant diphenylamine (DPA), applied to inhibit development of superficial scald, is also used frequently on CA-stored 'Empire' apples because it also prevents external CO₂ injury (Watkins et al., 1997; DeEll et al., 2005; Fawbush et al., 2008).

Despite the rapid adoption of 1-MCP based technology, little is known about its effects on the nutritional status of apples and its products. Water-soluble antioxidant activity was higher in peel extracts of 1-MCP treated 'Delicious' and 'Empire' apples compared with that of untreated fruit during cold storage (MacLean et al., 2003), while in peel tissues of 'Delicious' apples, total flavonoid concentrations were higher and chlorogenic acid levels lower in 1-MCP treated fruit than in untreated fruit (MacLean et al., 2006). While 1-MCP maintained anthocyanin concentrations, the flavonols and flavan-3-ols were unaffected by treatment (MacLean et al., 2006). Overall, the studies by MacLean et al. (2003, 2006) indicate that 1-MCP may affect the nutritional qualities of apples, but the research was restricted to peel tissues.

The focus of current research in the nutrition literature for apples is mostly towards phenolics. Although ascorbic acid may contribute to the health benefits of apples, the compound represents a minor part of the antioxidant activity of the fruit (Wang et al., 1996; Lee et al., 2003; Boyer et al., 2004). Nevertheless, ascorbic acid has critical enzymatic and non-enzymatic functions in tissues and is involved in detoxification of damaging oxygen radicals and their derivatives, the reactive oxygen species (ROS) (Davey et al., 2000). 1-MCP slows ascorbic acid loss in some fruit (Jiang et al., 2004; Liu et al., 2005), but 1-MCP treatment is also associated with lower ascorbic acid levels during storage of ‘Golden Smoothie’ apples and ‘Blanquilla’ pears (Larrigaudière et al., 2004; Vilaplana et al., 2006). Decreased ascorbic acid concentrations have been linked with development of physiological disorders such as core browning of pears (Larrigaudière et al., 2001; Franck et al., 2003; Franck et al., 2007), but no studies on relationships of this compound with physiological disorders of 1-MCP treated apple fruit are available. The objective of the current study was to investigate the effects of 1-MCP treatment during air and CA storage on antioxidant components and antioxidant activity of peel and flesh tissues of the ‘Empire’ apple.

2.2. Materials and methods

2.2.1. Fruit source, treatments and storage conditions

Fruit used in these experiments were picked during the optimum commercial harvest period for ‘Empire’ apples (Blanpied and Silsby, 1992) from mature trees at the Cornell University orchards at Lansing, NY. Fruit were randomly sorted into experimental units for either air or CA experiments.

For air storage, four replicates of 100 fruit were pre-cooled overnight at 0.5 °C and then they were either untreated or treated with 1 μ L L⁻¹ 1-MCP (SmartFresh

powder, 0.14% a.i., AgroFresh, Spring House, PA) for 24 h in sealed 135 L containers (Watkins et al., 2000). Fruit were stored for up to five months.

For CA storage, sets of four 55-60 fruit replicates were either untreated, or dipped in 1000 mg L⁻¹ diphenylamine (Shield DPA 15%, Pace International, Wenatchee, WA) for 1 min and allowed to dry for 1 h. Fruit were then cooled overnight at 0.5 or 2.2 °C, and either untreated or treated with 1µL L⁻¹ 1-MCP, to provide four treatments: 1) untreated; 2) DPA; 3) 1-MCP; and 4) DPA + 1-MCP-treated for each storage temperature. Four replicates of fruit for each treatment and temperature were stored in 850 L steel chambers, with 2 or 3 kPa O₂ (with 2 kPa CO₂). Final atmosphere regimes were established within 48 h and were maintained within 0.2 kPa of the target partial pressures using an Oxystat II CA system equipped with a GCS 660 Controller (David Bishop, England). CA storage was carried out for 9 months.

2.2.2. Fruit sampling

Ten fruit per treatment replicate were taken at harvest for assessment of internal ethylene concentration (IEC), flesh firmness, soluble solids content (SSC) and starch pattern indices. IEC and firmness were measured on 10 fruit replicates after 1, 2, 3, 4 and 5 months for the air stored fruit, and after 4.5 and 9 months for the CA stored fruit, plus 1 d at 20 °C. The IEC of each apple was measured on 1 mL of internal gas taken from the core. The gas was injected into a 6' x 1/8" stainless steel column packed with Alumina F-1 (0.318-0.423 mm) in a Hewlett Packard 5890, series II GC (Wilmington, DE) fitted with a flame ionization detector and using a Hewlett Packard 3395 integrator (Fawbush et al., 2008). Firmness was measured on opposite sides of each fruit with an EPT-1 pressure tester (Lake City Technical Products, Lake City, Canada) fitted with an 11.1 mm diameter probe. Additional sets of 5 fruit per

replicate were sampled as described below. At the last removal from storage, all remaining fruit were kept at 20 °C for 7 d and then assessed for disorders. Each fruit was sliced at least three times to reveal the presence or absence of internal disorders. The percentage of fruit with any visible injury was calculated.

For extraction of phenolic and other compounds, 10 fruits per treatment were taken on the day of removal of fruit from air storage and from the untreated and 1-MCP-treated fruit stored in 2 and 3 kPa O₂ at 0.5 or 2.2 °C. Fruit treated with DPA with or without 1-MCP were not sampled. Each fruit was peeled rapidly under subdued light (Rizzolo et al., 2002), and peel and flesh (without core tissues) were frozen immediately in liquid nitrogen. The frozen tissues were kept at -80 °C prior to extraction of total phenolics, flavonoids, anthocyanins, total antioxidant activity and total ascorbic acid. Measurements were performed on apple peel and flesh for both air and CA storage experiments, except for anthocyanin, which was measured only in peel samples.

2.2.3. Extraction for measurement of anthocyanins, flavonoids, phenolics, and total antioxidant activity

The frozen fruit were crushed into coarse pieces, and 10 g samples of peel or 50 g samples of flesh tissue were homogenized for 3 min with 100 mL or 200 mL, respectively, of 80% acetone using a commercial blender (Black & Decker, MD) as described previously (Dewanto et al., 2002; Sun et al., 2002). The homogenate was filtered through #1 Whatman paper. The filtrate was recovered and the acetone was evaporated off by a rotary evaporator (Labconco model 78820-00, Kansas City, MO) at 45 °C. The samples were then brought to 10 mL with deionized water, divided into several aliquots, and kept frozen at -80 °C until used for analysis.

2.2.4. Determination of total phenolic concentrations

The total phenolic concentration of the peel and flesh extracts was measured using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Meyers et al., 2003). Absorbance was measured at 750 nm versus a blank after 90 min at room temperature. The results were expressed as gallic acid equivalents on a fresh weight basis, g kg^{-1} .

2.2.5. Determination of total flavonoid concentrations

The total flavonoid concentration of the peel and flesh extracts was determined using a colorimetric assay (Meyers et al., 2003). The absorbance of the solution versus a blank at 510 nm was measured immediately. The results were expressed as catechin equivalents on a fresh weight basis, g kg^{-1} .

2.2.6. Determination of anthocyanin concentrations

The total anthocyanin concentration of the peel extracts was determined using a modified pH differential method described previously (Boyles and Wrolstad, 1993; Meyers et al., 2003; Wolfe et al., 2003). A spectrophotometer (Beckman-Coulter DU-7400 UV/Vis Spectrophotometer) was used to measure absorbance at 510 and 700 nm in buffers at pH 1.0 and 4.5. Absorbance readings were converted to mg cyanidin 3-glucoside, using the molar extinction coefficient of 26900 and absorbance of $A = [(A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}]$ (Meyers et al., 2003). Total anthocyanin was expressed as cyanidin 3-glucoside on a fresh weight basis, g kg^{-1} .

2.2.7. Quantification of the total antioxidant activity

The total antioxidant activity was determined using the PSC (peroxyl radical scavenging capacity) assay (Adom and Liu, 2005). Thermal degradation of ABAP (2,2'-azobisamidinopropane) produced peroxyl radicals (ROO•), which oxidized nonfluorescent dichlorofluorescein (DCFH) to fluorescent dichlorofluorescein (DCF). The degree of inhibition of DCFH oxidation, by antioxidants that scavenge peroxyl radicals, was used as the basis for calculating antioxidant activity. Fluorescence was monitored at 485 nm excitation and 538 nm emission with a fluorescent spectrophotometer (Fluoroskan Ascent FL, Thermo Electron Corporation, Asheville, NC). The results were calculated as vitamin C equivalents on a fresh weight basis, mmol kg⁻¹.

2.2.8. Extraction and measurement of ascorbic acid concentrations

The frozen peel or flesh tissues were crushed into coarse pieces, and 10 g samples were homogenized with 100 mL of buffer (2% metaphosphoric acid containing 2 mM EDTA) using a Waring commercial blender for 3 min. The slurry was centrifuged at 17,600 × g for 15 min at 4 °C. The ascorbic acid (AA) oxidase method was used to measure AA (Rao and Ormrod, 1995). The total amount of AA was determined by measuring the decrease in absorbance at 265 nm upon the addition of ascorbate oxidase to a mixture containing 100 mM phosphate buffer (pH 5.6) and neutralized extracts which were reduced by 20 mM dithiothreitol (DTT) in 50 mM 2-hydroxyethylpiperazine 2-ethanesulfonic acid (HEPES)-KOH buffer (pH 7.5). The amount of oxidized AA (or dehydroascorbic acid (DHA)) was determined by subtracting the reduced AA from the total AA (Rao and Ormrod, 1995). The concentrations were expressed as AA on a fresh weight basis, g kg⁻¹.

2.2.9. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model to determine main effects and interactions (Minitab, Release 15, State College, PA), and LSD values calculated for comparison of means. IECs were analyzed after transformation to logarithms. Pearson correlations were used to quantify the relationships among antioxidant contents, antioxidant activity and flesh browning.

2.3. Results

2.3.1. Maturity and quality at harvest

'Empire' apples stored in air and CA storage were all harvested on the same day. The IEC, flesh firmness, SSC and starch index of the fruit at harvest were $\log 0.43 \mu\text{L L}^{-1}$ ($2.66 \mu\text{L L}^{-1}$), 74.0 N, 12.5% and 5.7 units, respectively.

2.3.2. Air storage

2.3.2.1. Storage disorders

No storage disorders were observed in air stored fruit.

2.3.2.2. IEC and flesh firmness

The increase in IEC and softening in untreated fruit during storage was reduced greatly by 1-MCP treatment (Table 2.1).

Table 2.1 Log internal ethylene concentration (IEC; $\mu\text{L L}^{-1}$) and flesh firmness (N) of ‘Empire’ apples either untreated or treated with 1 $\mu\text{L L}^{-1}$ 1-MCP and stored in air at 0.5 °C for up to 5 months.

^aBack-transformed data are provided in parentheses

| Storage time (months) | Log IEC ($\mu\text{L L}^{-1}$) | | Firmness (N) | |
|-----------------------|----------------------------------|------------|--------------|---------|
| | - 1-MCP | + 1-MCP | - 1-MCP | + 1-MCP |
| 0 | 0.43 (2.7) ^a | | 74.0 | |
| 1 | 0.41 (2.6) | 0.27 (1.8) | 63.4 | 69.5 |
| 2 | 1.27 (18.6) | 0.23 (1.7) | 60.6 | 67.4 |
| 3 | 1.34 (21.9) | 0.39 (2.5) | 57.0 | 66.4 |
| 4 | 1.36 (22.9) | 0.38 (2.4) | 54.5 | 64.3 |
| 5 | 1.64 (43.7) | 0.35 (2.2) | 50.6 | 62.4 |
| LSD ($P=0.05$) | 0.422 | | 3.27 | |

2.3.2.3. Total Phenolic concentrations

The phenolic concentrations were higher in the peel than the flesh tissues (Figure 2.1). Overall, the phenolic concentration was higher (2.56 g kg⁻¹) in peel tissues of 1-MCP treated fruit than in those of untreated fruit (2.16 g kg⁻¹; $P = 0.012$). In the flesh, however, total phenolic concentrations of untreated fruit were higher at 0.90 g kg⁻¹ than the 0.84 g kg⁻¹ of 1-MCP treated fruit ($P = 0.023$). No significant effect of storage time was detected for either tissue type.

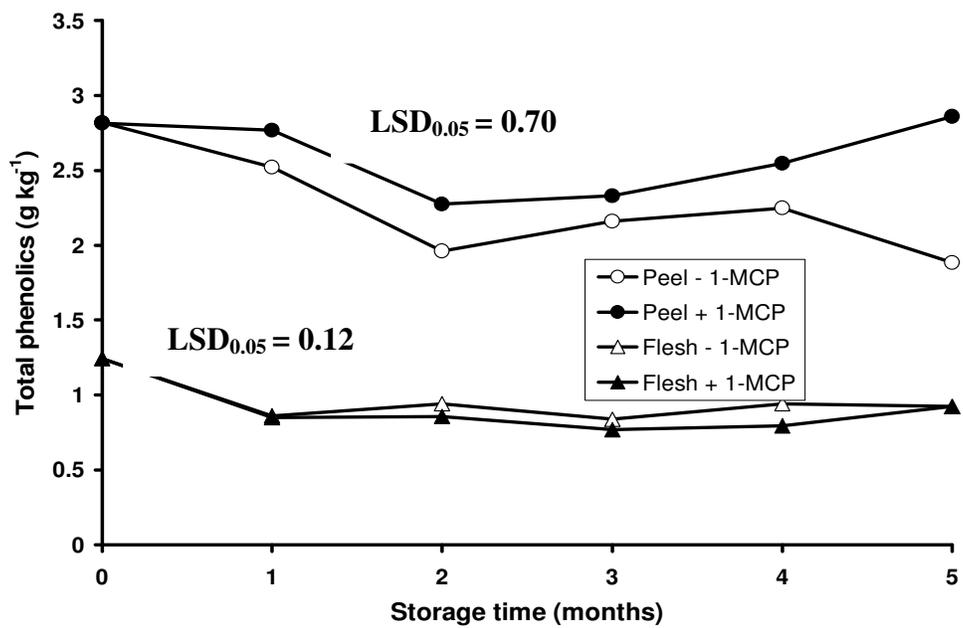


Figure 2.1 Total phenolic concentrations (gallic acid equivalents g kg^{-1}) in peel and flesh tissues of 'Empire' apples either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-MCP and stored at $0.5 \text{ }^{\circ}\text{C}$ in air for up to 5 months.

2.3.2.4. Total flavonoid and anthocyanin concentrations

The flavonoid concentrations in the peel declined from 1.01 g kg⁻¹ at harvest to a minimum of 0.80 g kg⁻¹ at month 2, but overall were affected inconsistently by storage time ($P=0.048$; data not shown). Total flavonoid concentrations in the flesh were 0.43 g kg⁻¹ at harvest, and no significant effects of either 1-MCP treatment or storage time were detected.

Anthocyanin concentrations were only measured in peel tissues. The concentration at harvest was 0.29 g kg⁻¹, and they were not affected by 1-MCP treatment (data not shown). Concentrations were affected by storage duration ($P = 0.028$), but inconsistently over time, being 0.38, 0.28, 0.32, 0.30 and 0.24 g kg⁻¹ at months 1, 2, 3, 4 and 5, respectively.

2.3.2.5. Ascorbic acid (AA) concentrations

Total AA concentrations declined in both untreated and 1-MCP-treated peel and flesh tissues during storage ($P \leq 0.001$; Figure 2.2). No significant effect of 1-MCP treatment on AA concentrations in peel tissues was detected. In flesh tissues, however, AA concentrations were slightly lower overall in 1-MCP-treated tissues than untreated tissues ($P = 0.021$), averaging 0.07 and 0.06 g kg⁻¹, respectively, although effects were evident at only some time points. DHA concentrations in the peel and flesh were not affected by 1-MCP treatment or storage time (data not shown).

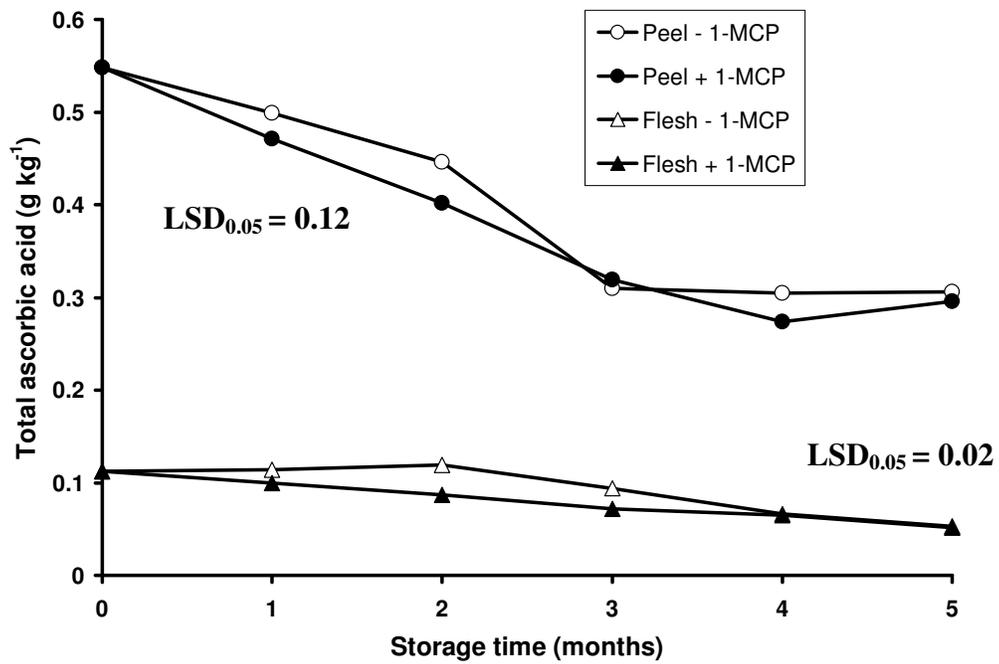


Figure 2.2 Total ascorbic acid concentrations (mg kg^{-1}) in peel and flesh tissues of 'Empire' apples either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-MCP and stored at $0.5 \text{ }^\circ\text{C}$ in air for up to 5 months.

2.3.2.6. Total antioxidant activity

The total antioxidant activity in peel tissue from 1-MCP treated fruit averaged 2.64 mmol kg⁻¹, compared with 2.12 mmol kg⁻¹ in untreated fruit ($P=0.004$), but differences between treatment were evident only at months 1 to 3 (Figure 2.3). Total antioxidant activity in flesh tissues was also higher in 1-MCP treated fruit than untreated fruit ($P=0.016$), averaging 1.14 and 0.95 mmol kg⁻¹, respectively.

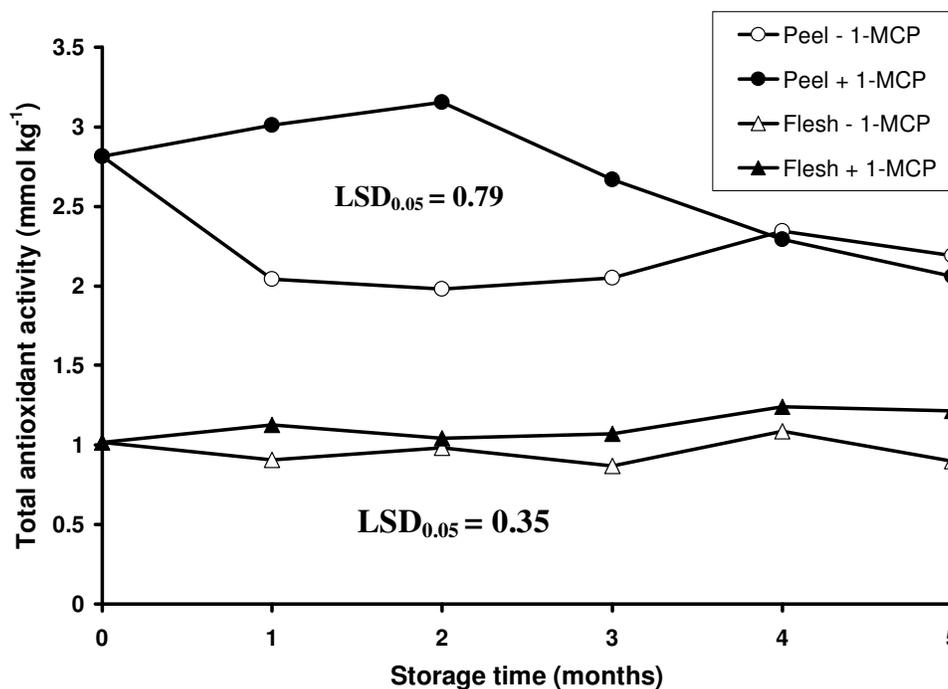


Figure 2.3 Total antioxidant activity (vitamin C equivalents, mmol kg⁻¹) in peel and flesh tissues of ‘Empire’ apples either untreated or treated with 1 μL L⁻¹1-MCP and stored at 0.5 °C in air for up to 5 months.

2.3.3. CA storage

2.3.3.1. Storage disorders

No external or internal disorders were detected after 4.5 months of CA storage, but a high incidence of flesh browning was observed in fruit after 9 months of storage (Table 2.2). Interactions were detected among all factors ($P \leq 0.001$), but the overall average in 1-MCP treated fruit was 84% compared with 77% in untreated fruit ($P = 0.001$), 83% in fruit stored at 0.5 °C compared with 79% at 2.2 °C ($P = 0.027$), and 86% in fruit stored at 2 kPa O₂ compared with 76% at 3 kPa O₂ ($P \leq 0.001$). No main effect of DPA was detected, and all subsequent analyses of antioxidant contents and activity were limited to 1-MCP treatment, O₂ partial pressure, temperature, and storage period.

Table 2.2 Incidence of flesh browning (%) of ‘Empire’ apples either untreated or treated with 1 µL L⁻¹ 1-MCP and 1000 mg L⁻¹ DPA, and stored at 0.5 or 2.2 °C in 2 or 3 kPa O₂ (2 kPa CO₂) for 9 months.

| DPA | 1-MCP | Flesh Browning (%) | | | |
|---------------------|-------|--------------------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa | 2 kPa | 3 kPa |
| - | - | 92 | 89 | 84 | 60 |
| - | + | 87 | 70 | 84 | 87 |
| + | - | 88 | 65 | 64 | 78 |
| + | + | 95 | 78 | 94 | 79 |
| LSD ($P=0.05$) | | 9.6 | | | |

2.3.3.2. IEC and flesh firmness

The IEC was much lower in 1-MCP treated fruit than in untreated fruit (Table 2.3), but treatment effects interacted with storage period and O₂ level ($P < 0.001$). Firmness was higher in 1-MCP treated fruit than in untreated fruit (Table 2.3), but treatment effects interacted with storage period and O₂ level ($P=0.002$), and with temperature and O₂ level ($P=0.022$).

Table 2.3 Log internal ethylene concentration (IEC; $\mu\text{L L}^{-1}$) and flesh firmness (N) of ‘Empire’ apples either untreated or treated with 1 $\mu\text{L L}^{-1}$ 1-MCP and stored at 0.5 or 2.2 °C in 2 or 3 kPa O₂ (2 kPa CO₂) for 4.5 or 9 months.

^aBack-transformed data are provided in parentheses

| Storage time (months) | 1-MCP | Log IEC ($\mu\text{L L}^{-1}$) | | | | Firmness (N) | | | |
|-----------------------|-------|----------------------------------|----------------|---------------|---------------|--------------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa | 2 kPa | 3 kPa | 2 kPa | 3 kPa | 2 kPa | 3 kPa |
| 4.5 | - | 2.26 (183) ^a | 2.27 (185) | 2.22 (167) | 2.33 (216) | 63.4 | 60.5 | 57.9 | 52.2 |
| | + | -0.10 (1.6) | -0.37 (0.4) | 0.07 (2.3) | 0.04 (1.5) | 68.8 | 69.4 | 68.0 | 68.5 |
| 9 | - | 2.22 (176) | 2.06 (116) | 2.37 (234) | 2.22 (170) | 59.4 | 52.1 | 52.9 | 40.0 |
| | + | -0.23 (1.8) | -0.34 (0.6) | 1.49 (32) | 1.74 (560) | 66.9 | 67.4 | 63.5 | 64.2 |
| LSD ($P=0.05$) | | 0.44 | | | | 2.64 | | | |

2.3.3.3. Total phenolic concentrations

No significant effect of 1-MCP on total phenolic concentrations in peel and flesh tissues was detected (Table 2.4). In peel tissues, total phenolics were affected only by storage temperature ($P=0.028$), overall concentrations being 2.53 g kg^{-1} at $0.5 \text{ }^{\circ}\text{C}$ compared with 2.21 g kg^{-1} at $2.2 \text{ }^{\circ}\text{C}$. In flesh tissues, however, the O_2 level was the only main effect detected ($P \leq 0.001$); total phenolics averaged 1.14 and 0.88 g kg^{-1} , at 2 and 3 kPa O_2 , respectively. An interaction was detected among all factors ($P=0.05$), but differences were inconsistent.

Table 2.4 Total phenolic concentrations (gallic acid equivalents, g kg^{-1}) in peel and flesh tissues of ‘Empire’ apples either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-MCP and stored at 0.5 or $2.2 \text{ }^{\circ}\text{C}$ in 2 or 3 kPa O_2 (2 kPa CO_2) for 4.5 or 9 months.

| Storage time (months) | 1-MCP | Peel | | | | Flesh | | | |
|-----------------------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa |
| 4.5 | - | 2.45 | 2.33 | 2.56 | 1.81 | 1.05 | 0.76 | 0.96 | 1.01 |
| | + | 2.85 | 2.47 | 2.47 | 2.27 | 1.50 | 0.57 | 0.90 | 1.12 |
| 9 | - | 2.36 | 2.80 | 2.37 | 2.12 | 1.59 | 0.91 | 1.08 | 0.66 |
| | + | 2.30 | 2.71 | 1.90 | 2.15 | 1.05 | 1.02 | 0.98 | 1.01 |
| LSD ($P=0.05$) | | 0.854 | | | | 0.356 | | | |

2.3.3.4. Total flavonoid and anthocyanin concentrations

The total flavonoid concentrations in peel tissues were not affected by 1-MCP treatment (Table 2.5), but overall, concentrations in these tissues were 1.30 g kg⁻¹ at 0.5 °C compared with 1.04 g kg⁻¹ at 2.2 °C ($P=0.001$), and 1.10 and 1.24 g kg⁻¹ at 4.5 and 9 months of storage ($P=0.048$), respectively. No other main effects or interactions were detected. In flesh tissues, total flavonoid concentrations were higher in 1-MCP treated than untreated fruit ($P=0.002$) at 2 kPa O₂ but not 3 kPa O₂.

Table 2.5 Total flavonoid concentrations (catechin equivalents, g kg⁻¹) in peel and flesh tissues of ‘Empire’ apples either untreated or treated with 1 µL L⁻¹ 1-MCP and stored at 0.5 or 2.2 °C in 2 or 3 kPa O₂ (2 kPa CO₂) for 4.5 or 9 months.

| Storage time (months) | 1-MCP | Peel | | | | Flesh | | | |
|-----------------------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa |
| 4.5 | - | 1.20 | 1.14 | 0.91 | 0.88 | 0.45 | 0.47 | 0.39 | 0.49 |
| | + | 1.38 | 1.15 | 1.01 | 1.14 | 0.53 | 0.45 | 0.56 | 0.42 |
| 9 | - | 1.49 | 1.38 | 1.12 | 1.17 | 0.48 | 0.50 | 0.49 | 0.44 |
| | + | 1.26 | 1.42 | 0.92 | 1.19 | 0.66 | 0.50 | 0.54 | 0.49 |
| LSD ($P=0.05$) | | 0.414 | | | | 0.118 | | | |

Total anthocyanin concentrations in peel tissues were not affected by 1-MCP treatment (Table 2.6), but they averaged 0.28 g kg⁻¹ at 0.5 °C compared with 0.22 mg kg⁻¹ at 2.2 °C ($P=0.004$). No other main effects or interactions were detected.

Table 2.6 Total anthocyanin concentrations (cyanidin 3-glucoside, g kg⁻¹) in peel tissues of ‘Empire’ apples either untreated or treated with 1 µL L⁻¹ 1-MCP and stored at 0.5 or 2.2 °C in 2 or 3 kPa O₂ (2 kPa CO₂) for 4.5 or 9 months.

| Storage time (months) | 1-MCP | 0.5 °C | | 2.2 °C | |
|-----------------------|-------|--------|-------|--------|-------|
| | | 2 kPa | 3 kPa | 2 kPa | 3 kPa |
| 4.5 | - | 0.28 | 0.22 | 0.20 | 0.18 |
| | + | 0.31 | 0.26 | 0.28 | 0.29 |
| 9 | - | 0.31 | 0.31 | 0.21 | 0.19 |
| | + | 0.25 | 0.33 | 0.20 | 0.24 |
| LSD (P=0.05) | | 0.119 | | | |

2.3.3.5. Total ascorbic acid (AA) concentrations

Overall, total AA concentrations in both peel and flesh tissues were not affected by 1-MCP treatment (Table 2.7). Although AA concentrations in peel tissues were higher ($P=0.006$) at 0.5 °C (0.39 g kg⁻¹) than at 2.2 °C (0.35 g kg⁻¹), they were affected differently by O₂ level and storage time at each temperature ($P=0.048$). In flesh tissues, total AA concentrations were unaffected by storage time at 0.5 °C, but decreased from 0.10 g kg⁻¹ at 4.5 months to 85 mg kg⁻¹ at 9 months at 2.2 °C ($P=0.018$). A temperature and O₂ level interaction was also detected ($P=0.048$). DHA concentrations were not affected by treatment in either peel or flesh tissues (data not shown).

Table 2.7 Total ascorbic acid concentrations (g kg^{-1}) in peel and flesh tissues of ‘Empire’ apples either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-MCP and stored at 0.5 or 2.2 °C in 2 or 3 kPa O_2 (2 kPa CO_2) for 4.5 or 9 months.

| Storage time (months) | 1-MCP | Peel | | | | Flesh | | | |
|-----------------------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa |
| 4.5 | - | 0.42 | 0.37 | 0.37 | 0.35 | 0.11 | 0.10 | 0.11 | 0.09 |
| | + | 0.44 | 0.37 | 0.35 | 0.31 | 0.11 | 0.10 | 0.10 | 0.09 |
| 9 | - | 0.39 | 0.43 | 0.38 | 0.33 | 0.11 | 0.11 | 0.10 | 0.09 |
| | + | 0.32 | 0.39 | 0.36 | 0.33 | 0.10 | 0.11 | 0.09 | 0.08 |
| LSD ($P=0.05$) | | 0.091 | | | | 0.015 | | | |

2.3.3.6. Total antioxidant activity

The total antioxidant activity in peel tissues was not affected by 1-MCP (Table 2.8), although only marginally ($P=0.054$). At 0.5 °C, antioxidant activity increased from $2.65 \text{ mmol kg}^{-1}$ at 4.5 months to $3.12 \text{ mmol kg}^{-1}$ at 9 months, but at 2.2 °C, decreased from $2.74 \text{ mmol kg}^{-1}$ at 4.5 months to $2.30 \text{ mmol kg}^{-1}$ at 9 months ($P \leq 0.001$). At 2 kPa O_2 , antioxidant activity decreased from $2.79 \text{ mmol kg}^{-1}$ at 4.5 months to $2.51 \text{ mmol kg}^{-1}$ at 9 months, while at 3 kPa O_2 , increased from $2.60 \text{ mmol kg}^{-1}$ at 4.5 months to $2.91 \text{ mmol kg}^{-1}$ at 9 months ($P=0.009$). No other main effects or interactions were detected.

In flesh tissues, the total antioxidant activity was higher in 1-MCP treated than untreated tissues, being 1.40 and $1.25 \text{ mmol kg}^{-1}$, respectively ($P=0.002$). Total antioxidant activity was higher in 3 kPa O_2 ($1.39 \text{ mmol kg}^{-1}$) than in 2 kPa O_2 ($1.26 \text{ mmol kg}^{-1}$), respectively ($P=0.012$). Interactions among all factors were detected, however, and overall trends were inconsistent (Table 2.8).

Table 2.8 Total antioxidant activity (vitamin C equivalents, mmol kg⁻¹) in peel and flesh tissues of ‘Empire’ apples either untreated or treated with 1 µL L⁻¹ 1-MCP and stored at 0.5 or 2.2 °C in 2 or 3 kPa O₂ (2 kPa CO₂) for 4.5 or 9 months.

| Storage time (months) | 1-MCP | Peel | | | | Flesh | | | |
|-----------------------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
| | | 0.5 °C | | 2.2 °C | | 0.5 °C | | 2.2 °C | |
| | | 2 kPa | 3 kPa |
| 4.5 | - | 2.87 | 2.51 | 3.04 | 2.66 | 1.11 | 0.91 | 1.32 | 1.52 |
| | + | 2.72 | 2.49 | 2.55 | 2.72 | 1.30 | 1.34 | 1.86 | 1.03 |
| 9 | - | 3.20 | 3.29 | 2.07 | 2.84 | 1.33 | 1.21 | 1.28 | 1.32 |
| | + | 2.84 | 3.14 | 1.91 | 2.37 | 1.74 | 1.62 | 1.19 | 1.16 |
| LSD (P=0.05) | | 0.656 | | | | 0.286 | | | |

2.3.3.7. Associations between individual phytochemical groups and total antioxidant activity, and browning

Pearson correlation coefficients were calculated for each phytochemical groups and total antioxidant activity for peel and flesh tissues from both air- and CA-stored fruit. In peel tissues from air-stored fruit, r values for phenolics and flavonoids, phenolics and anthocyanins, and flavonoids and anthocyanins, were 0.478 ($P=0.002$), 0.446 ($P=0.004$), and 0.585 ($P\leq 0.001$), respectively. However, only anthocyanins were correlated (0.551, $P\leq 0.001$) with total antioxidant activity. In peel tissues from CA-stored fruit, r values for phenolics and flavonoids, phenolics and anthocyanins, and flavonoids and anthocyanins, were 0.727 ($P\leq 0.001$), 0.595 ($P\leq 0.001$), and 0.670 ($P\leq 0.001$), respectively. Phenolics and anthocyanins were weakly correlated with total antioxidant activity, being 0.263 ($P=0.036$) and 0.314 ($P=0.012$), respectively.

No significant correlation for any individual phytochemical group and total antioxidant activity was detected for air- or CA-stored fruit.

Browning incidence in fruit stored in CA for 9 months was weakly correlated with phenolic concentrations in the flesh (0.359, $P=0.044$), but no significant correlations were detected for flavonoids, total antioxidant activity or total ascorbic acid.

2.4. Discussion

1-MCP inhibited ethylene production, as indicated by IEC, of 'Empire' apples stored in air and CA compared with untreated fruit (Tables 2.1 and 2.3). Although the firmness of treated fruit was consistently greater than untreated fruit, some softening occurred during storage, especially at the higher temperature in CA storage where IECs increased after 9 months (Table 2.3). 'Empire' is very responsive to 1-MCP, but the degree of ripening inhibition is affected by factors such as maturity, IEC at time of treatment, storage conditions and storage period (Watkins et al., 2000; DeEll et al., 2005; Watkins and Nock, 2005).

The effects of storage conditions, without 1-MCP treatment, on antioxidants has been well studied (Burda et al., 1990; Awad and de Jager, 2000; Awad and de Jager, 2003). In general, total phenolics, total antioxidant activity and radical scavenging capacity are stable or increase during storage. The results of our study also largely indicate that concentrations of total phenolics, flavonoids, anthocyanins and total antioxidant activity are relatively stable during air (Figures 2.1-2.3) and CA storage (Tables 2.4-2.8), but that the effects of 1-MCP on individual phytochemical groups were variable. In air stored fruit, total phenolic concentrations were higher in peel tissues, but lower in flesh tissues, of 1-MCP treated fruit compared with untreated

fruit (Figure 2.1). No effects of 1-MCP were found for total flavonoid or anthocyanin concentrations, except for those of flavonoids in flesh of CA-stored fruit.

Total antioxidant activity, assayed using the recently developed PSC method (Adom and Liu, 2005), was higher in both peel and flesh tissues of 1-MCP-treated fruit compared with untreated fruit stored in air (Figure 2.3), although CA-stored fruit only higher in the flesh tissues (Table 2.8). Higher total antioxidant activity (total oxyradical scavenging capacity; TOSC) in 1-MCP treated peel tissues of air-stored 'Empire' and 'Delicious' apples was also found by MacLean et al. (2003). The reason for higher antioxidant activity in air-stored 1-MCP-treated fruit are uncertain. Both total phenolic concentrations and total antioxidant activity were higher in peel tissues (Figures 2.1 and 2.3), and these two factors have been strongly correlated with each other in other studies (Kahkonen et al., 1999; Sun et al., 2002). In the current study, however, a significant correlation was found only between anthocyanin concentrations and total antioxidant activity, and only in peel tissues of air-stored fruit. MacLean et al. (2003) suggested that higher antioxidant activities in 1-MCP treated fruit might be a result of the ability of 1-MCP, through unknown mechanisms, to inhibit generation of free radicals. Although total antioxidant activity as measured by the 1-diphenyl-2-picrylhydrazyl (DPPH) method (Kondo et al., 2002) was not affected by 1-MCP treatment on 'Golden Smoother' apple flesh, peroxidase activity was much greater in 1-MCP treated fruit than in untreated fruit (Vilaplana et al., 2006). MacLean et al. (2006) found greater retention of anthocyanins, higher flavonoid concentrations, and lower chlorogenic acid levels in peel tissues of 1-MCP treated compared with untreated 'Delicious' apples stored in air. The focus of the current study was on total phytochemical groups. While individual compounds have different antioxidant potential (Lee et al., 2003), the reactions among them may be synergistic. Therefore, total antioxidant activities potentially provide a better estimate of the

overall contributions of antioxidant components (Wang et al., 1996; Vinson et al., 2001; Liu, 2003).

Ascorbic acid concentrations declined in both peel and flesh tissues during air storage (Figure 2.2), while in CA storage, patterns of change over time were inconsistent, depending on O₂ partial pressures and storage temperature (Table 2.7). Surprisingly little information is available concerning changes in ascorbic acid concentrations during storage, especially in CA, of apple fruit, and even less is known about the effects of 1-MCP. Vilaplana et al. (2006) found declining ascorbic acid concentrations in flesh tissues of 1-MCP treated fruit compared with untreated fruit after a month of air storage. The significance of decreased ascorbic acid concentrations during storage and to a greater extent on 1-MCP treated fruit in that study and this one is uncertain. Although ascorbic acid is generally considered to be important in nutrition (Davey et al., 2000; Boyer and Liu, 2004), it represents a minor component of the total antioxidant activity of apples (Eberhardt et al., 2000; Sun et al., 2002). Ascorbic acid, however, is a critical component of antioxidative processes in plant cells, interacting enzymatically and non-enzymatically with damaging oxygen radical and reactive oxygen species (Davey et al., 2000).

Core browning of pears, which results from low temperature and elevated CO₂ levels in the storage atmosphere, is associated with decreased ascorbic acid concentrations (Larrigaudière et al., 2001; Franck et al., 2003; Franck et al., 2007). The 'Empire' apple can be highly susceptible to flesh browning, and incidence can be higher in 1-MCP treated fruit (Watkins, 2008). The question arises as to whether or not ascorbic acid concentrations in the tissue may be related to disorder development. In the current study, no flesh browning was found in air stored fruit, but a very high incidence of the disorder was found in fruit after 9 months of CA storage (Table 2.2). The high disorder incidence was consistent with major commercial losses from flesh

browning in the year of this study. Flesh browning was not consistently higher in 1-MCP treated fruit than untreated fruit and no correlations with total ascorbic acid concentrations were detected. Therefore, these data do not support a hypothesis that susceptibility of fruit to flesh browning is correlated with loss of ascorbic acid; however, because all treatments resulted in high disorder incidence by 9 months, it is possible that earlier analyses when major differences in disorder incidence among treatments exist might reveal associations between the two factors.

In conclusion, 1-MCP delays fruit ripening of 'Empire' apples, but the effects of treatment on phytochemical groups are relatively small. Nevertheless, further research is warranted on the effects of 1-MCP on various non-enzymatic and enzymatic antioxidant systems to better understand how 1-MCP can enhance total antioxidant activity in air-stored fruit, and in flesh, but not peels of CA-stored fruit. In addition, information about the effects of 1-MCP on antiproliferation activity of apple extracts (Eberhardt et al., 2000; Wolfe et al., 2003) is lacking. Ethylene plays both direct and indirect roles in ripening processes of apples, but includes stimulatory effects on the activity of phenylalanine ammonia-lyase (PAL), the first enzyme in the flavonoid biosynthesis pathway and therefore anthocyanin production (Blankenship and Unrath, 1988), and peroxidases (Leja et al., 2003). Interestingly, MacLean et al. (2006) found higher flavonoid concentrations, while Vilaplana et al. (2006) found higher peroxidase activity, in 1-MCP treated fruit compared with untreated fruit, suggesting that 1-MCP may have complex effects on fruit metabolism.

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CHAPTER THREE

EXTERNAL CARBON DIOXIDE INJURY AND 1-METHYLCYCLOPROPENE (1-MCP) IN THE 'EMPIRE' APPLE

Abstract

The effects of several factors on the susceptibility of untreated and 1-MCP-treated 'Empire' apple (*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.) fruit to external CO₂ injury have been investigated. These factors include CO₂ partial pressure, the timing of elevated CO₂ exposure, delays between harvest and exposure to elevated CO₂, DPA concentration, and the timing of DPA treatment after exposure of fruit to 1-MCP. 1-MCP-treated fruit were more susceptible to external CO₂ injury than untreated fruit when stored in 5 kPa, but not in 1 kPa CO₂ (in 2 kPa O₂). 1-MCP did not increase the period of highest susceptibility to injury during controlled atmosphere storage. The greatest sensitivity to injury occurred 0–3 weeks after harvest. Sensitivity to injury decreased when untreated fruit were kept in air for up to 14 days before exposure to 5 kPa CO₂, but not for 1-MCP treated fruit. DPA treatment prevented development of CO₂ injury even at a level as low as 250 μL L⁻¹. DPA treatment could be delayed for 4 days after 1-MCP treatment while fruit were exposed to 5 kPa CO₂ in air without injury development. Overall the data indicate that 1-MCP enhances the risk of external CO₂ injury, but this injury can be prevented by treatment of fruit with DPA at harvest. In the absence of DPA treatment, handling procedures such as maintaining low CO₂ partial pressures in the storage atmosphere must be used.

Keywords: Apple; Storage; Ethylene; Carbon dioxide injury; *Malus x domestica*

3.1. Introduction

Carbon dioxide injury is a physiological disorder of apples (*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.), which can be manifested externally and/or internally, the type of injury and susceptibility being affected greatly by apple cultivar and growing conditions (Colgan et al., 1999; Elgar et al., 1998; Elgar et al., 1999; Fernandez-Trujillo et al., 2001; Volz et al., 1998; Watkins et al., 1997; Wilkinson and Fidler, 1973). Cultivars that are susceptible to injury under commercial conditions include 'Braeburn', 'Empire', 'Fuji', 'McIntosh' and 'Cortland'. The 'Empire' apple has been a focus of interest because of sporadic occurrence of injury that has resulted in major financial losses to storage operators in Michigan and New York (Burmeister and Dilley, 1995; Watkins et al., 1997).

In 'Empire' apples, CO₂ injury is usually external, affected areas being sunken, pebbly textured, sometimes with bronze discoloration, but always with an intact cuticle and epidermis (Watkins et al., 1997). Major losses of fruit to CO₂ injury were associated with recommendations to the industry in the early 1990s to eliminate postharvest treatment with diphenylamine (DPA), an antioxidant used routinely to prevent the physiological disorder superficial scald (Watkins, 2003), because of commercially negligible risk of scald developing during storage with 'Empire' fruit. Subsequently, it was shown that DPA reduces or prevents development of both external and internal CO₂ injury (Argenta et al., 2002; Burmeister and Dilley, 1995; Colgan et al., 1999; Fernandez-Trujillo et al., 2001; Watkins, 2003). Fruit susceptibility to CO₂ injury is greatest early in controlled atmosphere (CA) storage and risk of injury can be reduced by maintaining low CO₂ concentrations in the storage atmosphere during this period (Argenta et al., 2000; Elgar et al., 1998; Smock and Blanpied, 1972; Watkins et al., 1997). Injury can also be reduced markedly by

extending the length that fruit are in air storage before application of CA storage (Argenta et al., 2000; Colgan et al., 1999; Elgar et al., 1998; Watkins et al., 1997).

1-Methylcyclopropene (1-MCP) is thought to interact with ethylene receptors and thereby prevent ethylene-dependent responses (Sisler and Serek, 1997; Sisler and Serek, 2003). Inhibition of ethylene production is associated with maintenance of flesh firmness and other quality attributes in apples, especially during shelf life periods (reviewed by Watkins, 2006). 1-MCP has been commercialized as a new postharvest technology (Blankenship and Dole, 2003) and has become widely incorporated into apple industries around the world (Watkins 2006). In the US, the technology involves application of gaseous 1-MCP into sealed rooms, at a concentration of $1 \mu\text{L L}^{-1}$ for 24 hours. However, an apparent side effect of 1-MCP application noticed experimentally and by storage operators in New York has been higher incidences of CO_2 injury in treated 'Empire' apples (Watkins and Nock, 2004). DeEll et al. (2003) found much higher susceptibility of fruit to injury in 'Empire' and 'McIntosh' apples, while Zanella (2003) described higher incidence of a disorder likely to be CO_2 injury in 'Granny Smith' apples.

Depending on the specific operation, fruit are treated with 1-MCP in small lots or when the storage rooms are filled with fruit. AgroFresh (2007) recommendations for application of 1-MCP vary by cultivar and country, but for 'Empire' are within 7 days from harvest. DPA is applied to apples by drenching or dipping of bins or fruit on arrival at the storage facility, but thermofogging of DPA (Capon et al., 1987; Hurndall et al., 1994), a technology that has been used in Europe for some time, is only now going through registration processes in the USA (P. Sanderson, PACE International, pers. commun.). DPA thermofogging involves treatment of filled storage rooms and therefore questions about the relative urgency of DPA and 1-MCP applications to the fruit exist.

The objectives of this study were to investigate the effects of CO₂ partial pressure, the timing of exposure to elevated CO₂, delays between harvest and exposure to elevated CO₂, DPA concentration, and the timing of DPA treatment after exposure of fruit to 1-MCP, on external CO₂ injury of 'Empire' apples.

3.2. Materials and methods

3.2.1. Fruit

The experiments described here were carried out over 2 years, but each experiment was carried out only once. 'Empire' fruit were harvested from the same commercial orchard in western New York in both years. The orchard block was chosen because of a history of fruit susceptibility to CO₂ injury. Fruit of uniform size and free from blemishes were picked during the commercial harvest period (Blanpied and Silsby, 1992) and transported to the laboratory on the day of harvest. For each experiment, fruit were randomized to provide experimental units of 45 to 50 fruit per replicate.

3.2.2. 1-MCP, DPA and atmosphere treatments

Specific details are described within each experiment, but general conditions for 1-MCP, DPA and atmosphere treatments are as follows. Fruit were treated with 1 μL L⁻¹ 1-MCP for 24 hours after overnight cooling, either by releasing 1-MCP from SmartFresh powder (AgroFresh Inc., Rohm & Haas Company, Philadelphia, PA) into sealed 135 L containers as described by Watkins et al. (2000), or by treatment with SmartFresh tablets into 4000 L plastic tents using a release and fan system supplied by the manufacturers. DPA was applied as Shield DPA 15% (Pace International, Wenatchee, WA), by submerging fruit in the concentrations indicated below for 1 min.

Atmospheres were applied via a flow-through system. Untreated and 1-MCP treated fruit were divided into replicates that were each held in 19 L glass jars or plastic pails, sealed and connected to an atmosphere mixing system, which delivered the prescribed humidified premixed gas mixtures of CO₂ and O₂, balance N₂, or CO₂ in air. Flow rates of the gases were maintained at 200 ml.min⁻¹, and atmospheres were monitored at least once daily by gas chromatography (Fisher Gas Partitioner, model 1200, Fisher Scientific, Springfield, NJ) or by a portable analyzer (model 280 COMBO, David Bishop Instruments, Heathfield, UK). Atmospheres were maintained within 0.2 kPa of the desired partial pressures throughout the experiments. Fruit were stored for 20 weeks at 2 °C unless indicated otherwise, and transferred to a controlled temperature evaluation room maintained at 20 °C for 7 days.

3.2.3. Fruit quality assessments

Internal ethylene concentrations (IECs) were measured on 1 ml samples of internal gas extracted from each of 10 fruit per replicate (Watkins et al., 2000). The firmness of these fruit was then measured on opposite pored sides of each apple using an EPT-1 pressure tester (Lake City Technical Products, Lake City, Canada) fitted with an 11.1 mm diameter head. All fruit were then assessed for external CO₂ injury. Severity was also rated using a scale, where 1, 2, 3 and 4 represented slight (barely perceptible by eye), moderate (clear injury, but <25%), severe (26-50%) and very severe (>50%) injury, respectively. Each fruit was then sliced at least three times to reveal presence or absence of internal disorders. Depending on the experiment, these included internal CO₂ injury indicated by cavities, flesh browning and brown core. The percentage of fruit with injury was calculated.

3.2.4. Experiments

3.2.4.1. Effects of CO₂ concentration in 1-MCP treated fruit

The jars were connected to the flow through system that provided 1, 2.5 or 5 kPa CO₂ (in 2 kPa O₂) and stored at 2 °C. There were three replicates for each CO₂ atmosphere and treatment.

3.2.4.2. Effects of timing of exposure to elevated CO₂ partial pressures during storage

This experiment was carried out in parallel with experiment in section 3.2.4.1. Fruit were treated exactly the same except that fruit were exposed to a single 3 week-exposure of 2.5 or 5 kPa CO₂ (in 2 kPa O₂) during storage at 2 °C. When the fruit were not exposed to elevated CO₂ partial pressures, they were stored in 1 kPa CO₂ (in 2 kPa O₂). The periods of exposure were 0–3, 4–6, 7–9 or 10–12 weeks after harvest. Three replicates for either untreated or 1-MCP treated fruit were exposed to 2.5 or 5 kPa CO₂ for the first 3 weeks during storage and then transferred to 1 kPa CO₂. For the other exposure treatments, fruit were kept at 1 kPa CO₂ and then transferred to 2.5 or 5 kPa CO₂ for each 3-week period. Fruit were returned to 1 kPa after each exposure period the experiment ran for a total of 20 weeks.

3.2.4.3. Effects of delayed exposure to elevated CO₂

Fruit were cooled overnight and untreated or treated with 1-MCP. Untreated fruit were exposed to 5 kPa CO₂ (in 2 kPa O₂) at 1, 2, 7 or 14 days after harvest, while 1-MCP-treated fruit were exposed to 5 kPa CO₂ (in 2 kPa O₂) at 2, 7 or 14 days after harvest. Fruit were stored in these CA conditions and assessed after 10 weeks plus 7 days at 20 °C. There were four replicates per treatment.

3.2.4.4. Effects of exposure to elevated CO₂ before CA storage

Untreated or 1-MCP-treated fruit were exposed to 5 kPa CO₂ in air for 0, 1, 2, 4, 6 or 8 days. Additional untreated or 1-MCP treated fruit were dipped in DPA on the day of harvest and kept in 5 kPa CO₂ for 0 and 8 days. There were four replicates per treatment. At each time interval fruit were transferred to a commercial CA storage room maintained at 1.5 kPa CO₂ and 2.5 kPa O₂ at 2 °C for 16 weeks.

3.2.4.5. Effects of DPA concentration

Fruit were either untreated or treated with 250, 500 or 1000 µL L⁻¹ DPA on the day of harvest, cooled overnight, treated with 1-MCP, and exposed to 5 kPa CO₂ (in 2 kPa O₂) for 10 weeks. There were four replicates for each treatment. Only storage disorders were assessed.

3.2.4.6. Effects of timing of DPA treatment after 1-MCP treatment

Untreated or 1-MCP-treated fruit were exposed to 5 kPa CO₂ in air for 0, 1, 2, 4, 6 or 8 d. At each time interval fruit were dipped in DPA at 2 °C, allowed to drain dry for 15 min. and returned to 5 kPa CO₂ in air. All fruit were removed to 20 °C for evaluation of disorders after a total of 14 days of exposure to elevated CO₂. There were four replicates for each treatment.

3.2.5. Statistical analyses

Data were subjected to analysis of variance procedures, using balanced models, or to regression analysis (Minitab Release 13.1, State College PA). Variances were checked and where appropriate, data were transformed by logarithms for IEC data or arcsine for percentages. Percentage data are shown as back-transformed means.

3.3. Results

3.3.1. Effect of CO₂ concentration in 1-MCP treated fruit

Untreated and 1-MCP treated fruit were stored at 1, 2.5 or 5 kPa CO₂ for 20 weeks (Table 3.1). Overall, the log IEC and firmness values for untreated and treated fruit were 2.00 and -0.05 $\mu\text{L L}^{-1}$ and 61.0 and 65.1 N, respectively. No effect of CO₂ was detected.

Overall, external CO₂ injury incidence was greater in 1-MCP treated fruit than in untreated fruit, being 29% and 17%, respectively ($P=0.018$), and incidence increased with higher CO₂ partial pressure ($P<0.001$). However, a statistical significant effect of 1-MCP on injury was detected only at 5 kPa CO₂ (Table 3.1). Severity of injury was affected by CO₂ partial pressure ($P=0.001$) but not by 1-MCP, averaging 0.5, 1.2 and 1.5 units for 1, 2.5 and 5 kPa CO₂, respectively. Internal CO₂ injury also was not affected by 1-MCP treatment ($P=0.202$), but increased with higher CO₂ concentration ($P=0.001$). Flesh browning was also affected only by CO₂, incidence being higher in 2.5 and 5 kPa than at 1 kPa ($P=0.011$). Brown core incidence was greater in higher CO₂ partial pressures ($P=0.046$), and higher in 1-MCP treated fruit than in untreated fruit ($P=0.031$).

3.3.2. Effects of timing of exposure to elevated CO₂ concentrations during storage

Untreated and 1-MCP treated fruit were stored in 1 kPa CO₂ and transferred to either 2.5 or 5 kPa CO₂ for three-week intervals, and returned to 1 kPa CO₂. Different exposure times to either CO₂ partial pressure during storage did not affect log IEC or firmness values (Figures 3.1 A and B). Only 1-MCP affected these factors ($P<0.001$),

overall log IEC and firmness being 1.94 and $-0.01 \mu\text{L L}^{-1}$, and 62.6 and 64.8 N, respectively.

In contrast, there was a marked effect of timing of exposure to CO_2 on external and internal CO_2 injury (Figures 3.2 A and B); the incidence of either injury was highest when fruit were exposed to either 2.5 or 5 kPa CO_2 during the first three weeks of storage. Neither type of CO_2 injury was affected by 1-MCP treatment, nor by 2.5 kPa compared with 5 kPa CO_2 in the storage atmosphere. Severity ratings for external injury were 1.4, 0.6, 0.7 and 0.3 units for fruit exposed to 2.5 and 5 kPa CO_2 for weeks 0–3, 4–6, 7–9 and 10–12, respectively ($P < 0.001$). No effects of other factors or interactions among them were detected (data not shown).

Flesh browning was not affected by CO_2 , exposure time, or 1-MCP (data not shown). Statistically, brown core was affected by exposure time ($P = 0.024$), being 1.3, 0.6, 2.6 and 0.3% for 0–3, 4–6, 7–9 and 10–12 weeks, respectively, but these levels are low and of uncertain physiological significance.

3.3.3. Effects of delayed exposure to elevated CO_2

The IEC increased and firmness decreased in fruit without 1-MCP treatment as fruit were kept in air for increasing periods of time before they were exposed to 5 kPa CO_2 (Table 3.2). 1-MCP-treated fruit had lower IECs and were firmer than untreated fruit. The IEC of 1-MCP-treated fruit was not affected by delayed exposure to elevated CO_2 , while firmness of fruit increased during this time.

The incidence of external CO_2 injury of fruit without 1-MCP treatment was not affected by a 2 day delay before exposure to 5 kPa CO_2 compared with 1 day, but by 7 days was reduced by almost 50% of those measured in fruit-treated on day 1 (Table 3.2). A 14 days delay resulted in almost complete control of injury. In fruit that were

Table 3.1 Log internal ethylene concentration (IEC), flesh firmness, external CO₂ injury, internal CO₂ injury, firm flesh browning and core browning in ‘Empire’ apples, either untreated or treated with 1 µL L⁻¹ 1-MCP, and stored in 1, 2.5 or 5 kPa CO₂ (in 2% O₂) for 20 weeks at 2 °C. Fruit were assessed after 7 days at 20 °C.

| CO ₂ concentration in storage (%) | Log IEC (µL L ⁻¹) | | Firmness (N) | | Ext. CO ₂ injury (%) | | Int. CO ₂ injury (%) | | Flesh browning (%) | | Core browning (%) | |
|--|-------------------------------|--------|--------------|--------|---------------------------------|--------|---------------------------------|--------|--------------------|--------|-------------------|--------|
| | - 1-MCP | +1-MCP | - 1-MCP | +1-MCP | - 1-MCP | +1-MCP | - 1-MCP | +1-MCP | - 1-MCP | +1-MCP | - 1-MCP | +1-MCP |
| 1.0 | 2.11 | -0.01 | 61.4 | 66.1 | 2 | 2 | 1 | 0 | 1 | 3 | 1 | 3 |
| 2.5 | 1.96 | -0.04 | 59.9 | 65.0 | 21 | 30 | 4 | 6 | 6 | 10 | 4 | 7 |
| 5.0 | 1.93 | -0.10 | 61.5 | 64.2 | 28 | 56 | 8 | 13 | 7 | 4 | 5 | 10 |
| LSD (P = 0.05) | 0.278 | | 3.48 | | 17.2 | | 5.9 | | 4.9 | | 2.9 | |

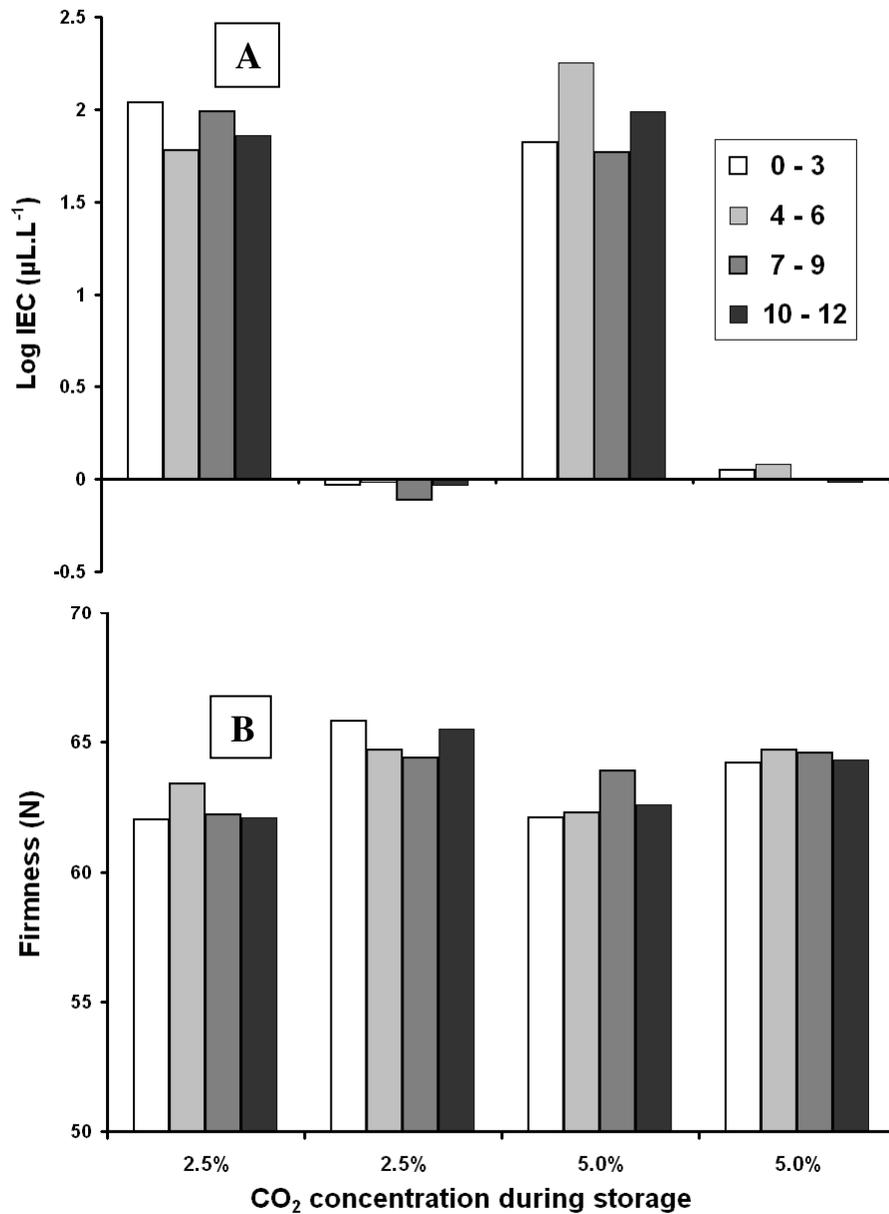


Figure 3.1 Log internal ethylene concentration ($\mu\text{L L}^{-1}$) (A); and flesh firmness (N) (B); in 'Empire' apples, either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-M CP, and stored in 1 kPa carbon dioxide (in 2 kPa oxygen) for up to 20 weeks at 2 °C. Fruit were exposed to 2.5 or 5 kPa carbon dioxide (in 2 kPa oxygen) at 0–3, 4–6, 7–9, 10–12 weeks. Fruit were assessed after 7 days at 20 °C. The vertical bars represent the LSD ($P=0.05$) for comparison of means.

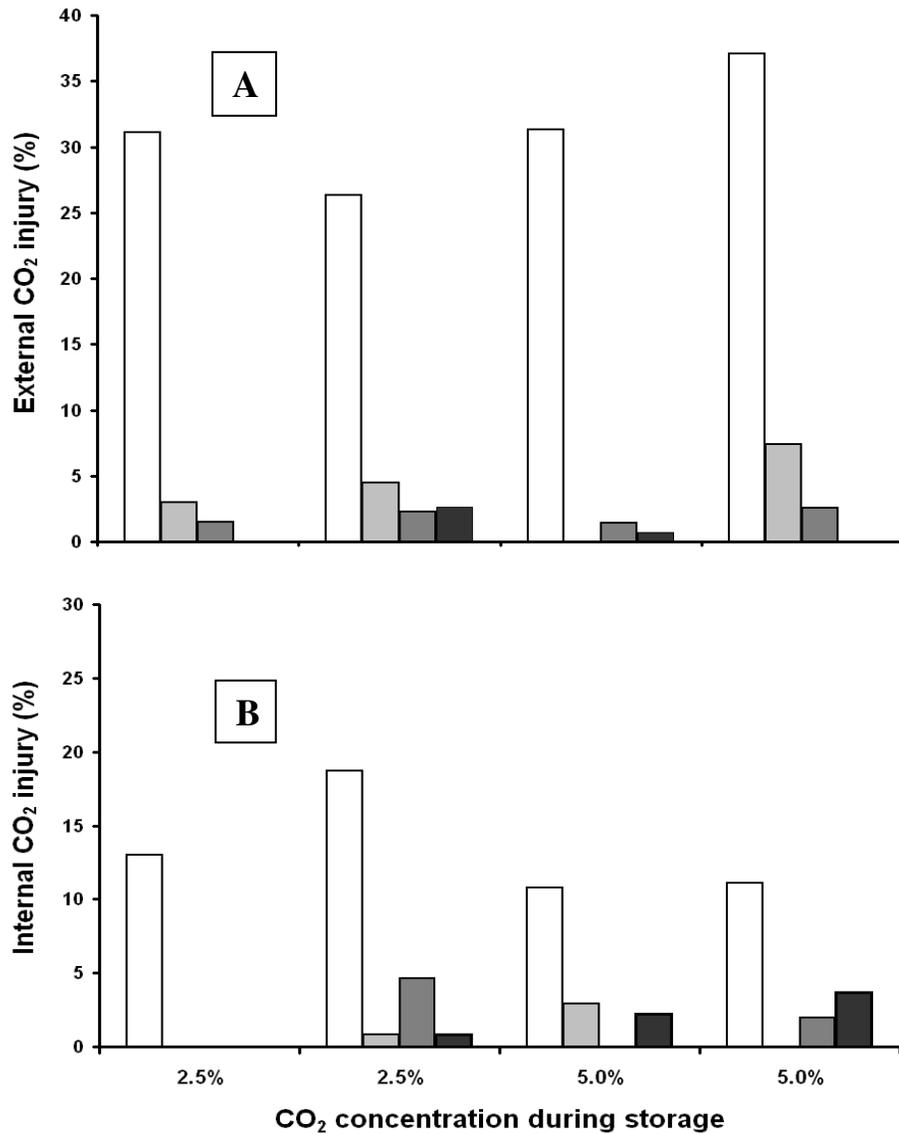


Figure 3.2 External (A) and internal (B) carbon dioxide injury (%) in ‘Empire’ apples, either untreated or treated with $1 \mu\text{L L}^{-1}$ 1-MCP, and stored in 1kPa carbon dioxide (in 2 kPa oxygen) for up to 20 weeks at 2 °C. Fruit were exposed to 2.5 or 5 kPa carbon dioxide (in 2 kPa oxygen) at 0–3, 4–6, 7–9, 10–12 weeks. Fruit were assessed after 7 days at 20 °C. The vertical bars represents the LSD ($P=0.05$) for comparison of means.

Table 3.2 Log internal ethylene concentration (IEC) ($\mu\text{L L}^{-1}$), flesh firmness (N) and incidence and severity of external CO_2 injury in ‘Empire’ apple fruit that were cooled overnight and exposed to 5 kPa CO_2 (in 2 kPa O_2) either 1, 2, 7 or 14 days after harvest, or cooled overnight, treated with 1 $\mu\text{L L}^{-1}$ MCP, and then exposed to 5 kPa CO_2 either 2, 7 or 14 days after harvest. Fruit were removed from storage after 1 weeks and kept at 20 °C for 7 days before evaluation.

¹Severity rating of 1 to 4 represents slight, moderate, severe and very severe, respectively

²Standard error of the mean

³L = linear, C = cubic; NS, *, *** represent non significant, or significant at $P < 0.05$ and < 0.001 , respectively

| Time after harvest (Days) | Log IEC ($\mu\text{L L}^{-1}$) | | Firmness (N) | | External CO_2 injury | | | |
|---------------------------|----------------------------------|---------|--------------|---------|-------------------------------|---------|--------------------------------|---------|
| | - 1-MCP | + 1-MCP | - 1-MCP | + 1-MCP | (%) | | (severity rating) ¹ | |
| | - 1-MCP | + 1-MCP | - 1-MCP | + 1-MCP | - 1-MCP | + 1-MCP | - 1-MCP | + 1-MCP |
| 1 | 2.30 | | 63.1 | | 69.6 | | 2.1 | |
| 2 | 2.36 | 0.35 | 60.8 | 66.6 | 68.7 | 71.1 | 2.2 | 2.2 |
| 7 | 2.30 | 0.31 | 62.3 | 68.9 | 34.1 | 70.7 | 1.3 | 1.8 |
| 14 | 2.43 | 0.23 | 58.8 | 69.9 | 0.6 | 58.9 | 0.3 | 1.7 |
| SEM ² | 0.040 | | 0.86 | | | | | |
| Regression ³ | L* | NS | L*, C* | L* | L*** | NS | L*** | L* |

treated to 1-MCP, however, the incidence of external CO₂ injury remained statistically unchanged by all delays. Severity of injury declined with increasing delays but to only a small extent. No other disorders were detected.

3.3.4. Effects of exposure to elevated CO₂ before CA storage

Untreated and treated fruit were kept in 5 kPa CO₂ in air for up to 8 days before being transferred to standard CA conditions. Overall, external CO₂ injury in 1-MCP-treated fruit averaged 58% compared with 24% in untreated fruit (Figure 3.3). The incidence of CO₂ injury increased with longer exposure to high CO₂, and was lowest in fruit exposed for only 1–2 days. No injury was detected in any DPA-treated fruit, exposed to 5 kPa CO₂ for either 0 or 8 days. Log IEC and firmness averaged 2.34 and 0.09 $\mu\text{L L}^{-1}$, and 60.8 and 69.7 N, in untreated and 1-MCP-treated fruit, respectively.

3.3.5. Effects of timing of DPA treatment after 1-MCP treatment

It was not possible to replicate thermofogging of fruit in these experiments and therefore fruit were removed briefly from elevated CO₂ partial pressures and dipped in DPA before being returned to atmospheres. No injury was detected in either untreated or 1-MCP-treated fruit when treated with DPA at 1, 2 or 4 days (Figure 3.4).

A low level of injury was observed in untreated fruit if they were not dipped with DPA until day 6, while more noticeable injury occurred in 1-MCP-treated fruit at this time. No other disorders were detected.

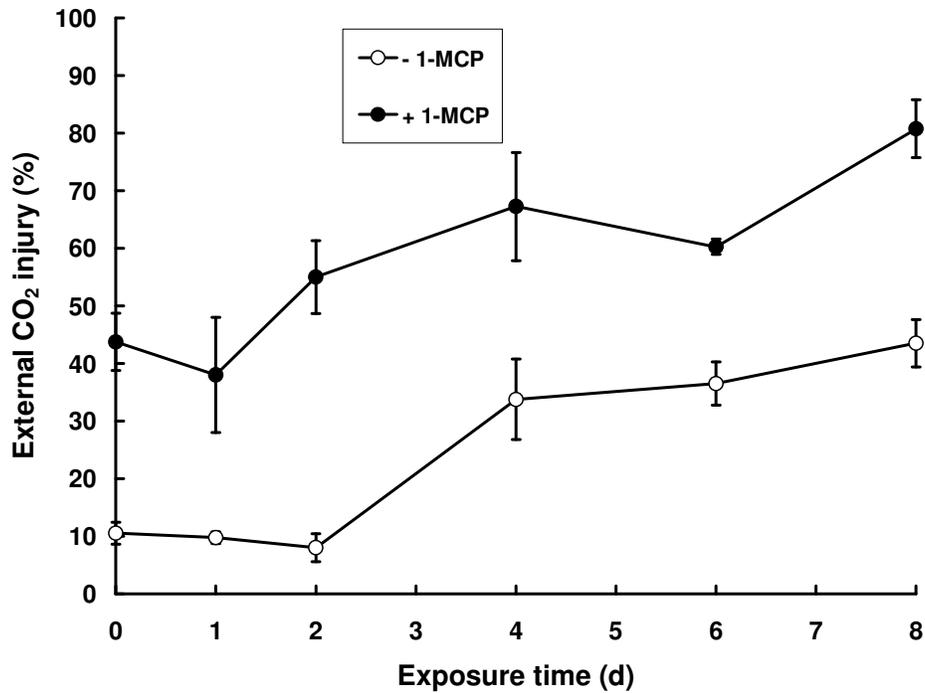


Figure 3.3 External CO₂ injury (%) in ‘Empire’ apples \pm S.E., either untreated or treated with 1 $\mu\text{L L}^{-1}$ 1-MCP, and stored in 5 kPa CO₂ (in air) up to 8 d at 2 °C, before being stored in CA storage. Fruit were evaluated after 16 weeks plus 7 days at 20 °C. The linear regressions of injury against time for untreated and 1-MCP-treated fruit were significant at $P < 0.001$ and $P = 0.01$, respectively.

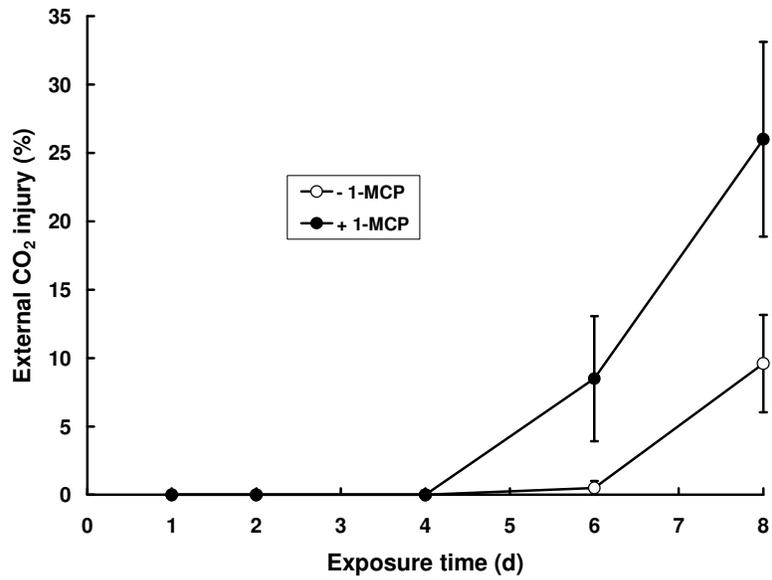


Figure 3.4 External CO₂ injury (%) in ‘Empire’ apples \pm S.E., either untreated or treated with 1 μ L L⁻¹ 1-MCP, and stored in 5 kPa CO₂ (in air) for 14 days at 2 °C. Fruit were removed from the storage atmosphere 1, 2, 4, 6, and 8 days, dipped in 1000 μ L L⁻¹ DPA before being returned to CO₂. Fruit were evaluated after 14 days plus 1 day at 20 °C.

3.3.6. Effects of DPA concentration

The IEC of the fruit was not affected by DPA treatment, but firmness of fruit declined slightly as DPA concentration increased (Table 3.3). External CO₂ incidence and severity was high in untreated fruit, but no injury was detected on fruit treated with any DPA concentration. Flesh browning (4%) was detected only in untreated fruit.

Table 3.3 Internal ethylene concentrations (IEC, $\mu\text{L L}^{-1}$), flesh firmness (N) and incidence and severity of external CO_2 injury in ‘Empire’ apple fruit either untreated or treated with 250, 500 or 1000 $\mu\text{L L}^{-1}$ diphenylamine (DPA), cooled overnight, treated with 1 $\mu\text{L L}^{-1}$ 1-MCP, and exposed to 5 kPa CO_2 (in 2 kPa O_2). Fruit were removed from storage after 10 weeks and kept at 20 °C for 7 days before evaluation.

¹Severity rating of 1 to 4 represents slight, moderate, severe and very severe, respectively

²Standard error of the mean

³L = linear; NS, *, *** represent non significant, or significant at $P < 0.05$ and < 0.001 , respectively

| DPA ($\mu\text{L L}^{-1}$) | IEC ($\mu\text{L L}^{-1}$) | Firmness (N) | External CO_2 injury | |
|------------------------------|------------------------------|--------------|-------------------------------|--------------------------------|
| | | | (%) | (severity rating) ¹ |
| 0 | 2.04 | 72.3 | 84.3 | 2.1 |
| 250 | 1.84 | 73.6 | 0 | 0 |
| 500 | 1.35 | 71.1 | 0 | 0 |
| 1000 | 1.80 | 70.1 | 0 | 0 |
| SEM ² | 0.426 | 0.51 | 4.18 | 0.18 |
| Regression ³ | NS | L* | L*** | L*** |

3.4. Discussion

1-MCP-treated fruit had low IECs and were firmer than untreated fruit in all the experiments reported here (Tables , Figure 3.1) confirming the inhibitory effect of 1-MCP on ripening of ‘Empire’ apples (Watkins and Nock, 2005; Watkins et al., 2000) as well as many other apple cultivars (Fan et al., 1999; Rupasinghe et al., 2000; Watkins, 2006). The primary focus of our study was to evaluate the effects of 1-MCP on external CO_2 injury of ‘Empire’ apples. To maximize the differences among treatments we used fruit harvested from an orchard block that is known to be highly susceptible to injury, and treated fruit with CO_2 partial pressures as high as 5 kPa.

Although CO₂ concentrations should be kept low while fruit are being loaded into rooms, undesirably high partial pressures approaching 5 kPa have been measured in some commercial operations where fruit are warm, ventilation poor, and lime added to rooms only at the end of room loading (Watkins, unpublished data). As would be expected for a disorder that is related to the presence of CO₂ (Burmeister and Dilley, 1995; Watkins et al., 1997) increasing CO₂ partial pressures in the storage atmospheres resulted in higher injury incidences (Table 3.1). In addition, 1-MCP increased the susceptibility of fruit to injury in 5 kPa CO₂ storage atmospheres. Although no effects of 1-MCP were detected at 1 and 2.5 kPa CO₂, statistically significant increases in injury incidence at 2 kPa CO₂ have been shown in other studies (unpublished data). These results confirm that 1-MCP increases external CO₂ injury shown by DeEll et al. (2003) for ‘McIntosh’ as well as ‘Empire’ apples.

A possible reason for increased susceptibility of 1-MCP-treated fruit to injury is that 1-MCP increases the period of highest susceptibility to injury during the storage period. A feature of apple fruit susceptibility to both external and internal CO₂ injuries is that injury occurs within the first several weeks of CA storage (Smock and Blanpied, 1972; Watkins et al., 1997; Elgar et al., 1998; Argenta et al., 2000). However, susceptibility of ‘Empire’ apples to injury was high in either 2.5 or 5% CO₂ only from weeks 0–3, regardless of whether or not fruit were 1-MCP-treated (Figure 3.2). Although injury appeared slightly higher in 1-MCP-treated fruit when exposed to elevated CO₂ for intervals later in the storage period, differences were not significant. Overall the data suggest that the current recommendation to maintain 1 kPa CO₂ or lower in the storage atmosphere for the first 4-6 weeks (Watkins and Nock, 2004) is appropriate, but the presence of a cumulative effect of CO₂ (Table 3.1) suggests that CO₂ partial pressures less than 2 kPa should be maintained throughout the storage period.

Delays in the establishment of CA storage can markedly reduce susceptibility of fruit to CO₂ injury (Watkins et al., 1997; Elgar et al., 1998; Colgan et al., 1999; Argenta et al., 2000). Development of CO₂ injury declined by 50% and was negligible if fruit were kept in air for 7 and 14 days, respectively, before exposure to 5 kPa CO₂ (Figure 3.3). However, injury remained high in 1-MCP-treated fruit, even after 14 days. Without 1-MCP, fruit quality decreases if fruit are kept in air for excessive periods and larger storage operations usually apply 'rapid CA' in which O₂ is reduced to 5 kPa within 5-7 days of harvest. Low IECs and high firmness of 1-MCP-treated fruit (Table 3.2) suggest that delays before CA storage may not result in loss of quality when 1-MCP technology is applied. Additional evidence that 1-MCP enhances early susceptibility of fruit to CO₂ injury is shown by the effects of 1-MCP when fruit were kept in a high CO₂ partial pressure in air prior to CA storage (Figure 3.3).

The reason that 1-MCP maintains susceptibility of fruit to CO₂ injury is not clear as little is known about the metabolic changes that occur during development of injury. It has long been thought that CO₂ injury was associated with accumulations of succinate in the tissue of CO₂-treated fruit (Hulme, 1956), but Fernandez-Trujillo et al. (2001) found similar levels of accumulation in fruit with and without DPA treatment. Burmeister and Dilley (1995) suggested that the CO₂ injury results from free radical catalyzed oxidation of susceptible amino acid residues in proteins essential for cell function. Accumulations of the fermentation products acetaldehyde and ethanol occurs in CO₂-treated fruit, but concentrations are not consistently related to injury and do not appear to be causal (Argenta et al., 2002; Fernandez-Trujillo et al., 2001; Volz et al., 1998). The observation that susceptibility of fruit to injury declines during air storage (Figure 3.3) suggests that some adaptation of fruit occurs after harvest that may be related to early ripening events. By preventing ripening of the fruit, 1-MCP appears to keep the fruit closer to its 'at harvest' state and thereby more susceptible to

injury. It is likely that 1-MCP treatment will maintain the risk of damage in other cultivars such as 'McIntosh' and 'Empire' (DeEll et al., 2003) as well as other cultivars such as 'Fuji' and 'Braeburn' (Elgar et al., 1998; Argenta et al., 2002) that are highly susceptible to CO₂ injury.

The powerful effect of DPA in preventing development of external and internal CO₂ injury (Argenta et al., 2002; Burmeister and Dilley, 1995; Colgan et al., 1999; Fernandez-Trujillo et al., 2001; Watkins et al., 1997) is confirmed here (Table 3.3). In addition, it is possible to use much lower DPA rates than required to control superficial scald, even with highly susceptible fruit and exposure to high CO₂ partial pressures. Because a primary action of DPA in preventing superficial scald is probably by its action as a free radical scavenger (Whitaker, 2004; Yeh et al., 2003), the results suggest that oxidative reactions may be involved in development of CO₂ injury. DPA is routinely used on 'Empire' apples, although many storage operators prefer to avoid the use of postharvest chemical drenches as they can increase decay, despite the presence of fungicides. If DPA is not used, extra caution must be taken to avoid accumulation of CO₂ in the storage atmosphere during fruit loading and during the first several weeks of CA storage. The probable advent of thermofogging technology for DPA application in the US raises an interesting question about the timing of DPA application relative to that of 1-MCP in CO₂ susceptible fruit. Our data show that injury developed on in fruit after 4 days of exposure to 5 kPa CO₂, though to a greater extent in 1-MCP-treated fruit than in untreated fruit (Figure 3.4). It is likely that in the case of less susceptible fruit and with control of CO₂ partial pressures in the storage, that the safe period to avoid injury is longer and perhaps closer to the registered 7 day maximum period after harvest for DPA thermofogging (Sanderson, pers. communication).

Few other disorders other than external and internal CO₂ injuries were detected in these studies, probably because the storage periods were generally short. An exception was flesh browning and brown core that was increased by elevated CO₂ partial pressures in storage (Table 3.1). The inhibitory effect of DPA on the disorder also suggests that it is aggravated by CO₂.

In conclusion, 1-MCP enhances risk of external CO₂ injury of 'Empire' apples. The reasons for increased sensitivity have not been established and require better understanding of the mechanisms of damage. Injury occurs in the first few weeks of storage, but while the period of high risk in storage is not increased by 1-MCP treatment, 1-MCP prevents the loss of sensitivity to CO₂ that occurs in fruit kept in air before CA storage. DPA completely controls CO₂ injury, even at low concentrations, and provides a safe and effective means to prevent injury development. Delaying DPA treatment after harvest, as would be necessary for thermofogging treatments, can be effective, but will be influenced by sensitivity of fruit to injury and control of CO₂ levels during loading, either with good ventilation or the addition of lime. External CO₂ injury can be avoided or reduced without DPA treatment, but special care is essential to avoid CO₂ accumulation during filling of rooms and during the first 4–6 weeks of storage.

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CHAPTER FOUR

SUMMARY

The effects of 1-methylcyclopropene (1-MCP) in postharvest preservation of physical quality and internal quality related to antioxidant status of 'Empire' apple were studied in this research project.

The second chapter focused on experiments investigating the effects of 1-MCP, in air and controlled atmosphere (CA) storage of 'Empire' apple on the concentrations of phytochemical groups that are of health benefits including vitamin C and their total antioxidant capacity. In the third chapter, experiments to study the effects of 1-MCP on the occurrence of physiological disorders in CA storage of apple were described particularly that of external carbon dioxide injury in CA-stored 'Empire' apple.

Fruit were stored in air for up to 5 months, and in controlled atmosphere (CA) in 2 and 3 kPa O₂ (2% CO₂) at 0.5 and 2.2 °C for 4.5 and 9 months. Ripening was delayed by 1-MCP treatment in both air and CA storage as indicated by internal ethylene concentrations and flesh firmness. Overall, total phenolic, flavonoid and anthocyanin concentrations as well as antioxidant activity are relatively stable during air and CA storage. Phytochemical concentrations and antioxidant activities were consistently higher in the 'Empire' apple peel tissue than in the flesh in both air and CA. There were no correlations found between total phenolics and antioxidant activity. 1-MCP effects on individual phytochemicals were variable. Moreover 1-MCP affects the peel and the flesh tissue differently. For fruit stored in air, total phenolic concentrations were higher in the peel but lower in the flesh. Variable

results were observed in CA storage, interactions among O₂ level, storage temperature and storage period interactions being complex. 1-MCP did not affect total flavonoid or anthocyanin concentrations except for flavonoid in the flesh of CA-stored fruit. Flavonoid concentrations were higher in the flesh of 1-MCP treated than untreated fruit kept in 2 kPa O₂ while anthocyanin concentrations were not affected by 1-MCP treatment. Ascorbic acid concentrations declined in both peel and flesh tissues of untreated and 1-MCP treated fruit stored in air. In CA the change was affected by these storage parameters but there were inconsistent pattern in the decline of ascorbic acid concentrations in CA-stored fruit. High incidence of flesh browning was observed at 9 months of CA storage in all treatments and this might have an effect on individual phytochemicals.

1-MCP was known to exacerbate certain physiological disorders after harvest. The effects of several factors such as CO₂ partial pressure, the timing of elevated CO₂ exposure, delays between harvest and exposure to elevated CO₂, DPA concentration, and the timing of DPA treatment after exposure of fruit to 1-MCP on the susceptibility of untreated and 1-MCP-treated 'Empire' apple fruit to external CO₂ injury have also been investigated and the highlights of the series of experiments for this study were described in chapter three. 1-MCP-treated fruit were more susceptible to external CO₂ injury than untreated fruit when stored in 5 kPa, but not in 1 kPa CO₂ (in 2 kPa O₂). 1-MCP did not increase the period of highest susceptibility to injury during controlled atmosphere storage. The greatest sensitivity to injury occurred 0–3 weeks after harvest. Sensitivity to injury decreased when untreated fruit were kept in air for up to 14 days before exposure to 5 kPa CO₂, but not for 1-MCP treated fruit. DPA treatment prevented development of CO₂ injury even at a level as low as 250 µL L⁻¹. DPA treatment could be delayed for 4 days after 1-MCP treatment while fruit were exposed to 5 kPa CO₂ in air without injury development. Overall the data indicate

that 1-MCP enhances the risk of external CO₂ injury, but this injury can be prevented by treatment of fruit with DPA at harvest. In the absence of DPA treatment, handling procedures such as maintaining low CO₂ partial pressures in the storage atmosphere must be used.

The lack of trends as of the effects of 1-MCP, storage factors (storage temperature, O₂ level and storage duration) and the lack of correlations between total phenolics and antioxidant capacity suggest that 1-MCP and storage factors may affect individual phytochemicals within the same class of total phenolics differently. It has been suggested that flavonoid biosynthesis is affected by ethylene, thus a study of the effects of 1-MCP treatment on the main individual flavonoid compounds which contribute differently to the antioxidant capacity of phytochemicals in apple could be of interest. Moreover, 1-MCP affects phytochemical compounds in apple peel and flesh differently and the locations of particular phytochemicals in the apple tissues are variable. Certain antioxidants are present in higher concentrations in the peel than in the flesh, and synergistic effects of the phytochemicals on antioxidant capacity could also exist. Highlights on the difference in phytochemical composition of the peel and the flesh can also be useful.

It may be worthwhile to look into the role of the ascorbate-glutathione cycle in relation to the occurrence of external CO₂ injury. The study of the effects of 1-MCP treatment of the fruit on antioxidative enzymes such as catalase and ascorbate peroxidase and metabolites such as ascorbate and glutathiones can be useful as they may be involved in the detoxification mechanism of the apple peel tissue where most symptoms of CO₂ injury disorder manifest. A study of the relationship between injuries that occur in CA storage such as flesh browning and CO₂ injury and antioxidant concentrations, and how they evolve over time during the storage duration can also be useful in order to estimate the apple optimal storage duration.

In conclusion, this study indicates that, despite the benefits of the use of 1-MCP technology in counteracting ethylene actions and thus delaying ripening and maintaining firmness even for long term storage, challenges arise from its application depending on the cultivar and the storage conditions used. More information is needed to further understand the effects of 1-MCP treatment of apple on phytochemical concentrations and antioxidant activity of stored fruit. Further study is needed regarding the effects of antioxidants on the occurrence of physiological disorders such as flesh browning and external CO₂ injury as affected by 1-MCP. For CO₂ injury susceptible fruit, DPA treatment is still the safest and most effective way to completely control the occurrence of this injury, and in case DPA is not used particular care should be taken to maintain CO₂ partial pressure in CA at a low level.