EFFECT OF NITRIC OXIDE ON METABOLISM OF FRESH-CUT APPLES AND LETTUCES IN RELATION TO SURFACE BROWNING

By

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DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

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PUBLICATIONS

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Journal paper

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Conference papers

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LIST OF ABBREVIATIONS

AAP	ascorbic acid-2-phospate
AATP	ascorbic acid-3-triphosphate
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
DETA	diethylenetriamine
DETANO	diethylenetriamine nitric oxide
DNA	deoxyribonucleic acid
EDRF	endothelium-derived relaxing factor
EDTA	ethylenediamine tetraacetic acid
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
$HO^{\bullet}$	hydroxyl radical
H_2O_2	hydrogen peroxide
HR	hypersensitivity response
IFPA	International Fresh-cut Produce Association
Inos	inducible NOS
LOX	lipoxygenase
mRNA	messenger RNA
MDA	Malondialdehyde
NiR	nitrite reductase
NO	nitric oxide
$NO^{\bullet}$	free radical nitric oxide
NO^{+}	nitrosonium cation
NO^{-}	nitroxyl anion
NO_2	nitrogen dioxide, nitrogen peroxide
N_2O	nitrous oxide
NOS	nitric oxide synthase
NR	nitrate reductase

1-MCP	1-methylcyclopropene
O_2	oxygen
$O_2^{\bullet-}$	superoxide anion
$OONO^-$	peroxynitrite ion
PAL	phenylalanine ammonia lyase
PBN	N-*tert*-butyl-α-phenylnitrone
POD	peroxidase
PPO	polyphenol oxidase
ROS	reactive oxygen species
RNA	ribonucleic acid
SNAP	S-nitroso-N-acetylpenicillamine
Sin^{-1}	3-morpholinosyl-nonomone
SNP	sodium nitroprusside

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ABSTRACT

Surface browning is an important cause of deterioration of fresh-cut produce during postharvest handling. Nitric oxide (NO) has recently been found to delay the onset of surface browning in fresh-cut apples and iceberg lettuce.

Effectiveness of NO applied as NO gas and the NO donor compound 2,2'-(hydroxynitrosohydrazino)-bisethanamine (diethylenetriamine nitric oxide, DETANO) dissolved in phosphate buffer (pH 6.5) solution on the physiological parameters of ethylene production, respiration and water loss, and biochemical parameters of total phenol content, PPO activity, ion leakage and lipid peroxide level were investigated. Granny Smith apple slices treated with 10 $\mu l.l^{-1}$ NO gas and 10 $mg.l^{-1}$ DETANO showed delayed development of surface browning and also resulted in a lower total phenol content, inhibition of PPO activity, reduced ion leakage and reduced rate of respiration but had no significant effect on ethylene production, water loss or lipid peroxide level as measured by malondialdehyde and hydrogen peroxide levels. The two control treatments of phosphate buffer (pH 6.5) and water dips also had significant effects on all parameters compared to untreated slices. The relative effectiveness treatments on postharvest life, apple physiology and biochemistry was DETANO > NO gas > phosphate buffer > water > untreated. The NO donors, sodium nitroprusside (SNP) and Piloty's acid dissolved in water also inhibited development of surface browning but were not as effective as DETANO.

Apple slices dipped in chlorogenic acid dissolved in water showed surface browning within an hour of treatment. Dipping in DETANO solution negated the effect of chlorogenic acid whether applied before or after dipping in chlorogenic acid solution while the buffer and NO

gas were also effective. A UV-scan of chlorogenic acid dissolved in water showed a marked decreased in absorbance over the eight day storage period suggesting that chlorogenic acid was oxidised by aerial oxygen. The addition of NO gas and DETANO accelerated the loss of chlorogenic acid.

It is suggested that browning development of fresh-cut produce can be inhibited by action taken soon after cutting. The concentration of phenols on the surface could be the rate limiting steps in browning development with non-enzymatic oxidation of phenols by atmospheric oxygen a contributor to browning.

NO gas, DETANO and SNP inhibited the surface browning of green oak lettuce slices. The optimum concentration of DETANO or SNP (500 $mg.l^{-1}$) and NO gas (100 $\mu l.l^{-1}$) resulted in approximately 60% and 30% increase in postharvest life over untreated slices respectively.

1. INTRODUCTION

Fresh-cut produce is cleaned, cored, peeled, chopped, sliced, or diced and packaged fresh fruits and vegetables that are marketed as a value-added agricultural commodity ready for consumer use. Fresh-cut produce is defined as "any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state" (IFPA and PMA, 1999). Fresh-cut produce is gaining popularity in countries where there is an efficient and reliable cool chain and distribution system (Martın-Belloso et al., 2006).

Fresh-cut fruits and vegetables in USA and Europe accounted for 12% and 18%, respectively, of the total fresh market sales in 2006 (Rogers, 2006). Sales in the USA increased from US$3.3 billion in 1994 to US$11 billion in 2000 and were projected to reach US$15 billion in 2005 (IFPA, 2005). In Australia, annual sales of fresh-cut produce were AUD$5 million in 1995 (Fox, 1995) and the market share for fresh cut produce is around 3% of total (AUD$ 4.439 billion) fruit and vegetable sales in 2006 (Rogers, 2006). Sales of fresh-cut fruit are estimated to be about US$2 billion in global value sales in 2009 (Euromonitor International, 2009).

Fresh-cut produce is more perishable than intact produce and quality is adversely impacted from physical stress during peeling, cutting, slicing, shredding and trimming which involve removal of protective epidermal cells (Watada et al., 1996). This leads to reduced shelf-life due to the onset of factors such as excessive tissue softening and browning on the cut surface. For example, fresh-cut apple slice quality deteriorates due to rapid oxidative browning of apple flesh, microbial development and physiological stress during transport and storage (Abbott et al., 2004). Any change in colour is an easily recognized sign of loss of quality and hence of economic value (Reiner et al., 2008).

Browning of fresh-cut produce can be initiated by both enzymatic and non-enzymatic pathways. Enzymatic browning occurs due to the oxidation of ortho-phenolic compounds to quinones by the action of polyphenol oxidase (PPO) enzymes and which then polymerise to brown pigments (Oktay et al., 1995; Milani and Hamedi, 2005). PPO is generally associated with the plastid and while phenolic substrates are located in the vacuoles of cells. Browning develops following cell destruction and intracellular compartmentation disruption allowing the substrates to mix (Landrigan et al., 1996). PPO activity in apples and its association with browning has been reported by many authors (e.g Nicolas et al., 1994; Hu et al., 2007; Toivonen et al., 2005). Non-enzymatic browning occurs due to a wide number of reactions such as Maillard reaction between amino acid and reducing sugar, caramelisation of sugar and ascorbic acid degradation, chemical oxidation of phenols and maderisation (Manzocco et al., 2001). Moreover, polyphenols, ascorbic acid and other carbonyl compounds can also take part in the Maillard reaction (Rizzi, 1994; Yaylayan, 1997). Maillard reaction between amino acid and reducing sugar which is major cause of browning in apple juice (Garcia and Barrett, 2002; Burdurlu and Karadeniz, 2003).

Various physical treatments and chemical additives are used to inhibit browning of fresh-cut produce (Martinez and Whitaker, 1995). Physical treatments include exposure to low temperature, heat treatment, modified/controlled atmospheres, edible coatings and gamma irradiation (Garcia and Barrett, 2002; Rojas- Grau et al., 2008). The use of sulphite is the most common chemical approach to inhibit browning (Iyengar and McEvily, 1992) whilst the use of chemicals such as ascorbic acid, isoascorbic acid, or citric acid, or an inhibitor of PPO such as cysteine or 4-hexylresorcinol also delay browning (Siddiq et al., 1994; Buta et al., 1999).

Nitric oxide (NO) is a small, highly diffusible molecule that initially attracted attention as an environmental pollutant involved in ozone destruction in the stratosphere and acid rain formation (Howard, 1980; Stamler and Feelisch, 1996). NO was first identified as a signalling molecule in the cardiovascular and nervous systems of mammals (del Rio et al., 2004), but has now been shown to have effects on numerous biological processes in animals (Lloyd-Jones and Bloch, 1996; Wink and Mitchell, 1998; Ignarro, 2000). NO emission from plants was first reported in soybean plants treated with herbicides (Klepper, 1979) but has since been claimed to have a role in many plant processes, such as growth, ripening and disease resistance (Lesham, 2000; Neill et al., 2003).

NO can be applied to plants and animals as the gaseous form or by release from an NO donor compound. NO donors divide into three broad categories: clinical nitrovasodilators, *S*-nitrosothiol and diazeniumdiolates (Thomas et al., 2002). Among these NO donors, the diazeniumdiolates are commonly used compound in biomedical research for their versatility, spontaneous generation of NO and known quantity of NO generation at controllable rates (Keefer et al., 1996; Arnold et al., 2002; Keefer, 2003).

Postharvest horticulture studies with NO was first reported by Leshem et al. (1998) who found short term fumigation with NO gas extended the storage life of some horticultural produce. A range of studies on various produce have since shown NO either applied as a gas or donor compound delayed senescence and extended postharvest life (Lesham et al., 1998 ; Wills et al., 2000; Flores et al., 2008; Pristijono et al., 2008; Zhang et al., 2008). NO gas has also been shown to extend postharvest life by delaying the browning of fresh-cut apple slices and lettuce slices (Pristijono et al., 2006 ; Wills et al., 2008).

Diethylenetriamine/nitric oxide (DETANO) is a slow release diazeniumdiolate that is stable as a solid and quantitatively releases NO in solution at various rates with pH control (Hrabie et al., 1993). The first use of DETANO on plant tissues was by Noritake et al. (1996) who found it induced accumulation of the phytoalexin rishitin in potato tubers. Bowyer et al. (2003) reported postharvest application of DETANO increased the vase life of carnation flowers. Sodium nitroprusside (SNP), a transition metal NO complex has been found to inhibit internal browning in longan and plum (Duan et al., 2007; Zhang et al., 2008).

This study has focused on the surface browning problem in Granny Smith apple (*Malus x domestica* Borkh) slices and different fancy lettuce slices (Green oak, Green coral, Baby cos and Butter) treated with NO gas and the NO donor compounds DETANO and SNP. Another NO donor compound, Piloty's acid was also examined on apple slices. The application of NO gas and DETANO solution was also investigated on apple slices for their effect on physiological factors (respiration, ethylene production and water loss) and biochemical changes (total phenol content, PPO activity, ion leakage, lipid peroxide level, hydrogen peroxide production). A study was also made of the effect of chlorogenic acid and its interaction with DETANO and NO gas on browning. The effect of controlled atmospheres in conjunction with DETANO solution on surface browning of apple slices was also examined.

2. LITERATURE REVIEW

2.1. Fresh-cut fruits and vegetables

Fresh-cut produce can be defined as any fruit or vegetable or combination of produce that has been physically altered from its original form whilst remaining fresh. Fresh-cut produce are unique among the food products in that they remain metabolically active but their shelf-life and storage stability are shortened as consequences of the processes. In many countries, such as the USA, with efficient commercial distribution systems, the fresh-cut produce industry has become the fastest growing part of the food retail market, by offering consumers convenient, ready-to-eat produce (Martin-Belloso et al., 2006). Fresh-cut produce preparation involves unit operations such as, washing, peeling, slicing and packaging as well as various chemical treatments to inhibit deterioration (Wiley, 1994).

In fresh-cut produce, many cells and membranes are disrupted by mechanical damage incurred in preparation, leading to alterations in tissue metabolism and although these alterations differ between produce, many show an increase in respiration, ethylene evolution, water loss, alterations in flavour and aroma and enhanced surface browning during storage (Rolle and Chism, 1987; Brecht, 1995; Art´es et al., 1998; Saltveit, 2003). In order to have a reasonable shelf-life, fresh-cut produce require special care during the whole handling process from harvest to consumption to overcome these problems.

2.1.1. Physiology of fresh-cut fruits and vegetables

Respiration, ethylene production, water loss and discolouration are basic physiological processes in fresh-cut produce, which have been extensively studied (Watada et al., 1996; Gunes et al., 2001; Degl'Innocenti et al., 2007).

2.1.1.1. Ethylene production

Ethylene is synthesized by all plants and has an important role in growth regulation and metabolism including during the postharvest behaviour of fruit and vegetables (Wills et al., 2007).

In fresh-cut produce, physical damage or wounding caused by slicing or peeling results in an increased rate of ethylene production within minutes (Abe and Watada, 1991; Abeles et al., 1992). Lamikanra (2002) reported that enhanced ethylene production is localized to tissue in close vicinity to the wound or cutting injury. This wound ethylene can increase the permeability of membranes and perhaps reduce phospholipid biosynthesis, which can upset the dynamic processes of cellular structure and membrane integrity (Watada et al., 1990). Villas-Boas and Kader (2007) reported that this enhanced ethylene production can accumulate within a package of produce to the detriment of shelf-life.

Many produce including iceberg lettuce and apples, have shown a large increase in ethylene production due to cutting (Ke and Salveit, 1989; Lopez-Galvez et al., 1996; Soliva-Fortuny et al., 2002). Raybaudi-Massilia et al. (2007) found higher concentration of ethylene (28.7 $\mu l.l^{-1}$) in water dipped fresh-cut ripe Fuji apples sealed in polypropylene bags at 5°C for 30 days due to cutting. Hu et al. (2007) found that ethylene production was induced by cutting wound in fresh-cut apple and increased rapidly from 100 $nl.g^{-1}hr^{-1}$ to 150 $nl.g^{-1}hr^{-1}$ at 5ºC on the first 2 day and then decreased gradually during the storage period.

Muharrem et al. (2007) reported ethylene production of fresh-cut cantaloupe cubes was approximately 4-5-fold higher than intact fruit. Agar et al. (1999) reported that physical tissue damage of kiwi fruits caused by slicing and peeling resulted in increased ethylene

production within 2 to 6 hr at 20°C and 1 to 3 days at 2°C which was 2 to 4 times higher than that of unpeeled slices.

An ethylene response in some produce due to cutting injury is not consistent. For example, Hoffman and Yang (1982) found increased ethylene production in fresh-cut of cantaloupe after cutting, while Luna-Guzman et al. (1999) found a reduction in ethylene production. The difference in ethylene production could be due to the stage of maturity of the fruit (Toivonen and DeEll, 2002).

Vilas-Boas and Kader (2007) reported that the ethylene inhibitor, 1- methylcyclopropene (1-MCP) delayed softening and browning in fresh-cut Kent and Keitt mango slices and delayed browning of apple slices (Jiang and Joyce, 2002; Bai et al., 2004; Rupasinghe et al., 2005). Fan and Mattheis (2000) reported that exposure of 0.9 $\mu l.l^{-1}$ 1-MCP to iceberg lettuce leaves for 4 hr at 6°C delayed the development of russet spotting which induced by ethylene stimulation of phenylpropanoid metabolism. Optimal 0.1 $\mu l.l^{-1}$ of 1-MCP for 1 hr extended the storage life of shredded iceberg lettuce at 5°C (Wills et al., 2002).

2.1.1.2. Respiration

Toivonen and DeEll (2002) reported that cutting of fruits and vegetables results in an increase in respiration which might be due to enhanced aerobic mitochondrial respiration. The increased respiration of fresh-cut fruits and vegetables would be expected to result in a reduced postharvest life (Rolle and Chism, 1987).

Respiration rate varies among produce in response to cutting injury. For example, Mao et al. (2007) found that respiration of kiwi slices increased immediately after cutting whereas Gorny et al. (2000) found no immediate effect of cutting on the respiration of pear slices although respiration increased after six days at 10ºC. Hu et al. (2007) found that initial respiration rate in fresh-cut apple at 20ºC increased markedly from 50.1 $mg.kg^{-1}$ hr^{-1} to 150 $mg. kg^{-1}$ hr^{-1} following cutting. Raybaudi-Massilia et al. (2007) found that apples showed an increase in respiration immediately after cutting. Smyth et al. (1998) found that respiration in iceberg lettuce slices increased after 24 hr at 5^{o}C. Watada et al. (1996) found that respiration in fresh-cut lettuce stored 10 days increased about 190% compared to that of whole lettuce stored at 5^{o}C.

2.1.1.3. Water loss

A major problem facing fresh-cut produce is the loss of water, which can cause reduction in crispness, loss of nutritional quality and undesirable changes in colour (Wills et al., 2007). The water loss of fruits and vegetables is determined by many factors (Bartz and Brecht, 2003) but a major factor is surface area to volume ratio (Wills et al., 2007). One effect of cutting is to increase the surface area to volume ratio which leads to an increased rate of water loss and appearance changes such as wilting, shrivelling and textural changes (Lamikanra, 2002; Toivonen and Brummell, 2008). The rate of water loss in fresh-cut produce can be minimized by proper handling techniques including temperature and relative humidity control (Garcia and Barrett, 2002).

Olivas et al. (2007) found that Gala apple slices lost around 20% of the initial weight after 2 days storage at 5ºC while Rocha and Morais (2000) reported that water loss was about 140% greater in Jonagored apple slices stored in air compared to in controlled atmosphere storage.

Ryall and Lipton (1972) reported that textural breakdown can cause more than 5% water loss of lettuce slices. Martinez et al. (2008) found that water loss increased about 5.5 % by storage time in fresh-cut butter head lettuce stored at 5°C for 17 days.

2.2. Postharvest browning of horticultural produce

Both enzymatic and non-enzymatic oxidation are considered the key factors in postharvest browning of horticultural produce including apple and lettuces (Walker and Wilson, 1975; Degl'Innocenti et al., 2007).

2.2.1. Enzymatic browning

Enzymatic browning involves endogenous polyphenolic compounds containing an *o*-dihydroxy group being oxidized in the presence of oxygen to their corresponding *o*-quinone by oxidative enzymes such as polyphenol oxidase (PPO) with subsequent reactions leading to the formation of brown polymeric pigments known as melanins (Robards et al., 1999; Salcini and Masantini, 2005). Depending on the reactivity of the *o*-quinone and the environment of the oxidation reaction, polymeric pigments have different hue and intensity (Fulcrand et al., 1994; Richard-Forget et al., 1995).

Peroxidases (PODs) can also contribute to the process of browning (Cantos et al., 2001; Campos-Vargas and Saltveit, 2002) as these are also able to catalyse the oxidation of phenols to quinones. PODs have been reported to contribute to enzymatic browning in apples (Nicolas et al., 1994), pears (Richard-Forget and Gauillard, 1997), and pineapples (Teisson, 1972). However, the involvement of PODs has been questioned due to the high affinity of

PPO for its natural substrates and the low levels of hydrogen peroxide (H_2O_2) in fruit (Richard-Forget and Gauillard, 1997; Subramanian et al., 1999).

2.2.1.1. Polyphenol oxidase

2.2.1.1.1. Action of PPO

While polyphenol oxidase (E.C 1.14.18.1) enzymes, are well known to catalyze the *o*-hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones (Vámos-Vigyázó, 1981), the actual role of PPO in plants remains obscure (Zawistowski et al., 1991; Sherman et al., 1995) although the role of PPO in plant resistance against disease has been well documented (Bonner, 1957; Vámos-Vigyázó, 1981; Ray and Hammerschmidt, 1998). Li and Steffens (2002) suggested that PPO was involved in stimulating plant resistance against insect attack by inducing anti-nutritive defences. Recent studies provide evidence that PPOs may be involved in other processes. For example, aureusidin synthase, a PPO homolog has been shown to catalyse reactions involved in the development of flower petal colouration (Nakayama et al., 2000).

2.2.1.1.2. PPO activity in apples

PPO activity in apples and its association with browning is well documented. PPO in apple is located in the chloroplast, mitochondria, and cytoplasm and the activity of PPO is greater in earlier harvested than in commercially ripe fruits (Mayer, 1987). Du and Bramlage (1995) reported that PPO activity at commercial maturity was relatively low and remained constant during low temperature storage. Murata et al. (1993) found the highest level of PPO in the core followed by skin tissue while Wakayama (1995) reported the highest PPO activity in the cortex. Goupy et al. (1995) reported that the degree of apple surface browning was related to

PPO activity whereas Nicolas et al. (1994) found no clear correlation between PPO activity and apple browning. Coseteng and Lee (1987) found no set pattern in the degree of surface browning during maturation and storage and PPO activity with different cultivars showing different patterns.

2.2.1.1.3. PPO activity in lettuces

Phenylalanine ammonia lyase (PAL) initiated synthesis of phenylpropanoid compounds and their subsequent oxidation by PPO was reported by Ke and Saltveit (1989) to induce browning in wounded lettuce. Peiser et al. (1998) reported that browning was correlated with enhanced PAL activity in wounded lettuce tissues. An increased PAL activity was also found by Degl'Innocenti et al. (2007) and Couture et al. (1993) to be correlated with the susceptibility of fresh-cut lettuce to browning. Heimdal et al. (1995) and Hisaminato et al. (2001) found no correlation between browning and PPO activity in iceberg lettuce however they showed a correlation between browning and PAL. They also indicated PAL was induced by cutting, polyphenols such as dicaffeoyltartaric acid and 5- caffeoylquinic acid then being synthesised and these polyphenols are successively oxidized by PPO.

2.2.1.2. Peroxidase (POD)

2.2.1.2.1. Action of POD

Peroxidases (POD) such as hydrogen peroxide oxidoreductase (E.C. 1.11.1.7), are an indicator of quality deterioration such as flavour loss and various biodegradation reactions, but are also relevant to the postharvest browning of a wide variety of fruits and vegetables during processing (Vámos-Vigyázó, 1981; Jang and Moon, 2011**).** POD catalyzes the oxidation of phenolic compounds to *o*-quinone using either hydrogen peroxide or organic

peroxides (Garcia et al., 2007; Gajhede, 2001; Kader et al., 2002). There are two possible mechanisms for POD-catalysed browning reactions. The first involves the generation of H_2O_2 during the oxidation of some phenolics, while the second involves the use of quinonic forms by POD (Lamikanra, 2002).

In pear, the relative significance of PPO and POD in the browning of pear flesh appeared to be strongly affected by the nature of the oxidised phenol and therefore by the stability of the corresponding *o*-quinones (Richard-Forget and Gauillard, 1997). Rouet-Mayer et al. (1990) and Teisson (1972) claimed that PODs are not involved in enzymatic browning following mechanical injury, as the internal level of H_2O_2 is limited.

2.2.1.2.2. POD activity in apples

POD activity was reported to be higher in the peel of Red Delicious, Cox's Orange Pippin, Santana, Goudreinet and Granny Smith apples while the pulp of Goudreinet, Royal Gala and Golden Delicious apples showed higher POD activity (Garcia et al., 2007). POD activity increased with increased ripening (Gorin and Heidema, 1976), with a peak in activity around the climacteric stage of ripening in the peel and pulp tissues (Lamikanra, 2002). Significantly more soluble and ionically bound POD activity was found in the peel than in the cortex of apple slices (Moulding et al., 1987). Although it has been known that PPO is the main enzyme associated with enzymatic browning on fresh cut apples, it is also required to know the changes in POD as they can contribute to discoloration in fresh-cut products. Jang and Moon (2011) found that apple slices stored at 10°C for 12 days, showed fluctuation with the highest value in the POD activity on the 4th day of storage.

2.2.1.2.3. POD activity in lettuces

Lamikanra (2002) found POD activity in fresh-cut lettuces indicative of the relative level of oxidative stress which is essentially a regulated process, the equilibrium between the oxidative and antioxidative capacities determining the fate of the plant. In iceberg lettuce, POD activity was found to be higher in green tissue than in mid-rib vascular tissue (Martin-Diana et al., 2005b). In fresh-cut lettuces, POD activity was found to be higher with an increase in peroxide induced by cutting (Degl'Innocenti et al., 2007). Chen et al. (2010) found that in asparagus lettuce stored at 4°C, POD activity decreased in the first 4 days then increased to day 8. Martin-Diana et al. (2005a) found synergistic activity in iceberg lettuce between POD and PPO enzymes. Castañer et al. (1999) found that a decrease in POD activity in lettuces can decrease the production of substrate available for PPO because the products of POD are used by the PPO.

2.2.2. Non-enzymatic browning

Non-enzymatic browning can arise from different mechanisms such as metal ion interaction with phenol that involves direct intervention of phenols as substrate, pyrolysis of food carbohydrates, ascorbic acid degradation and by a Maillard reaction (Vámos-Vigyázó, 1981; Robards et al., 1999). Though the exact mechanism of browning via metal ion interaction is not well understood (Oszmianski et al., 1996) but it probably involves the ability of phenolic compounds to form metal complexes (Cheng and Crisosto, 1997). Several different metal ions such as copper, iron, tin, magnesium, calcium and potassium have been involved in polyphenol interactions in wine (Beveridge, 1997; Mathew and Parpia, 1971). However, Oszmianski et al. (1996) found that the degradation of phenolic compounds (catechin) in wine increased in the presence of iron. Copper and iron are associated with increased rates of

oxidation of catechin and other phenolics to their brown products possibly by copper oxidation of phenolics to quinones which are then thought to be involved in polymerisation reactions to form brown pigments (Robards et al., 1999). Nicoli et al. (2000) studied the enzymatic and chemical oxidation on the antioxidant capacity of catechin model systems and apple derivatives and found both enzymatic and chemical oxidation of catechin promoted an initial increase in the chain-breaking activity which was associated with the development of browning. They also reported that the initial increase and the following decrease in the radical scavenging properties in blanched apple puree can be a result of chemical oxidation of phenol.

Non-enzymatic browning can also result from pyrolysis of food carbohydrate due to heat treatment above the melting point of the sugar under alkaline or acidic conditions (Namiki, 1988). Ascorbic acid degradation is the spontaneous thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions even in the absence of an amino-compound (Wedzicha, 1984).

Maillard reaction involves reaction of a carbonyl group with an amine group to form an unstable Schiff's base that rearranges to a more stable aminoketose that has chelating, reducing and oxygen-scavenging properties (Hodge, 1953; Tan and Harris, 1995). The degradation of Amadori products results in the production of deoxyosuloses or key -dicarbonyl Maillard reaction transient intermediates, which generate numerous compounds via several concurrent mechanisms. The generation of short chain sugar fragment carbonyl compounds is known to participate in browning mechanisms (Weenen and Tjan, 1994).

Stamp and Labuza (1983) reported that non-enzymatic browning in fruit is mostly associated with brown colour development during thermal processing. Atrooz (2008) studied Maillard reactions in apple and claimed that non-enzymatic browning was not directly related to the antioxidant activity. The antioxidant activity is the ability of a compound to reduce oxidative damage due to free radicals and oxygen species by capturing electrons and modifying its own chemical structure to a stable form (Roginsky**e** and Lissi, 2005). No published article was found on non-enzymatic browning in fresh-cut produce.

2.2.3. Phenolic compounds

Phenolic compounds are a major class of secondary metabolites in plants with a range of structures and functions. They generally possess an aromatic ring bearing one or more hydroxy substituents. Plant phenols are classified based on the number of constitutive carbon atoms in conjunction with the basic phenolic skeleton, as benzoquinones, phenolic acids, phenylacetic acids, cinnamic acids, naphthoquinones, xanthones, stilbenes, flavonoids, lignins and biflavonoids (Robards et al., 1999).

Phenolic compounds are widely distributed in fruits and vegetables and may play an important part in their physiology and metabolism. Phenolics act as substrates for browning but they also act as antioxidants (Rice-Evans et al., 1996) and are implicated in pathogen resistance (Piretti et al., 1996; Mayr et al., 1997). Hung et al. (2004) and Halliwell (1994) reported that regular consumption of foods rich in phenolic compounds (fruits, vegetables, whole grain cereals, red wine, tea) is associated with reduced risk of cardiovascular diseases, neuro degenerative diseases, and certain cancers. Huang et al. (2006) reported that phenolic compounds are thought to deliver health benefits by several mechanisms, including free radical scavenging, protection and regeneration of other dietary antioxidants (e.g. vitamin E)

and chelating of pro-oxidant metal ions. The antioxidant properties of phenolics act through being a reducing agent, hydrogen donor and singlet oxygen quencher (Rice-Evans et al., 1997).

2.2.3.1. Phenolic compounds of apples

The many different phenolic compounds of apple have been studied extensively (Chinnici et al., 2004). The main two sub-types of phenolic compound in apple are the flavonoids and the acids. Markowski and Plocharski (2006) claimed that the most common acids are chlorogenic acid, caffeic acid and p-coumaric acid. Robards et al. (1999) reported that the major phenolic compounds in apple fruit were benzoquinones, phenolic acids, cinnamic acids and flavonoids with chlorogenic acid, caffeic acid and p-coumaric acid classified as cinnamic acids. Amiot et al. (1992) also reported that the cinnamic acids are the dominant phenolic compound in apple cortex. A phenolic compound classification is presented in Table 1.

Hydroxycinnamic acids such as chlorogenic acid, caffeic acid, p-coumaric are considered as the most important substrates for PPO and their oxidation products enhance the oxidation of other phenolics such as flavan-3-ols by a coupled mechanism (Amiot et al., 1992 ; Shahidi and Naczk, 2004).

Table 2.1 Classes of phenolic compounds and characteristic compounds in apples.

Class	Characteristic compounds
Benzoquinones	Catechol, 4-methyl catechol
Phenolic acids	Hydroxybenzoic acid, *p*-hydroxybenzoic acid, 3-4-Dihydrobenzoic acid, salicylic acid
Cinnamic acids	Chlorogenic acid, hydrocaffeic acid, caffeic acid, ferulic acid, *p*-coumaric acid, 4-coumaroylquinic acid, sinapic acid
Flavonoids such as :	
Flavonols	Quercetin, quercetin glycoside, dihydroquercetin, kaempferol, kaemferol glycosides, rutin
Anthocyanins	Cyanidin-3-galactoside, leucoanthocyanidins, procyanidin, procyanidin B2, cyanidin glycosides including acylated derivatives
Flavanols	(+)-Catechin, (-)-epicatechin, flavan-3-ols
Chalcones (dihydrochalcones)	Phloretin derivatives notably phloridzin

Source : Adapted by Pristijono (2007) from Robards et al. (1999) and Alonso-Salces et al. (2001)

Chlorogenic acid, is an ester of polyphenolic caffeic acid and (-)-quinic acid [1L-1(OH), 3,4/5-tetrahydroxycyclohexanecarboxylic acid] (Moridani et al., 2001; Clifford et al., 2003). Chemical structure of chlorogenic acid is illustrated in Fig 2.1. Friedman and Jurgens (2000) found that chlorogenic acid was not stable at high pH but was stable in the acid pH of 3.5-4 of apple juice and cider. Lee et al. (2003) determined the average concentration of chlorogenic acid in six apple cultivars was about 9 mg/100 g of fresh weight.

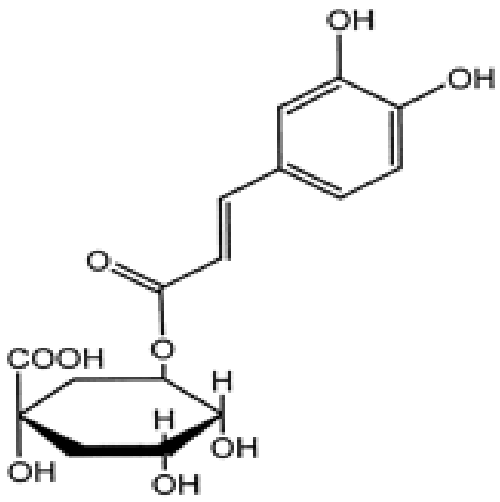

Fig 2.1 Chemical structure of chlorogenic acid (Moridani et al., 2001)

Catechin and epicatechin in apples have also been identified as substrates of PPO and play a role in browning (Rocha and Morais, 2001). Dihydrochalcones have also been claimed to have a significant influence on colour development (Goupy et al., 1995). Awad et al. (2001) stated that the level of phenolic compounds varies greatly with cultivar, maturity and season and between individual apples of the same cultivar. For example, Van der Sluis et al. (2001)

found a 10-30% variation in the content of chlorogenic acid, phloridzin, epicatechin and quercetin glycosides among individual Jonagold apples.

2.2.3.2. Phenolic compounds of lettuces

In red, iceberg and romain lettuces, the major phenolic compounds are reported to be caffeoyltartaric, chlorogenic, decaffeoyltartaric and 3',5'-decaffeoyltartaric acids (Ke and Salveit, 1989; Ferreres et al., 1997; Cantos et al., 2001). DuPont et al. (2000) detected a number of quercetin conjugates including quercetin 3-(6-malonylglucoside), quercetin 3-glucoside, quercetin 3-glucoronide and quercetin 3-rhamnoside in red and green leaf lettuces.

When lettuce tissue is wounded, an increase in the amount of phenolic acids was reported in the stem of red pigmented (Ferreres et al., 1997) and iceberg lettuce leaves (Saltveit, 2000; Cantos et al., 2001; Kang and Saltveit, 2003) with an accumulation of phenolic compounds found in cells up to 2 cm from the site of the wound and associated tissue browning (Peiser et al., 1998; Campos-Vargas and Saltveit, 2002). Cantos et al. (2001) reported that chlorogenic acid is the main phenolic compound accumulated in the mid-rib of six processed lettuces after wounding. Peiser et al. (1998) claimed that wounding of lettuce produces a signal that migrates through the tissue and induces the synthesis of enzymes in the phenylpropanoid pathway and that ultimately leads to increases the production of phenolic compounds which enhance browning. However, the increased activity of phenylalanine ammonia-lyase (PAL) in wounded lettuce leaves, has been found to exhibit no correlation between browning and an increase of phenolic acids (Tomas-Barberan et al., 1997; Cantos et al., 2001). By contrast, Couture et al. (1993) found that increased PAL activity was correlated with the susceptibility to browning of fresh cut lettuce. Saltveit and Loaiza-Velarde (2000) and Saltveit (2000) reported that wounding induces the *de novo* synthesis of PAL, which initiates the reactions to

increase the level of phenolic compounds and browning in lettuce. A modified version of Saltveit (2000) system is given in Figure 2.2.

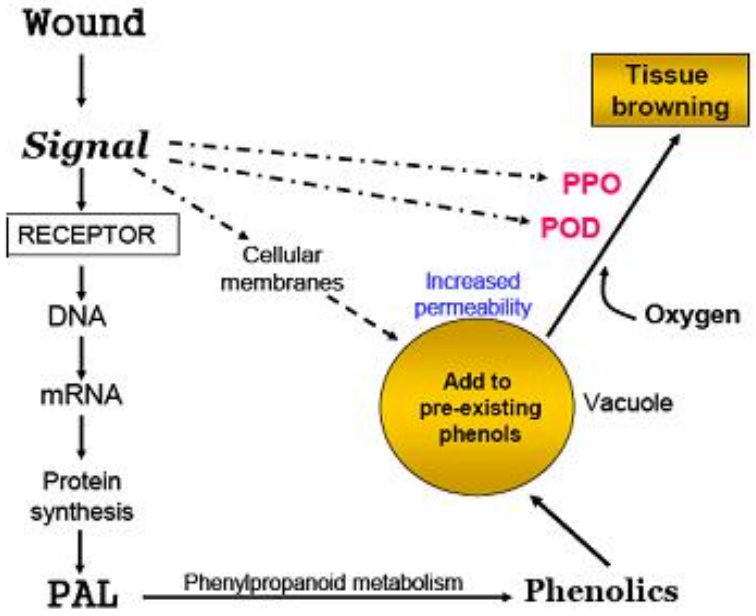

Fig 2.2 Interrelationships between wounding of lettuce leaf tissue and subsequent induced changes in phenolic metabolism that leads to tissue browning. Adapted from Saltveit, (2000).

2.3. Methods for inhibiting browning on fresh-cut produce

A range of physiological and chemical treatments have been developed to inhibit browning and generally increase shelf life. Low temperature, modified atmosphere packaging (MAP) with low temperature storage, heat treatment, edible coatings and ascorbic acid are the more common methods for inhibiting browning of fresh-cut produce (Lamikanra and Watson, 2001; Rojas-Graü et al., 2008; Koukounaras et al., 2008).

2.3.1. Temperature

Keeping fresh-cut produce close to the optimum temperature of 0^{o}C or 5^{o}C and sometimes 10^{o}C for chilling sensitive produce is the most important factor in maintaining quality and maximizing postharvest life (Garcia and Barrett, 2002; Watada et al., 1996). Temperature has a great effect on respiration and fresh-cut produce generally have higher respiration than the same intact produce due to enhanced physical stress (Lamikanra, 2002). Since respiration increases exponentially with temperature (Watada et al., 1996), fresh-cut produce benefits greatly if stored at low temperature, due to retarded metabolism as seen in a reduced respiration, ethylene production, colour changes and softening (Perez et al., 2004).

The firmness values of Granny Smith apple slices were below the minimum firmness standard of 6.35 Kgf at 13 and 20ºC while the firmness values were at or above the minimum standard at 1 and 5ºC stored for 3 weeks (Toivonen and Hampson, 2009). Moreira et al. (2006) reported that weight loss in romain lettuce slices was 0.6, 1.0 and 1.6 g / 100 g at 0, 8 and 15°C respectively for approximately 200 hr which indicated that weight loss increased with increased storage temperature. They also reported that reduced weight loss of fresh vegetables can be attributed to evaporation of surface water remaining on the product after washing, dehydration and respiration.

Short exposure to temperature in the range of 40 to 60°C can produce heat shock proteins by the plant tissue which can prevent undesirable metabolic processes occurring (Brecht et al., 2004). For example, heat-shock at 45°C for 90 sec was found to inhibit wound-induced browning of iceberg (Loaiza-Velarde and Saltveit, 2001) and romaine lettuce (Saltveit, 2000). Similar results on iceberg lettuce were obtained by Loaiza-Velarde et al. (1997). Apple slices

prepared from whole apple treated with heat shock at 45^{o}C for 105 sec resulted less browning on the cut surface compared to those prepared from non-treated apple (Kim et al., 1993).

2.3.2. Modified atmosphere

Storage in modified atmospheres (MA) with reduced oxygen and elevated carbon dioxide levels can reduce spoilage, decrease respiration and ethylene production, delay ripening, retard textural softening, which results in extended storage life in a range of fresh-cut fruits and vegetables at low temperature (Beaudry 1999; Das et al., 2006).

Since O_2 can be the oxidant for browning reactions, a modified atmosphere with low O_2 and elevated CO_2 levels can contribute positively to inhibit browning in fresh-cut produce (Rojas-Grau et al., 2009).

Carbon dioxide gas in a MA also plays an important role in inhibition of microbial growth (Hendricks and Hotchkiss, 1997; Al-Ati and Hotchkiss, 2002). Farber (1991) reported that atmospheres in the range of 3–8% CO_2 and 2–5% O_2 are generally recommended for MA storage of fruits and vegetables. High carbon dioxide levels (10-30%) can benefit various fresh-cut fruit and vegetables by delaying browning (Kader and Saltveit, 2003), but excessive levels of carbon dioxide and oxygen will induce anaerobic respiration and development of off-odours.

For fresh-cut apple slices, the use of modified atmosphere packaging (MAP) has been shown to inhibit enzymatic browning (Gorny, 1997). An atmosphere of 2.5% O_2 and 7% CO_2 can increase the shelf life of apple slices due to inhibition of ethylene production (Rojas-Grau et al., 2007) and inhibition of browning of the cut surface (Soliva-Fortuny et al., 2001). Rocha

and Morais (2001) found inhibition of browning of the cut surface of Jonagored apples with 2% O_2 and 12% CO_2**.** Surface browning of Spartan apple slices was inhibited by pretreating the whole fruit with 100 kPa O_2 as compared with those from the air pretreatment at 1^{o}C (Lu and Toivonen, 2000). .

A storage atmosphere with 10-30% CO_2 has been found to inhibit browning of lettuces (Gil et al., 1998; Heimdal et al., 1995) while Beltran et al. (2005) showed that storage of iceberg lettuce in 4 % O_2 and 12 % CO_2 delayed in browning. Lopez-Galvez et al. (1996) found that an atmosphere of 3% O_2 + 10% CO_2 for iceberg and romain lettuce pieces maintained a better visual quality score than air stored lettuce slices for 12 days at 5ºC. Use of 80 % O_2 and 10-20% CO_2 was reported by Escalona et al. (2006) to reduce respiration and avoid fermentation of fresh-cut lettuce.

A range of edible coatings, such as chitosan, starch, cellulose, alginate, gluten, whey, carnauba, beeswax and fatty acids have been shown to preserve the quality of fresh-cut produce by decreasing respiration and senescence while protecting the aroma, texture, colour and water loss of produce throughout storage (Gonza´lez-Aguilar et al., 2010 ; Raybaudi Massilia et al., 2008).

2.3.3. Chemical agents

A range of chemical agents are able to inhibit browning (Friedman, 1996). Sulphites, are very effective anti-browning agents but are banned in most countries on fresh fruits and vegetables due to potential harmful health effects (FDA, 1989; Iyengar and McEvily, 1992). Anti-browning agents approved for use on fruits and vegetables include reducing agents, acidulants, chelating and complexing agents.

2.3.3.1. Reducing agents

In a review of reducing agents, Iyengar and McEvily (1992) reported that they inhibited browning by reducing *o*-quinones back to *o*-diphenols and also through an effect on PPO activity. Reductants are generally irreversibly oxidized during the reaction which means the protection they provide is only while some reductant is present (Garcia and Barrett, 2002). The most effective reducing agents used for inhibiting browning in fruits and vegetables are ascorbic acid, calcium ascorbate, and cysteine (Abbott et al., 2004).

Ascorbic acid has been widely used as an anti-browning agent for fresh-cut fruits and vegetables. A 1% solution of ascorbic acid was able to inhibit browning of Liberty apple slices (Son et al., 2001) with an inhibitory effect of 0.5% ascorbic acid on PPO activity found in lettuce slices (Altunkaya and Gökmen**,** 2008). Combinations of ascorbic acid with an acidic polyphosphate were also highly effective on cut surfaces of apple (Sapers et al., 1989) while ascorbic acid in combination oxalic acid (Son et al., 2000), N-acetylcysteine (Rojas-Graü et al., 2006) and calcium chloride (Ponting et al., 1972) increased the effectiveness in inhibiting browning. Roura et al. (2008) found that browning of iceberg lettuce slices was inhibited by a combination treatment of ascorbic acid (1g/100 g) in chlorinated water and heat treatment (50^{o}C for 2 min).

The combination of antioxidant agents such as N-acetylcysteine and glutathione into alginate- and gellan-based coatings has been shown to reduce browning in fresh-cut apples, pears and papayas (Tapia et al., 2005, Rojas-Graü et al., 2007; Rojas-Graü et al., 2008).

The proprietary product, NatureSeal™ (calcium ascorbate) patented by Chen et al. (1999) with Red Delicious apple slices treated with 3.8% calcium ascorbate dip having delayed

browning at 2-5°C in a sealed polyethylene bag. Subsequently, use of 5% or 7% calcium ascorbate (NatureSeal™) reduced browning of the cut surface of Gala, Empire, Honeycrisp, Granny Smith and Golden Delicious apples (Fan et al., 2005a; 2005b; Harte et al., 2003; Karaibrahimoglu et al., 2004; Wang et al., 2007).

2.3.3.2. Acidulants

Acidulants, such as citric, oxalic, tartaric, malic, phosphoric and hydrochloric acids delay browning by lowering the pH of fresh-cut produce to minimize the PPO activity (Suttirak and Manurakchinakorn, 2010). Citric acid (0.25 - 0.5 %) is most common acidulent used to inhibit browning of sliced apple (Santerre et al., 1988). Browning of fresh-cut apple is also inhibited by citric acid combined with ascorbic acid, sodium monohydrate and sodium chloride (Vamos-Vigyazo, 1995; Rocha et al., 1998; Zuo et al., 2008).

Altunkaya and Gökmen (2009) found that 0.05% citric acid had positive effect for the inhibition of browning and also decreased the rate of degradation of phenolic compounds in lettuce slices within 24 hr of storage.

2.3.3.3. Chelating and complexing agents

Chelating agents inhibit browning either by binding with copper in the active site of PPO or reducing its availability for incorporation into the holoenzyme (Iyengar and McEvily , 1992; Lamikanra, 2002). Sporix, an acidic polyphosphate chelator has been used for inhibiting browning of fresh-cut fruits and vegetables (Garcia and Barrett, 2002). The combination treatment of Sporix with ascorbic acid was found to inhibit browning of apple slices (Sapers

and Hicks, 1989) while Sapers et al. (1989) found that apples slices treated with ascorbic acid-2-phospate (AAP) and ascorbic acid-3-triphosphate (AATP) reduced browning.

Garcia and Barrett (2002) stated that complexing agents are capable of entrapping and forming complexes with PPO reaction products. They also mentioned that the central cavity of cyclodextrin can form inclusion complexes with phenolics and hence depleting PPO substrates whereas Sapers and Hicks (1989) found that β- cyclodextrin was not effective in inhibiting browning of diced apple, probably due to its low diffusion. EDTA (Ethylenediamine tetraacetic acid), a complexing agent, was used to inhibit browning of iceberg lettuce stored for 24 hr at 20ºC (Castaner et al., 1996**).**

2.4. Nitric oxide

Nitric oxide (NO) was initially known as an industrial pollutant that, among other effects, caused reduction of ozone in the stratosphere and contributed to the formation of acid rain (Stamler et al., 1992; Lamattina et al., 2003). However, in recent years NO has generated considerable interest due to its synthesis in both animals and plants and its effect as a regulator of many cellular functions. (Crawford, 2006; Badhiyan et al., 2004). NO was named "Molecule of the Year" in 1992 by the journal *Science* (Koshland, 1992; Delledonne, 2005) and three US scientists received the 1998 Nobel Prize in human physiology and medicine for the discovery of NO's signalling role in cardiovascular and nervous systems (Leshem, 2000). NO is now recognized as a key signalling molecule in many animal species and also in plants where it is known to orchestrate a wide range of processes such as stomatal closure and growth and development (Neill et al., 2002; Guo et al., 2003; Pagnussat et al., 2003; Wilson et al., 2008).

2.4.1. Chemistry of nitric oxide

NO is a colourless gas which due to its low density is able to diffuse into the hydrophilic parts of the cell such as the cytoplasm, but can also cross freely through the lipid phase of membranes (Neill et al., 2003; Tuteja et al., 2004).

NO is very reactive and will react with oxygen to form both nitrite and nitrate (Wendehenne et al., 2001; Arasimowicz and Floryszak-Wieczorek, 2007; Wilson et al., 2008). The chemical nature of NO is illustrated in Figure 2.3. As a free radical, NO is suitable for electron transfer and it can exist as three interchangeable forms: the radical (NO•), the nitrosonium cation (NO^+) and the nitroxyl anion (NO^-) (Wojtaszek, 2000; Wendehenne et al., 2001).

NO reacts with superoxide anions or H_2O_2 to produce the very potent peroxynitrite ion (OONO─) which is toxic to many cells (Delledonne et al., 2001; Cevahir et al., 2007) and deleterious to lipids, proteins and DNA (Lipton et al., 1993).

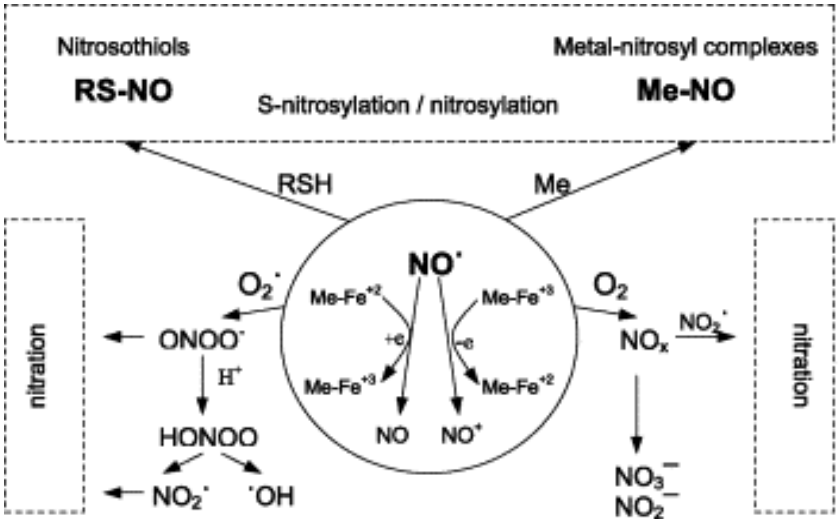

Fig. 2.3. Chemistry of nitric oxide (NO). Reproduced from Arasimowicz and Floryszak-Wieczorek (2007).

2.4.2. Nitric oxide in mammalian systems

NO was discovered as the endothelium-derived relaxing factor (EDRF) by Ignarro et al. (1988) but is now considered as a major signaling molecule which regulates diverse physiological and pathophysiological mechanisms in maintaining blood pressure in the cardiovascular system, neural transmission in the brain and immunological systems (Feldman et al., 1993; Jaffrey and Snyder, 1995; Lloyd-Jones and Bloch, 1996; Mayer and Hemmens, 1997). As an endothelium–derived relaxing factor, NO contributes to the regulation of local and systemic vascular resistance, distribution of blood flow and oxygen delivery and arterial pressure in the vascular system (Umans and Levi, 1995). Feldman et al. (1993) showed that NO in the blood vessel also contributes to decreased aggregation and adhesion of blood platelets to endothelial cells which leads to a decreased propensity of blood to clot.

In the central nervous system, NO acts as a neurotransmitter in the cerebellum where it functions as a regulator of synaptic plasticity and may be essential for development of memory (Gartwaite and Boulton, 1995), and in the peripheral nervous systems including the respiratory tract, digestive system, urinary tract, cerebral vasculature and male sexual function (Feldman et al., 1993; Rand and Li, 1995).

NO plays many more roles in the immune system, inflammation, and immunity (Karpuzoglu and Ahmed, 2006) and is responsible for the cytostatic activity of macrophages (Stuehr, 1999). Bogdan (2001) found that inducible NO synthase (iNOS) is responsible for high levels of NO production and shows potent antimicrobial effects to control infection and hence is critical in immune defence. Activated macrophages produce high levels of NO and other reactive oxygen species to kill microorganisms such as viruses, bacteria, protozoa, and fungi,

by decreasing glutathione, increasing DNA breaks and via oxidation of lipids and DNA (Pacelli et al., 1995).

2.4.3. Nitric oxide in plants

NO emission from plants and its role in plant growth were first studied in the 1970s. Anderson and Mansfield (1979) reported that NO can either enhance or reduce growth of tomato plants depending on the variety, soil fertility and NO concentration. The first study showing that plants can produce NO was in soybean plants treated with herbicides where NO was emitted from chemical reactions of accumulated nitrite with plant metabolites such as salicylate derivatives or the chemical decomposition of HNO_2 (Klepper, 1978). Ambient NO concentration was proposed as a determining factor of ozone toxicity in plants by Neighbour et al. (1990) who found that leaf injury caused by ozone only occurred when >0.002 $\mu l.l^{-1}$ NO was added.

Leshem and Haramaty (1996) studied NO and plant signalling and found that adding the NO-releasing compound S-nitroso-N-acetylpenicillamine (SNAP) to pea leaves resulted in a greater emission of NO than ethylene, and both increased with the addition of the ethylene precursor 1-aminocyclopropene-1-carboxylic acid (ACC). They suggested that NO may regulate the ethylene production in growing plants.

Leshem et al. (1998) suggested that NO may delay senescence by limiting ethylene emission. NO emission appeared to be lower in ripe fruits such as banana and strawberry than in unripe fruits with an associated increase in ethylene production (Leshem and Pinchasov, 2000).

There have been various studies showing the involvement of NO in plant growth, development and physiological processes including xylogenesis, programmed cell death, pathogen defence responses, flowering and stomatal closure (Leshem et al., 1998; Lamattina et al., 2003; Neill et al., 2003; Delledonne, 2005; Lamotte et al., 2005; Cevahir et al., 2007). NO also acts as a mediator in the biological effects of primary messenger molecules such as hormones (Neill et al., 2002). NO can also act as an antioxidant and an anti-stress agent against biotic and abiotic stress condition in plants, such as in wounds, infections, drought, extreme temperatures, ultra-violet radiation and ozone exposure (Garces et al., 2001; Neill et al., 2003). NO as a key messenger molecule regulates the expression of genes involved in the synthesis of jasmonic acid (Jih et al., 2003), pathogen responses (Durner et al., 1998) and stimulation of the hypersensitivity response (HR) (Delledonne et al., 1998; Romero-Puertas et al., 2004). NO is also involved in reproductive mechanisms that operate during pollen recognition by the stigma (Hiscock et al., 2007).

2.5. Effects of NO gas on postharvest life of horticultural produce

Postharvest studies with NO gas was first reported in a preliminary study by Leshem et al. (1998) who found short term fumigation of NO gas on strawberry, broccoli, cucumber, Chinese broccoli, kiwi fruit and mushroom in a nitrogen atmosphere followed by storage in air at 20^{o}C resulted in 70-180 % extension of postharvest life compared to control produce. A more detailed study by Wills et al. (2000) on strawberry stored at 20^{o}C and 5^{o}C in humidified air containing 0.1 $\mu l.l^{-1}$ ethylene, found an increase in postharvest life with the most effective concentration being 5 to 10 $\mu l.l^{-1}$ NO at both temperatures. Ku et al. (2000) reported that rate of water loss was reduced by about 20% from a range of fruits, vegetables and flowers following a 24 hr exposure to NO. After 45 days storage at 5°C, ethylene production of

control peaches was significantly higher than those fumigated with 15 μl. l^{-1} NO gas (Zhu et al., 2009).

Pleiffer et al. (1999) found the half life of NO in air increased from 24 sec to 7 hr with decreasing NO concentration from10,000 $\mu l.l^{-1}$ to 10 $\mu l.l^{-1}$. The rate of oxidation of NO also decreases with increasing temperature due to the decrease in intermolecular interaction- a collision between two NO molecules and one O_2 molecule results in formation of a transient complex which leads to the formation of two molecules of NO_2 (Tsukahara et al., 1999). At 27^{o}C, the rate constant *k* for the oxidation of NO was reported as 7.0 x 10^3 $l^2.mol^{-2}.s^{-1}$ and decreased by 0.04 x 10^3 $l^2.mol^{-2}.s^{-1}$ per degree of rise in temperature. Soegiarto et al. (2003) examined the use of 40 – 100 $\mu l.l^{-1}$ NO gas in air to fumigate flowers, fruits and vegetables. They showed that the rate of NO loss was much faster in the presence of produce than in air due to the rapid uptake of NO by produce but the rate of NO uptake varied greatly among produce. They concluded that NO fumigation in air at low concentration had a sufficiently long half life to make it feasible to treat produce.

Fumigation of pear with 10 $\mu l.l^{-1}$ NO gas in air for 2 hr showed decreased ethylene production while fumigation with 10 and 50 $\mu l.l^{-1}$ NO in air for 12 hr delayed skin yellowing (Sozzi et al., 2003). Zhu et al. (2006) found that peaches treated with 5 and 10 μl. l^{-1} NO gas in air for 3 hr had ripening delayed through reduced ethylene biosynthesis. They also reported that NO inhibited lipoxygenase (LOX) activity in peaches and the decrease in LOX activity might be a collateral process with the inhibition of ethylene biosynthesis.

However, most research has been on the effect of NO on non-climacteric produce. NO fumigation with 1000 μl. l^{-1} for 5 hr delayed yellowing and retarded the onset of chlorophyll

degradation in broccoli florets (Eum et al., 2009). Soegiarto and Wills (2006) also reported that fumigation with 10 and 100 $\mu l.l^{-1}$ NO gas combined with a modified atmosphere with 2 and 5% O_2 in air, increased the postharvest life by 250% for strawberry and 100% for iceberg lettuce compared with control. Fumigation with 10- 4000 $\mu l.l^{-1}$ NO gas in air for 2 hr extended the postharvest life of green bean by 14% and more than 50% for broccoli (Soegiarto and Wills, 2004) while a 30% increase in postharvest life of carnation flowers was obtained when fumigated with 1 and 5 $\mu l.l^{-1}$ NO gas in air (Bowyer et al., 2003). Surface browning of cut slices of Granny Smith apple (Pristijono et al., 2006) and iceberg lettuce (Wills et al., 2008) was inhibited by postharvest fumigation with NO gas.

2.6. Nitric oxide donor compounds

NO donor compounds have a chemical structure that under specific conditions quantitatively degrade to release NO and there is considerable medical interest in using NO donors. Hou et al. (1999) reported that NO donor compounds can be classified into six categories based on the atom to which the NO releasing moiety is attached, namely, *C*-NO, *N*-NO, *O*-NO, *S*-NO, heterocyclic-NO and transition metal-NO donors. The more important classes of NO donor compounds for biological purposes are *N*-NO, *S*-NO, and transition metal-NO compounds. While it is not known if NO-donor compounds would be approved for food use, they would seem to be safe as they are used for human therapeutic purposes. The ongoing development of research on novel NO donors shows a perspective trend in the search for effective drugs in the near future.

2.6.1. SNAP

S-nitroso-N-acetylpenicillamine (SNAP) of the S-NO category has been used as a drug to treat cardiovascular disease (Megson and Webb, 2002). In plant research SNAP has been used to investigate the effect of NO on the life cycle, plant pathogen interactions and plant responses to stresses. Seed germination, inhibition of hypocotyl elongation, inhibition cell death and preservation of chlorophyll have been stimulated by SNAP (Beligni and Lamattina, 2000, 2002).

2.6.2. DETANO

NO donors of the N-NO category can be classified as N-nitrosamines, N-hydroxy-N-nitrosamines and diazeniumdiolates (Hou et al., 1999). According to Fitzhugh and Keefer (2000), diazeniumdiolates have been widely used for biomedical applications. Diazeniumdiolates are synthesized by exposing nucleophiles such as primary, secondary or polyamines to NO gas under pressure to generate relatively stable solid donor compounds (Arnold et al., 2002). Decomposition of diazeniumdiolates in solution generates up to two molar equivalents of NO, follows first-order kinetics, and the reaction is highly dependent on solution temperature and pH, and the nucleophilic adduct (Hou et al., 1999; Lemairè et al., 1999; Megson and Webb, 2002).

The most stable diazeniumdiolate (1-substituted diazen-1-ium-1,2-diolate) NO donor compound is 2,2'-(hydroxynitrosohydrazino)-bisethanamine (diethylenetriamine-nitric oxide, DETANO) with a half life in solution of 20 hr at 37^{o}C and pH 7.4 (Lemairè et al., 1999; Fitzhugh and Keefer, 2000; Srinivasan et al., 2001). The rate of NO release, as an NO$^{\bullet}$ free radical (Hou et al., 1999), is pH dependent with a shorter half-life in more acidic solution. The half-life of an 0.10 mM DETANO solution ranges from 24 sec in solutions buffered to

pH 2.04 up to 24.1 hr in solutions buffered to pH 7.46 (Davies et al., 2001). The quality of water used in commercial operations is variable and the instability of DETANO at even mildly acidic pH (Hrabie et al., 1993) would require the use of a buffered solution to ensure a near neutral pH is retained throughout dipping to prevent premature degradation of DETANO. Noritake et al. (1996) first reported DETANO action on plants with induction of the phytoalexin rishitin in potato tubers while Scherer and Holk (2000) found DETANO stimulated betalaine biosynthesis in *Amaranthus caudatus*.

The postharvest application of DETANO was initially studied by Bowyer et al. (2003) who found that cut carnation flowers had an extended postharvest life of about 50% compared to control flowers. Eight types of cut flower treated with 10 and 100 $mg.l^{-1}$ DETANO in a solution buffered to pH 6.5 followed by storage in air containing 0.1 $\mu l.l^{-1}$ ethylene had an extended vase life ranging between 10 to 200 % over control flowers (Badiyan et al., 2004). Pristijono et al. (2008) found that dipping in 10 mg l^{-1} DETANO in pH 6.5 phosphate buffer was effective in inhibiting surface browning of apple slices stored at 0°C compared to untreated slices and extended the postharvest life of apple slices by about 170%. For iceberg lettuce slices, dipping in 500 mg l^{-1} DETANO in water for 5 min was found to be effective for extending postharvest life with DETANO more effective at inhibiting browning than fumigation with NO gas (Wills et al., 2008).

2.6.3. Sodium nitroprusside (SNP)

Sodium nitroprusside (SNP) (Na_2 [Fe $(CN)_5$ NO] $2H_2O$) is a transition metal NO complex class of donor compound that is used clinically to reduced blood pressure (Pitkanen et al., 1999) and in relaxation of vascular muscle and dilation of peripheral arteries and veins (Wang et al., 2002; Marks et al., 1991). SNP is light sensitive and releases the NO^+ cation

(Huo et al., 1999). SNP is soluble in water and relatively stable in solution, resisting oxidation at a neutral or slightly acidic pH (Verner, 1974).

In plants, Murgia et al. (2002) reported that SNP induced the accumulation of ferritin transcript and protein in *Arabidopsis* leaves. Lamattina et al. (2001) found that SNP increased resistance to chilling, heat stress and UV irradiation damage in plants. Pagnussat et al. (2003) found that SNP stimulated adventitious root development in cucumber plants while Zhang et al. (2009) reported that foliar application of SNP reduced malondialdehyde (MDA) content and membrane permeability in *Arabidopsis* leaves and hence inhibited oxidative damage.

SNP has been found to inhibit ethylene production, respiration rate, the activity of aminocyclopropane-1- carboxylic acid (ACC) synthase and reduce the content of ACC in strawberry (Zhu and Zhou, 2007). SNP also inhibited pericarp browning in intact longan fruit (Duan et al., 2007) and flesh browning in whole plum fruits (Zhang et al., 2008) during storage.

2.6.4. Piloty's acid

Piloty's acid (*N*-hydroxybenzenesulfonamide) is a *S*-nitrosothiols NO donor compound that decomposes to release the nitroxyl anion (NO^-) and this decomposition reaction occurs very slowly in aqueous solution at around pH 7 (Hughes and Cammack, 1999). Zamora et al. (1995) reported that the oxidative breakdown of Piloty's acid led to formation of the NO free radical and they suggested that $NO^{\bullet}$ rather than NO^- may account for the vasorelaxing and anti-platelet activity in isolated vascular tissue and platelet-rich plasma of rat. The oxidative

degradation of Piloty's acid is strongly pH-dependent and high pH favours decomposition (Zamora et al., 1995).

No published article was found on the postharvest use of Piloty's acid in plants.

3. MATERIALS AND METHODS

3.1. Produce

Granny Smith apples (*Malus x domestica* Borkh) of similar size, shape and colour were harvested from a commercial orchard in Orange, NSW, transported to the laboratory and stored in air 0ºC for up to 6 months. During this period, apples were periodically selected for inclusion in a series of experiments.

The required number of apples in each experiment were removed to 20ºC and after 2 hr each apple was hand-cut longitudinally into six unpeeled slices using a sharp stainless steel knife and slices were placed into 4 L containers with a sealable lid. Each container contained six slices, each from a different apple with a total weight of 100 ± 10 g and this comprised a treatment unit. Each experiment was repeated at least three times on different occasions and there were three treatment units in each replicate.

The fancy lettuces (*Latuca sativa* L.) (Green oak, Green coral, Butter and Baby cos) were purchased from a local supermarket when required and transported to the laboratory. After discarding the outer damaged and wilted leaves, lettuce leaves were selected for uniformity of size, shape and colour, and without any defects or mechanical injury. The mid-rib section of lettuce was cut horizontally into pieces of about 1 × 3 cm using a sharp stainless steel knife. Slices from a single leaf were evenly distributed into the required number of 4 L containers. For each treatment unit, 25-30 g of lettuce slices from 4-5 different lettuce heads were placed in a container.

3.2. Treatment with NO

3.2.1. NO gas

NO gas was obtained from a cylinder of 4500 $\mu l.l^{-1}$ NO in nitrogen (BOC Gases, Sydney). A unit of fresh cut apple or lettuce slices was sealed in a 4 L container and NO gas was applied through an injection port in the lid of the container to give the desired concentration of NO. The NO gas concentration was calculated from the formula:

$$\text{Volume of NO injected (ml)} = \frac{\text{Desired concentration } (\mu l.l^{-1}) \times \text{Vol. of container (ml)}}{\text{Standard NO concentration } (\mu l.l^{-1})}$$

For concentrations higher than 100 $\mu l.l^{-1}$, NO gas from the cylinder was flushed directly through the inlet at a flow rate of 1 $l.min^{-1}$. The flushing time was determined according to the formula:

$$\text{Flushing time (min)} = \frac{\text{Desired concentration } (\mu l.l^{-1}) \times \text{Vol. of container (L)}}{\text{NO flow rate } (l.min^{-1}) \times \text{standard NO concentration } (\mu l.l^{-1})}$$

After application of NO gas for the desired time, the lids of the containers were opened to atmospheric air and the lid replaced but an injection port (4 mm diameter) in the lid was opened to prevent CO_2 gas accumulation inside the container. A beaker (50 ml) with water was provided in each container to maintain a high humidity. All treated and control containers were then stored at 5˚C.

3.2.2. Donor compounds

DETANO was supplied by Dr. M. C. Bowyer, University of Newcastle, as a powder that had been prepared by reaction of diethylenetriamine (DETA) with NO gas at a pressure of 400-

500 kPa for 24 hr (Hrabie et al., 1993). The DETANO was placed in a sealed jar and stored at -18^{o}C before use. After each use of DETANO, the jar was flushed with nitrogen gas for 10 to 15 min to remove oxygen and resealed.

SNP and Piloty's acid were purchased from Ajax Chemicals, Victoria and Cayman Chemical, Ann Arbor, Michigan, respectively. SNP was stored in the dark at 20˚C and Piloty's acid was stored at -18^{o}C.

The desired concentration of DETANO was dissolved in 0.01 M buffer phosphate at pH 6.5. 1.4 g sodium dihydrogen phosphate (Chem-Supply, Beverley, South Australia) was dissolved in approximately 900 ml distilled water, 8.05 g sodium chloride (Sigma Aldrich, Sydney) was added and the volume made up to 1 L with distilled water. SNP and Piloty's acid were dissolved in water.

A unit of fresh-cut slices was placed in a stainless steel mesh strainer and dipped into a donor solution for 5 min at 20^{o}C. After draining, the slices were allowed to dry for 2-5 min before placing in a 4 L container and storing at 5^{o}C. The lid of the container had an open pore to avoid CO_2 gas accumulation and a beaker (50 ml) of water to maintain a high humidity atmosphere.

3.3. Assessment of postharvest life

3.3.1. Apple

The browning of apple slices was assessed on the colour of the cut surface using a colorimeter (Minolta CR-300, Osaka) to measure the L value (lightness). Six readings were

taken from cortex tissue of each apple slice, three from each side of a slice with the colorimeter measuring head placed along the longitudinal axis on the midpoint between the core and skin at 1/3, 1/2, and 2/3 from the calyx. The colorimeter was calibrated with a white tile plate (Calibration Plate CR-A43) before measurement.

To show the relationship between L value and a visual score of browning, a preliminary experiment was conducted at 20^{o}C. Visual scoring of browning on 30 slices was made each day using a 1 to 5 scale, where 5 = no browning, 4 = slight browning of the cut surface, 3 = moderate browning of the cut surface, 2 = severe brown discoloration of the cut surface, and 1 = complete discolouration of the cut surface. The L value measurement with the colorimeter was also taken on each slice daily.

The mean L value when each of the 30 slices attained a browning score of 5, 4, 3, 2 and 1 was calculated. Figure 3.1 shows the relationship between L value and visual score for browning of the cut slices was a linear function. The time taken for browning to attain an L value of 75.6 which was equivalent to a visual score of 3 was taken as the postharvest life for apples in all subsequent studies.

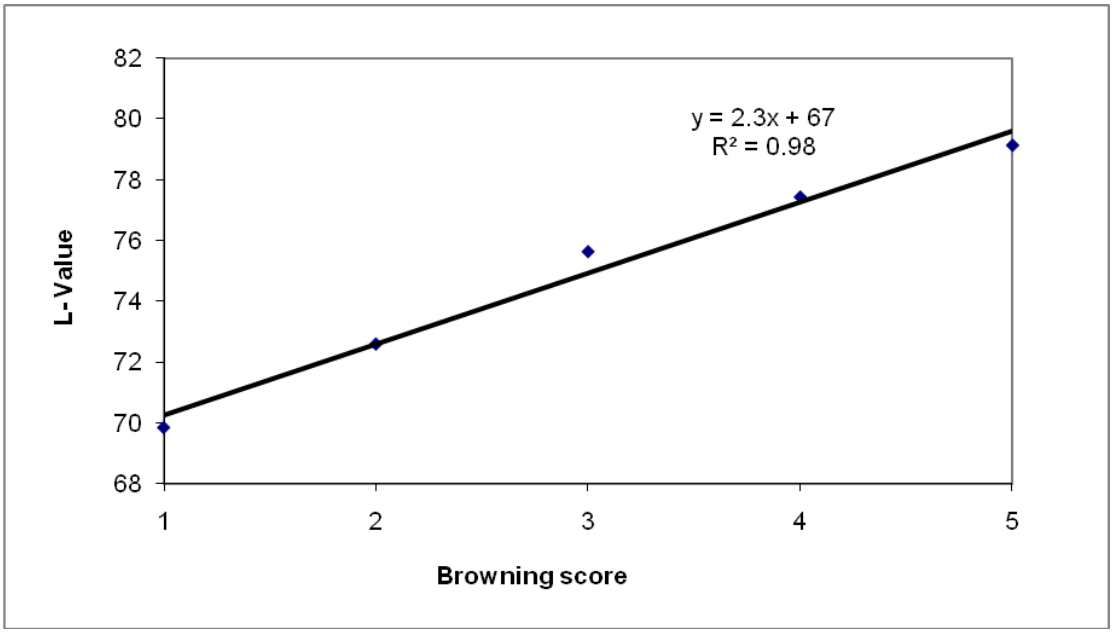

Fig 3.1 Relationship of Minolta L value to subjective score of browning for Granny Smith apple slices.

Values are the mean L value for the 30 apples to attain each browning score.

In apple experiments, the L value of each apple slice in a unit was measured daily and the mean L value of all slices in a unit was plotted against time. A linear regression was used to show the time taken for the L value to decline to 75.6 which was taken as the postharvest life. The mean postharvest life of all six slices in a treatment unit was expressed as the postharvest life for the treatment unit.

3.3.2. Fancy lettuces

Postharvest life was assessed on the colour of the cut surface of each lettuce slice that was assessed visually with browning ranked from 1 to 5, where 5 = fresh without any browning/pink colour, 4 = slight browning/pink discolouration of the cut surface, 3 = moderate browning of the cut surface, 2 = severe browning / pink discoloration of the cut surface, and 1 = complete browning / pink discoloration of the cut surface. The cut surface of

a lettuce leaf was too small to allow use of the colorimeter. The daily mean score for all lettuce slices in a treatment unit was calculated. The daily mean of score was plotted against time and the time taken for browning to attain a score of 3 was accepted as the postharvest life.

3.4. Physiological assessment of apple and lettuce slices

3.4.1. Respiration

Fresh cut slices of Granny Smith apples and fancy lettuces were weighed before treatment. A treated unit of produce was placed into a sealed 800 ml plastic container at various days after treatment. After 4 hr in the sealed container, a gas sample (1 ml) was collected from the sealed container in a syringe.

The concentration of CO_2 in the gas sample was determined by injecting into a thermal conductivity gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with two stainless steel columns (60 cm x 1 mm i.d.) in series. The column for CO_2 contained Haysep N (80-100 mesh) (Altech, Sydney) and the operating temperatures for the detector, injector, column and current detector were 110^{o}C, 50^{o}C, 110^{o}C and 80^{o}C, respectively. The carrier gas was high purity argon (BOC Gases, Sydney) at a flow rate of 25 ml.min^{-1}. The gas chromatograph response to a standard gas mixture containing 5.07 ± 0.1% CO_2 in nitrogen and 4.99 % + 0.1 % oxygen in air (BOC Gases, Sydney) was used to quantify the test sample concentrations. Respiration was calculated from the formula:

$$\text{Respiration (ml } CO_2.kg^{-1}.hr^{-1}) = \frac{\%\ CO_2 \times \text{Vol. of container (ml)}}{\text{Initial produce weight (kg)} \times 100 \times \text{time (hr)}}$$

3.4.2. Ethylene

A gas sample was collected from the atmosphere of fresh-cut produce in a sealed container as for respiration (Section 3.4.1) and the concentration of ethylene was measured by injecting the gas sample into a flame ionisation gas chromatograph (Varian Star CX-3400, Walnut Creek, CA) fitted with a stainless steel column (2 m x 3.2 mm o.d. x 2.2 mm i.d.) packed with Porapak Q (80 - 100 mesh) (Altech, Sydney). 150^{o}C, 90^{o}C and 70^{o}C were the operating temperatures for the detector, column and injector, respectively. Nitrogen, hydrogen and air were used as carrier and combustion gases at a flow rate of 15 ml.min^{-1}, 20 ml.min^{-1} and 50 ml.min^{-1}, respectively. The ethylene production rate was calculated from the formula :

$$\text{Ethylene production } (\mu l\ C_2H_4.kg^{-1}.hr^{-1}) = \frac{C_2H_4\ (\mu l.l^{-1}) \times \text{Vol. of container (L)}}{\text{Initial produce weight (kg)} \times \text{time (hr)}}$$

3.4.3. Water loss

Water loss from the fresh cut pieces was measured by the change in weight of the samples over a storage period. The weight of fresh cut produce was recorded before treatment, immediately after treatment then daily during storage in the sealed container with lid holes and a beaker of water at 5ºC for 4 days. Water loss was calculated from the formula:

$$\text{Water loss (\%)} = \frac{\text{Water loss (g)}}{\text{Initial produce weight (g)}} \times 100$$

3.5. Biochemical assessment of apple slices

For biochemical assessment, five apple slices from five different apples were combined to provide a treatment unit. Treated units of five apple slices were stored in a 4 L container at 5^{o}C. One apple slice was removed from each container at various times up to 8 days of storage to measure a range of biochemical factors. Each factor was assessed on a different batch of fruit.

3.5.1. Total phenol content

Total phenol content was determined according to the Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965). For each apple slice, a section of tissue (1g) was cut from the outside of the core area to just below the skin at a point halfway from the calyx to core and the sample was immediately placed at -18ºC for 30 min. The frozen tissue was then homogenized with a pestle in a cold mortar with cold 75% methanol (5ml) and the homogenate centrifuged (Beckman J2-MC, PaloAlto, CA) at 13,000 g for 5 min at room temperature. Extracting into cold methanol minimized the interference of non-phenolic compounds to the assay. The supernatant was filtered through filter paper (Whatman 1, 7.0 cm) and diluted with distilled water (1:10). A diluted sample (0.5 ml) was added to diluted 2N FC reagent (1:10) (2.5 ml). After 3-4 min, 7.5% sodium carbonate solution (2 ml) was added to the mixture and covered for 2 hr at room temperature.

The absorbance of the solution at 765 nm was determined by using a Varian Cary 50 UV-spectrophotometer (Varian Australia, Clayton, Victoria). Gallic acid was used as a calibration standard and a standard curve was prepared using concentration 10, 25, 50 and 100 ppm. The data was expressed as ppm (mg/l) gallic acid equivalents.

3.5.2. PPO activity

Frozen apple tissue (1 g) obtained as described above was homogenized in a mortar and pestle with a cold solution (4ºC) containing 100 mM sodium phosphate buffer (pH 7.0) and 0.25 g polyvinylepolypyrrolidone (10 ml). The homogenate was centrifuged at 17,000 g for 15 min at 4ºC and extracts were filtered through three layers of cheese cloth.

Enzyme activity in the supernatant was determined according to the method described by Yingsanga et al. (2008). Supernatant (1 ml) combined with sodium phosphate buffer (100 mM, pH 7.0) (1 ml) and pyrocatechol (50 mM) (1 ml) was taken into a 3 ml cuvette and one unit of PPO activity was measured spectrophotometrically as a 0.01 unit change in absorbance per min at 410 nm at 25ºC.

3.5.3. Lipid peroxidation

3.5.3.1. Malondialdehyde

Malondialdehyde (MDA) was considered to be a suitable biomarker for lipid peroxidation caused by reactive oxygen species (ROS) which are purported as a major cause of membrane deterioration in plant tissues (Mittlar, 2002; Zheng et al., 2007).

MDA was measured by the method described by Heath and Packer (1968). Fresh apple tissue (1 g) was homogenized in a mortar and pestle with a solution (3 ml) containing 0.25% thiobarbituric acid and 10% trichloroacetic acid. The homogenate was incubated in a water bath at 90°C for 1 hr and then cooled and centrifuged at 10,000 g for 15 min at room temperature. The absorbance of the supernatant was determined at 560 and 600 nm.

Subtracting the absorbance at A_{600} from A_{560} to correct for the unspecific turbidity of the samples and using the MDA molar extinction coefficient (155 mM^{-1}. cm^{-1}), the content of MDA was calculated as:

MDA (µm) content $= A_{560} - A_{600} /155$

3.5.3.2. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) was used as an alternative biomarker for oxidative stress generated by reactive oxygen species (ROS) in plant tissues during normal metabolism (Lu et al., 2009).

H_2O_2 was determined by the method of Sun et al. (2010). A section of fresh apple tissue (2 g) was homogenized in a mortar and pestle with cold (4ºC) acetone (10 ml). The homogenate was centrifuged at 15000 g for 15 min at 4º C. An extract (1 ml) was mixed with 5% titanium dioxide in 20% (v/v) H_2SO_4 (0.1 ml) and 25% ammonia solution (Chem-Supply, Beverley, South Australia) (0.2 ml) and then centrifuged at 10000 g for 10 min. The supernatant was discarded and the precipitate was dissolved in 2M H_2SO_4 (3 ml) and the absorbance was recorded at 415 nm. H_2O_2 was calculated using the extinction coefficient 0.28 $\mu mol^{-1}.cm^{-1}$ (Hung and Kao, 2007).

3.5.4. Ion leakage

Ion leakage from cells was measured using the method described by Song et al. (2006). A 0.5 cm thick section of fresh apple flesh (2 g) was placed in a beaker containing de-ionized water (40 ml) and incubated for 2 hr at 25°C. The conductivity of the solution was then measured with a conductivity meter (Model 4071, Jenway, Staffordshire, UK) as the initial reading. The solution with the sample was then boiled for 15 min and after cooling in room

temperature, the conductivity was measured as the final reading. The percentage of the ion leakage was calculated as follows:

$$\text{Percent (\%) ion leakage} = \frac{\text{Initial reading}}{\text{Final reading}} \times 100$$

3.6. Treatment with chlorogenic acid

3.6.1. Effect of browning

The effect of chlorogenic acid on the development of browning of fresh-cut apple slices was examined in conjunction with dipping in DETANO solution. Apple slices were dipped into 10 $mg.l^{-1}$ DETANO dissolved in 6.5 pH phosphate buffer (0.01M), in phosphate buffer only or water for 5 min and allowed to drain. Slices were also fumigated with 10 $\mu l.l^{-1}$ NO gas for 1 hr at 20^{o}C. Five minutes after completion of the treatment slices were then dipped into different concentrations (0.1, 0.01 and 0.001%) of chlorogenic acid (Sigma Aldrich, Sydney) dissolved in water for 10 sec. In another experiment the order was reversed with apple slices dipped into chlorogenic acid then treated with NO. Treated slices were stored in a 4 L container at 5^{o}C as previously stated. The L value of each slice was measured daily and the mean postharvest life of all six slices in a treatment unit was determined as previously stated.

3.6.2. Spectral change of chlorogenic acid

To observe the oxidation of chlorogenic acid, solutions of 0.001% chlorogenic acid dissolved in water, DETANO (10 $mg.l^{-1}$) and pH 6.5 phosphate buffer (0.01 M) were prepared. In addition an aqueous solution of chlorogenic acid that was fumigated with NO gas for 1 hr (10 $\mu l.l^{-1}$) was also prepared. All solutions were scanned periodically over 8 days

at 20ºC with a UV-spectrophotometer where the scan speed was medium over the wavelength range of 200 to 800 nm.

3.7. Controlled atmosphere (CA) storage

The effects of CA storage on apple slices was examined at 20^{o}C with specific gas mixtures generated using a gas flow board and control valves, gas supply inlet, gas mixing apparatus and gas analyser. Air from a compressor and CO_2 or O_2 from a cylinder (BOC Gases, Sydney) were suppled at a pressure of 400 kPa and then regulated to a flow at a constant pressure of 15 kPa using pressure regulators. The flow of air and CO_2 was further controlled by 'Hoke' needle valves and glass capillaries to generate the required flow of gas at the required concentrations of CO_2 (2, 4 and 6%) and O_2 (1, 2 and 5%) which were then humidified by bubbling through a jar of water. The humidified gas mixture was passed through a flow board with a manifold fitted with glass capillaries to obtain multiple gas outlets at a uniform low flow rate of 25 ml/min each of which was passed into a jar containing fruit slices. The concentration of CO_2 and O_2 in gas streams were measured with an infra red gas analyser (ICA 61, Kent, UK).

Three slices from three different apples (100 ±10 g) were treated by submerging in distilled water and 10 mg.l^{-1} DETANO dissolved in phosphate buffer for 60 sec**.** A unit of slice**s** was stored at 20ºC in a sealed glass jar (1.8 L) and a required gas mixture of CO_2 in air and O_2 in N_2 was passed into the glass jar through above stated process and exhausted through an outlet tube.

Postharvest life was assessed based on daily visual scoring of browning (from 1 to 5) observed each day to a mean score of 3.0 for all the slices in a treatment unit. The

concentration of ethylene was measured by collecting a gas sample (1 ml) from the outlet tube of the sealed glass jar (sealed for 4 hr) in a syringe and injecting into a gas chromatograph. The water loss was measured by the change in weight of the samples over a given storage period.

3.8 Statistical analysis

Statistical procedures were performed using SPSS for Microsoft version 18.0 software package (SPSS Chicago, IL). Analysis of variance was conducted on mean values for treatments and storage times for every parameter. Where ANOVA analysis showed a significant difference for treatment or storage time, the LSD was calculated from the mean values at P = 0.05 by the following formula : $LSD = t \sqrt{2MS/R}$, where t = degrees of freedom, MS = error using the mean square value, R = number of replicates in the mean value. For every parameter, each replicate has 3 treatment units but the mean of these three units was taken as a single value for that replicate. Linear regression equations were calculated to determine the relationship between postharvest life and the applied treatment.

4. RESULTS

4.1. Granny Smith Apple

4.1.1. Effect of NO gas and DETANO- Season -1

All experiments during a season were conducted from late March to late September on a batch of apples harvested in March and stored at 0ºC until required. Studies determined effects of NO gas and DETANO on browning development and physiological and biochemical changes of apple slices.

4.1.1.1. Preliminary study

A preliminary study was conducted soon after the 2009 harvest to gauge the likely effect of the various treatments on apple physiology and constituents. NO treatments applied were apples slices fumigated with 10 $\mu l.l^{-1}$ NO gas in air for 1 hr at 20^{o}C and dipped in 10 $mg.l^{-1}$ DETANO dissolved in pH 6.5 phosphate buffer for 5 min with water-dipped fruit and untreated slices being control treatments. After treatment all fruits were stored at 5^{o}C. Biochemical and physiological changes were assessed daily from 0-4 days of storage.

4.1.1.1.1. Browning development

The initial experiment confirmed that DETANO inhibited browning ($P<0.001$) with a significantly longer postharvest life of 9.0 days than all other treatments while apples fumigated with NO gas produced a longer postharvest life of 6.5 days than those dipped in water and untreated (4.8 and 4.5 days, respectively) which were not significantly different.

4.1.1.1.2. Effect on apple physiology

The effect of NO and DETANO on the ethylene production, respiration and water loss of Granny Smith apple slices is given in Table 4.1.

The respiration rate of apple slices was significantly different between treatments ($P<0.05$) with slices treated with NO gas and DETANO significantly lower than untreated or water-dipped slices, which were not significantly different over the whole storage period. There was no significant difference between NO gas and DETANO-treated slices. There was a significant effect of time on respiration ($P<0.001$) with the rate higher at 0 days in all treatments than all other days.

NO gas and DETANO showed no significant effect on ethylene production or water loss of apples slices at any storage time compared to untreated and water-dipped slices.

4.1.1.1.3. Effect on apple biochemistry

The levels of total phenol, PPO activity and MDA and ion leakage during 0-4 days at 5ºC are given in Table 4.2.

Total phenol content of fresh-cut apple slices, was significantly different between treatments ($P<0.001$) with the lowest level in DETANO-treated slices. Total phenol content in NO gas-treated slices was significantly less than those in water which was less than in untreated slices. There was a significant effect ($P<0.001$) of storage period on total phenol content increasing throughout the storage period.

Table 4.1 Respiration, ethylene production and water loss of Granny Smith apple slices fumigated with 10 µl.l^{-1} NO gas for 1 hr or dipped in 10 mg.l^{-1} DETANO in pH 6.5 phosphate buffer solution for 5 min at 20^{o}C, followed by storage at 5^{o}C in air

Treatment	day 0	1	2	3	4	**Mean**
			Respiration (ml CO_2.kg^{-1}.hr^{-1})			
Untreated	9.5	6.6	6.7	6.5	6.9	**7.2**
Water	8.9	6.8	7.2	6.4	6.9	**7.2**
NO gas	8.3	5.6	6.4	6.1	6.1	**6.5**
DETANO	8.1	5.7	6.1	6.1	6.5	**6.5**
LSD (5%) n=3	*1.65*				*n=12*	***0.82***
			Ethylene production (ml.kg^{-1}. hr^{-1})			
Untreated	3.1	1.2	1.5	1.3	1.3	**1.7**
Water	3.4	1.3	1.6	1.2	1.0	**1.7**
NO gas	2.8	1.4	1.4	1.2	1.4	**1.6**
DETANO	2.9	1.2	1.3	0.9	1.1	**1.5**
LSD (5%) n=3	*0.65*				*n=12*	***0.33***
			Water Loss (%)			
Untreated	0.2*	0.6	1.0	1.6	2.1	**1.1**
Water	0.4	0.8	1.2	1.7	2.1	**1.2**
NO gas	0.2	0.7	1.1	1.6	2.0	**1.1**
DETANO	0.4	0.8	1.3	1.8	2.2	**1.3**
LSD (5%) n=3	*1.27*				*n=12*	***0.63***

Values are the mean of 3 replicates with 3 treatments units in each replicate
*Water loss at day 0 was assessed 2 hr after treatment.

PPO activity over the storage period showed no significant difference between DETANO, NO gas and water-treated slices but NO gas and DETANO-treated slices were significantly lower than untreated ($P<0.01$). Storage period showed a significant difference ($P<0.001$) with PPO activity of all treatments increasing at day 4.

Lipid peroxide levels as represented by malondialdehyde (MDA) showed no significant difference between treatments. There was a significant effect ($P<0.001$) of storage period with the level of MDA decreasing with increasing time.

ANOVA analysis for ion leakage showed that there was no significant difference between treatments. Storage period showed a significant difference ($P<0.01$) with ion leakage of all treatments increasing at day 1 and decreasing at 2 to 4 days.

Table 4.2 Total phenol, PPO activity, MDA content and ion leakage of Granny Smith apple slices fumigated with 10 $\mu l.l^{-1}$ NO gas for 1 hr or dipped in 10 $mg.l^{-1}$ DETANO in pH 6.5 phosphate buffer solution for 5 min at 20°C, followed by storage at 5°C in air

Treatment	day 0	1	2	3	4	**Mean**
Experiment 1[a]		*Total Phenol (Gallic acid equivalents ppm)*				
Untreated	189	183	193	200	224	**198**
Water	162	169	180	89	199	**179**
NO gas	156	162	171	180	186	**171**
DETANO	152	158	164	170	174	**164**
LSD (5%) n=3	*9.89*				*n=12*	***4.94***
Experiment 2[b]		*PPO activity (ΔAbs/min)*				
Untreated	0.035	0.043	0.046	0.056	0.060	**0.048**
Water	0.038	0.041	0.042	0.044	0.052	**0.043**
NO gas	0.032	0.041	0.040	0.040	0.046	**0.040**
DETANO	0.039	0.045	0.040	0.038	0.042	**0.041**
LSD (5%) n=5	*0.012*				*n=20*	***0.006***
Experiment 3 [c]		*Malondialdehyde (MDA) (µmol)*				
Untreated	2.36	2.18	2.03	1.77	1.77	**2.02**
Water	2.32	2.04	2.03	1.91	1.83	**2.03**
NO gas	2.22	2.04	2.04	1.92	1.69	**1.98**
DETANO	2.45	1.93	1.81	1.85	1.75	**1.96**
LSD (5%) n=4	*0.37*				*n=16*	***0.18***
Experiment 4 [c]		*Ion leakage (%)*				
Untreated	47.8	55.7	52.8	51.2	50.3	**51.6**
Water	46.4	53.1	51.9	50.5	48.9	**50.1**
NO gas	43.2	51.0	49.3	47.7	48.0	**47.8**
DETANO	44.6	51.0	49.1	48.3	46.8	**48.0**
LSD (5%) n=4	*7.47*				*n=16*	***3.73***

Values are the mean of [a] 3 replicates, [b] 5 replicates and [c] 4 replicates with 3 treatment units in each replicate

4.1.1.2. Main study

Changes in apple composition were investigated over the period 0-8 days as the preliminary study found that the postharvest life of DETANO-treated apple slices was extended to about 8 days. Dipping slices in a pH 6.5 phosphate buffer solution was added as an additional control.

4.1.1.2.1. Browning development

The data in Table 4.3 confirmed that DETANO-treated slices showed a greater postharvest life than those fumigated with NO gas and that DETANO and NO gas showed a greater postharvest life than their respective control treatments of phosphate buffer and untreated. Slices dipped in buffer showed a significantly longer postharvest life than untreated slices. The effect of DETANO in retarding browning development is shown in Fig 4.1.

Comparison with the postharvest life obtained in the preliminary study showed a similar extension achieved by DETANO and NO gas. Thus, NO was equally effective on fresh and 6 months stored fruits.

4.1.1.2.2. Effect on apple physiology

The effect of NO and DETANO on the respiration of Granny Smith apple slices during 0-8 days at 5ºC is given in Table 4.4.

Over the whole storage period (0-8 days), respiration was significantly different between treatments ($P<0.001$) with the respiration of DETANO < NO gas < phosphate buffer < water < untreated. A difference between treatments was apparent from day 0 when the respiration

Table 4.3 Postharvest life of Granny Smith apple slices dipped in DETANO in pH 6.5 phosphate buffer solution and fumigated with NO gas at 20^{o}C followed by storage at 5^{o}C in air.

Treatment	Postharvest life (Days)
Untreated	3.5
Water for 5 min	3.9
Phosphate buffer for 5 min	5.5
10 µl.l^{-1} NO gas for 1 hr	6.9
10 mg.l^{-1} DETANO for 5 min	8.6
LSD (5%) n=3	*1.64*

Values are the mean of 3 replicates with 3 treatments units in each replicate

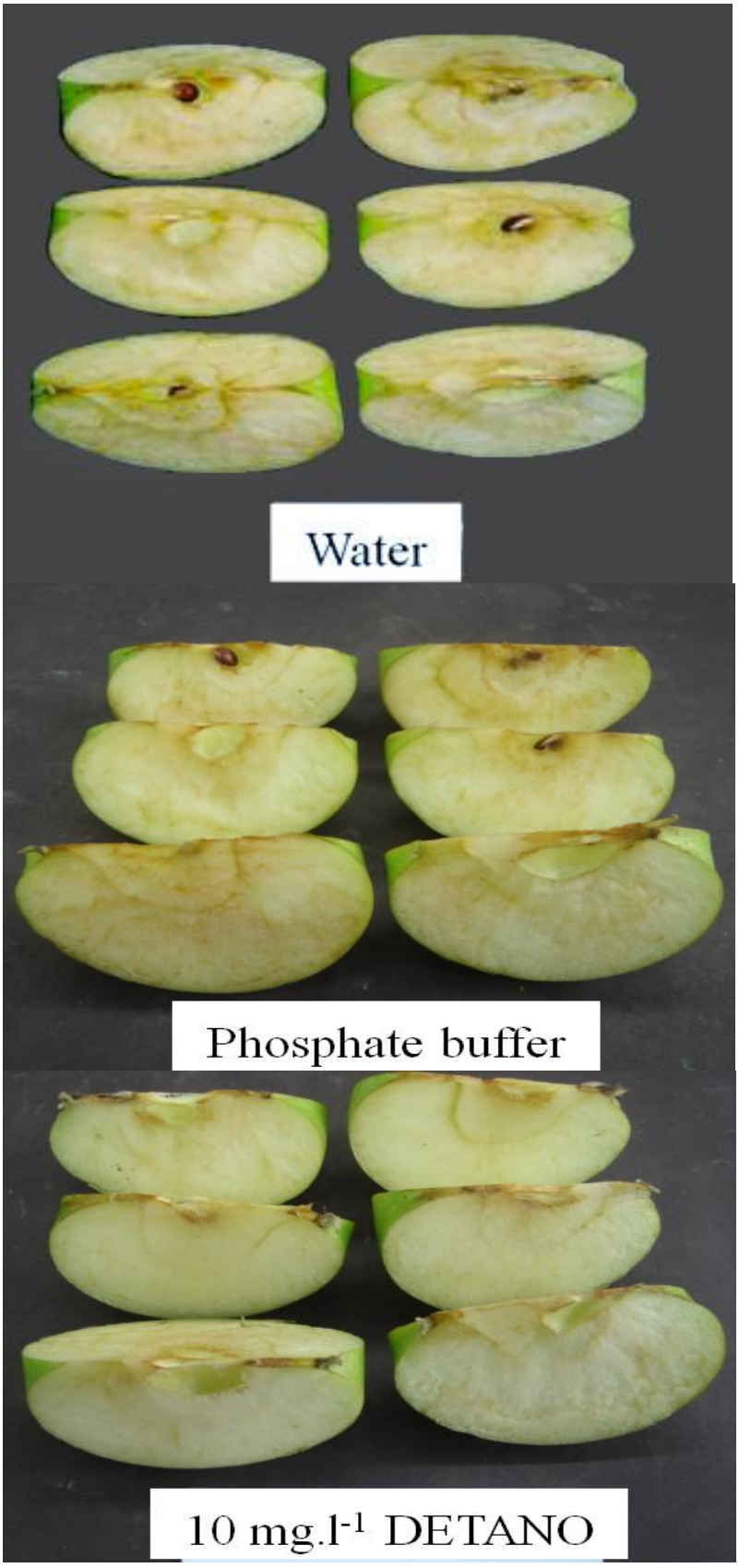

Fig. 4.1 Apple slices dipped in water, phosphate buffer and 10 mg.l^{-1} DETANO in pH 6.5 phosphate buffer solution for 5 min that had been stored for 8 days at 5ºC in air

of DETANO-treated slices was lower than all other treatments except for NO gas with NO gas lower than water and untreated slices. Storage period had a significant effect ($P<0.001$) on respiration for all treatments with significant reduction in respiration from day 0 to day 2 then increasing to day 8.

Ethylene production and water loss of apple slices were not examined as the preliminary experiment showed that NO had no effect on these attributes.

4.1.1.2.3. Effect on apple biochemistry

The levels of total phenol, PPO activity, MDA and ion leakage during 0-8 days at 5ºC are given in Table 4.5.

4.1.1.2.3.1. Total phenol

Statistical analysis for total phenol showed that there was a significant difference ($P<0.001$) between treatments with the total phenol of DETANO < phosphate buffer, NO < untreated and untreated > all other treatments but there was no significant difference between DETANO and NO gas. Storage time had a significant effect ($P<0.001$) with total phenol content increasing during storage. The results are similar to those obtained in preliminary study.

4.1.1.2.3.2. PPO activity

There was a significant effect of treatment ($P<0.001$) on PPO activity with DETANO < NO gas < water < untreated. Unfortunately phosphate buffer was not included as a treatment in this study. PPO activity increased ($P<0.001$) in all treatments with increased storage time.

Table 4.4 Respiration of Granny Smith apple slices fumigated with 10 $\mu l.l^{-1}$ NO gas for 1 hr and dipped in 10 $mg.l^{-1}$ DETANO in pH 6.5 buffer phosphate for 5 min at 20°C, followed by storage at 5°C in air

Treatment	Respiration (ml $CO_2.kg^{-1}.hr^{-1}$)					
	day 0	2	4	6	8	**Mean**
Untreated	14.0	11.6	13.1	15.1	16.7	**14.1**
Water	13.6	10.8	12.5	13.8	15.6	**13.2**
Phosphate buffer	12.1	10.1	11.0	12.5	14.4	**12.0**
NO gas	11.2	9.1	10.4	11.2	13.4	**11.1**
DETANO	10.2	8.3	9.9	10.7	13.1	**10.4**
LSD (5%) n=3	*1.15*				*n=15*	***0.52***

Values are the mean of 3 replicate with 3 treatments units in each replication

The greater effectiveness of the treatments than in the preliminary study was due to extending the study to 8 days as a greater difference between treatments occurred at the longer storage times.

4.1.1.2.3.3. Lipid peroxide

Statistical analysis of MDA levels showed that there was no significant difference between treatments nor with storage time similar to the preliminary study.

4.1.1.2.3.4. Ion leakage

Statistical analysis showed a significant effect of treatment ($P < 0.001$) with DETANO < NO gas < phosphate buffer = water < untreated over the whole storage period. Storage time had a significant effect on ion leakage ($P<0.001$) while increased at day 2 then decreased at 4 days and increased again at 6 to 8 days.

Table 4.5 Total phenol, PPO activity, MDA content and ion leakage of Granny Smith apple slices fumigated with 10 $\mu l.l^{-1}$ NO gas for 1 hr or dipped in 10 $mg.l^{-1}$ DETANO in pH 6.5 phosphate buffer for 5 min at 20°C, followed by storage at 5°C in air

Treatment	Day 0	2	4	6	8	**Mean**
Experiment 1		*Total Phenol (Gallic acid equivalent in ppm)*				
Untreated	149	159	170	178	186	**168**
Water	145	151	159	170	178	**161**
Buffer	140	145	156	165	172	**155**
NO gas	135	140	152	158	166	**150**
DETANO	133	138	148	155	161	**147**
LSD (5%) n=3	*14.64*				*n=15*	***6.54***
Experiment 2			*PPO activity (ΔAbs/min)*			
Untreated	0.031	0.038	0.047	0.054	0.060	**0.046**
Water	0.028	0.034	0.042	0.049	0.056	**0.042**
Buffer	-	-	-	-	-	-
NO gas	0.026	0.029	0.038	0.044	0.053	**0.038**
DETANO	0.025	0.027	0.034	0.042	0.047	**0.035**
LSD (5%) n=3	*0.006*				*n=12*	***0.003***
Experiment 3			*Malondialdehyde (MDA) (µmol)*			
Untreated	1.86	2.09	2.03	1.92	2.04	**1.99**
Water	1.77	1.98	1.92	1.87	2.01	**1.91**
Buffer	1.94	2.04	1.98	1.89	1.97	**1.97**
NO gas	1.93	2.03	1.98	1.98	1.86	**1.97**
DETANO	2.21	2.19	2.02	1.91	1.92	**2.05**
LSD (5%) n=3	*0.23*				*n=15*	***0.10***
Experiment 4			*Ion leakage (%)*			
Untreated	52.0	56.0	50.5	52.1	55.1	**53.1**
Water	49.2	52.8	48.6	49.8	51.8	**50.5**
Buffer	48.1	51.5	46.9	48.5	50.2	**49.0**
NO gas	45.8	50.1	45.0	46.1	47.5	**46.9**
DETANO	45.1	48.5	42.7	44.5	45.6	**45.3**
LSD (5%) n=3	*3.38*				*n=15*	***1.51***

Values are the mean of 3 replicates with 3 treatments units in each replicate

4.1.2. Effect of NO gas and DETANO- Season - 2

All experiments were conducted from late March to middle September 2010 on a batch of apple harvested in March 2010 and stored at 0ºC until required. Studies determined the effects of NO gas and DETANO solution on browning development and physiological and biochemical changes of apple slices in order to confirm the findings obtained in Season-1.

4.1.2.1. Browning development

Statistical analysis of effects on NO gas and DETANO on browning development of apple slices (Table 4.6) showed that there was a significant difference ($P<0.001$) between treatments with a greater postharvest life for DETANO-dipped slices than those fumigated with NO gas and with both treatments having a greater postharvest life than their respective control treatments of phosphate buffer and untreated. Phosphate buffer showed an extension in postharvest life over water-dipped or untreated slices.

4.1.2.2. Effect on apple physiology

The effect of NO gas and DETANO on the respiration and ethylene production of Granny Smith apple slices is shown in Table 4.7.

Statistical analysis showed that there was a significant difference ($P<0.001$) between treatments with the respiration rate of slices treated with DETANO < NO gas < phosphate buffer < water < untreated. Storage period also had a significant effect ($P<0.001$) on respiration with a significant reduction in respiration for all treatments from day 0 to day 2 then increasing to day 8.

There was no significant effect on ethylene production of apples fumigated with NO or dipped in DETANO at any time during storage.

Table 4.6 Postharvest life of Granny Smith apple slices fumigated with NO gas or dipped in DETANO and phosphate buffer pH 6.5 at 20°C, followed by storage at 5°C in air

Treatment	Postharvest life (Days)
Untreated	3.4
Water for 5 min	4.2
Phosphate buffer for 5 min	6.1
10 µl.l^{-1} NO gas for 1 hr	7.2
10 mg.l^{-1} DETANO for 5 min	8.6
LSD (5%) n=4	*0.77*

Values are the mean of 4 replicates with 3 treatment units in each replicate

Table 4.7 Respiration and ethylene production of Granny Smith apple slices fumigated with 10 µl.l^{-1} NO gas for 1 hr or dipped in 10 mg.l^{-1} DETANO and buffer phosphate buffer pH 6.5 for 5 min at 20°C, followed by storage at 5°C in air

Treatment	day 0	2	4	6	8	**Mean**
			Respiration (ml CO_2.kg^{-1}.hr^{-1})			
Untreated	14.7	13.2	14.1	16.0	17.9	**15.2**
Water	13.0	11.6	13.3	14.5	16.0	**13.7**
Buffer	11.9	10.1	11.7	13.4	14.5	**12.3**
NO gas	10.1	8.6	10.2	11.9	12.7	**10.7**
DETANO	9.0	6.5	8.3	10.4	11.9	**9.2**
LSD (5%) n=3	*2.23*				*n=15*	***0.99***
			Ethylene production (ml.kg^{-1}.hr^{-1})			
Untreated	2.8	2.6	1.9	2.6	2.2	**2.4**
Water	2.8	2.6	2.1	2.2	2.0	**2.3**
Buffer	2.7	2.9	1.7	1.8	1.6	**2.1**
NO gas	3.0	2.6	1.8	2.4	1.9	**2.3**
DETANO	3.0	2.7	1.6	2.0	1.6	**2.2**
LSD (5%) n=3	*0.67*				*n=15*	***0.29***

Values are the mean of 3 replicates with 3 treatment units in each replicate

4.1.2.3. Effect on apple biochemistry

The effect of NO gas and DETANO on total phenol, PPO activity, ion leakage and hydrogen peroxide level is given Table 4.8.

4.1.2.3.1. Total phenol

Total phenol content showed a significant difference ($P<0.001$) between treatments. Slices treated with NO gas and DETANO had a significantly lower phenol content than all other treatments. Slices treated with phosphate buffer showed a significantly lower phenol content compared to water-dipped slices which was less than in untreated slices. Storage period showed a significant difference ($P<0.001$) with total phenol content of all treatments increasing throughout storage.

4.1.2.3.2. PPO activity

PPO activity was significantly different ($P<0.001$) between treatments with the PPO activity of DETANO < NO gas < phosphate buffer < water < untreated. Storage period had a significant effect ($P<0.001$) on PPO activity with the level in all treatments increasing during storage.

4.1.2.3.3. Hydrogen peroxide level

In the previous season NO and DETANO had no effect on lipid peroxide level as measured by MDA. To further check possible involvement of ROS in oxidative stress, hydrogen peroxide level was measured. Statistical analysis showed there was no significant difference between treatments. Storage time had a significant effect ($P<0.001$) on hydrogen peroxide with the level at day 0 and 2 lower than at 4 to 8 days.

4.1.2.3.4. Ion leakage

Statistical analysis of ion leakage showed a significant difference ($P<0.001$) between treatments. A lower percentage of ion leakage was found in NO gas and DETANO-treated apple slices than in phosphate buffer which was less than in water which was less than in untreated slices. Storage time had a significant effect ($P<0.001$) on ion leakage. It was greater at day 2 compared to 0 days in each treatment and decreased at day 4 then increasing up to day 8.

4.1.2.3.5. Comparison between Season -1 and 2

Browning development showed consistent results between treatments in fruit from season-1 and season-2 and with fruit treated soon after harvest and after prolonged storage.

A reduced rate of respiration was found in DETANO-dipped and NO gas-fumigated slices compared to other treatments during both season-1 and season-2. Storage period showed a significant effect on respiration during both season-1 and season-2 with an initial decrease followed by a continuing rise during storage. There was no effect on ethylene production or water loss in either season.

Treatment with NO gas and DETANO solution had no significant effect on MDA level in season-1 nor on hydrogen peroxide level in season-2.

A significantly lower total phenol content was found in NO gas and DETANO-treated slices during both seasons and storage period also showed a consistent increase in total phenol content in both seasons.

Table 4.8 Total phenol, PPO activity, hydrogen peroxide level and ion leakage of Granny Smith apple slices fumigated with 10 µl.l^{-1} NO gas for 1 hr or dipped in 10 mg.l^{-1} DETANO and phosphate buffer pH 6.5 for 5 min at 20^{o}C, followed by storage at 5^{o}C in air

Treatment	day 0	2	4	6	8	**Mean**
			Total phenol (Gallic acid equivalent in ppm)			
Untreated	150	163	172	186	196	**174**
Water	137	147	158	167	177	**157**
Buffer	128	134	147	155	165	**146**
NO gas	105	116	128	140	150	**128**
DETANO	88	104	118	130	141	**116**
LSD (5%) n=3	*22.62*				*n=15*	***10.12***
			PPO activity (ΔAbs/min)			
Untreated	0.061	0.064	0.070	0.076	0.083	**0.071**
Water	0.058	0.060	0.064	0.071	0.078	**0.066**
Buffer	0.053	0.056	0.061	0.068	0.074	**0.062**
NO gas	0.050	0.053	0.058	0.064	0.069	**0.059**
DETANO	0.045	0.048	0.053	0.059	0.064	**0.054**
LSD (5%) n=3	*0.007*				*n=15*	***0.003***
			Hydrogen peroxide (µmol)			
Untreated	6.6	6.2	7.5	6.9	7.3	**6.9**
Water	6.6	6.4	7.5	7.3	6.7	**6.9**
Buffer	5.9	6.5	6.9	7.4	6.8	**6.7**
NO gas	5.9	6.8	7.0	7.6	6.6	**6.8**
DETANO	6.0	6.3	6.9	7.5	6.9	**6.7**
LSD (5%) n=3	*1.30*				*n=15*	***0.58***
			Ion leakage (%)			
Untreated	55.5	61.3	57.6	65.4	70.3	**62.0**
Water	53.2	58.3	54.8	62.5	67.4	**59.2**
Buffer	50.6	55.8	52.3	59.8	64.8	**56.7**
NO gas	48.9	53.4	49.1	57.5	61.7	**54.1**
DETANO	45.9	50.5	46.6	54.0	58.0	**51.0**
LSD (5%) n=3	*5.38*				*n=15*	***2.40***

Values are the mean of 3 replicates with 3 treatment units in each replicate

PPO activity in NO gas and DETANO-treated slices was significantly lower than other treatments in the main trial conducted in both seasons and storage period showed an increase in PPO activity.

The main study in seasons-1 and 2 showed that DETANO and NO gas-treated slices had lower ion leakage than all other treatments. There was also a consistent effect of storage period in both seasons with the level decreasing from 0 days to 2 days then increasing up to 8 days.

4.1.3. Combined data from season -1 and season 2

Since similar findings for all factors were obtained in fruit harvested in season 1 and season 2, the data for both seasons were combined and the mean values are presented in Table 4.9 and 4.10 and in Fig 4.2 for factors which showed a significant effect of treatment.

Statistical analysis of postharvest life (Table 4.9) showed a significant difference ($P<0.001$) between all treatments with the greatest inhibition of browning development for DETANO-dipped slices then slices fumigated with NO gas. Both treatments had a greater postharvest life than their respective control treatments of phosphate buffer and untreated. Phosphate buffer showed an extension in postharvest life over water-dipped slices which was greater than in untreated slices.

For respiration and total phenol content there was a significant difference ($P<0.001$) between treatments with the level in slices treated with DETANO < NO gas < phosphate buffer < water < untreated (Table 4.10). Storage period also had a significant effect ($P<0.001$) with total phenol content of all treatments increasing throughout storage while respiration showed a significant reduction for all treatments from day 0 to day 2 then increasing to day 8.

Table 4.9 Postharvest life of Granny Smith apple slices on season-1 and 2 that were fumigated with NO gas or dipped in DETANO and phosphate buffer pH 6.5 at 20^{o}C, followed by storage at 5^{o}C in air

Treatment	Postharvest life (Days)
Untreated	3.4
Water for 5 min	4.1
Phosphate buffer for 5 min	5.8
10 µl.l^{-1} NO gas for 1 hr	7.1
10 mg.l^{-1} DETANO for 5 min	8.6
LSD (5%) n=7	*0.62*

Values are the mean of 7 replicates with 3 treatment units in each replicate

Examination of the data for PPO activity was complicated by the phosphate buffer not being included in season-1. Analysis of the data for the other four treatments showed that PPO activity was significantly different ($P<0.001$) between treatments with DETANO < NO gas < water < untreated. Storage period had a significant effect ($P<0.001$) on PPO activity with the level in all treatments increasing during storage.

Ion leakage showed a significant difference ($P<0.001$) but not between all treatments. A significantly lower percentage of ion leakage was found in NO gas and DETANO-treated apple slices compared to those of buffer and water control slices but the difference between DETANO and NO gas was not significant. Ion leakage of buffer-treated slices was significantly lower than untreated but neither was significantly different to water-treated slices. Storage time had a significant effect ($P<0.001$) on ion leakage. It was greater at day 2 compared to 0 days in each treatment and decreased at day 4 then increasing up to day 8.

From Fig. 4.2 and Table 4.10 it can be seen that all treatments had an effect on all the significantly affected physiological and biochemical factors soon after treatment. The magnitude of differences between treatments did not change on further storage.

Since differences between treatments were constant over the 8 day storage period, the mean value can be used to represent the level for each physiological and biochemical factor for each treatment. Linear regression analysis of the data showing the relationship of each factor with the respective postharvest life for each treatment is presented in Fig. 4.3. All factors show an inverse linear relationship with postharvest life indicating that the greater the postharvest life of a treatment the lower was the rate of respiration, total phenol content, PPO activity and ion leakage.

Regression equations between storage time and the mean value of respiration, total phenol, PPO activity and ion leakage for each treatment are presented in Table 4.11. The intercept of equations for the different treatments are significantly different for respiration, total phenol, PPO activity and ion leakage. However, while the slope of the equations for total phenol and PPO activity are significantly different indicating a significant effect of storage time, the slope for respiration and ion leakage are not significantly.

The relationship between postharvest life and the intercept of a regression equation of total phenol content, PPO activity, ion leakage and respiration of individual treatments generated in Table 4.11 is presented in table 4.12, The data show that the intercept of all equations was significantly different ($p<0.05$) but there was no significant difference in the slope for any factor. This indicates that the level of total phenol, PPO activity, ion leakage and respiration were affected soon after treatment and the relative magnitude of the differences were sustained over the storage period.

Table 4.10 Respiration, total phenol, PPO activity and ion leakage of Granny Smith apple slices on season 1 and 2 that were fumigated with NO gas for 1 hr or dipped in DETANO and phosphate buffer pH 6.5 for 5 min at 20°C, followed by storage at 5°C in air

Treatment	day 0	2	4	6	8	**Mean**
			Respiration (ml $CO_2.kg^{-1}.hr^{-1}$)			
Untreated	14.4	12.4	13.6	15.6	17.3	**14.7**
Water	13.1	11.2	12.9	14.2	15.8	**13.4**
Buffer	12.0	10.1	11.3	13.0	14.5	**12.8**
NO gas	10.6	8.9	10.3	11.5	13.1	**10.9**
DETANO	9.5	7.4	9.1	10.5	12.5	**9.8**
LSD (5%) n=6	*1.24*				*n=30*	***0.56***
			Total phenol (Gallic acid equivalent in ppm)			
Untreated	149.6	161.2	171.1	181.7	191.3	**171.0**
Water	141.0	149.0	158.4	168.6	177.1	**159.0**
Buffer	134.3	139.5	151.1	159.8	168.3	**150.0**
NO gas	120.3	128.0	139.8	149.2	158.2	**139.0**
DETANO	110.5	121.4	132.6	142.4	151.0	**131.6**
LSD (5%) n=6	*16.64*				*n=30*	***7.44***
			PPO activity (ΔAbs/min)			
Untreated	0.046	0.051	0.058	0.065	0.072	**0.058**
Water	0.043	0.047	0.053	0.060	0.067	**0.054**
Buffer	-	-	-	-	-	-
NO gas	0.038	0.041	0.048	0.054	0.061	**0.048**
DETANO	0.035	0.038	0.043	0.051	0.056	**0.045**
LSD (5%) n=6	*0.003*				*n=24*	***0.001***
			Ion leakage (%)			
Untreated	53.75	58.64	54.02	58.73	62.72	**57.57**
Water	51.21	55.51	51.71	56.15	19.59	**54.84**
Buffer	49.33	53.64	49.61	54.15	57.50	**52.85**
NO gas	47.32	51.75	47.05	51.80	54.58	**50.50**
DETANO	45.50	49.51	44.64	49.26	51.78	**48.14**
LSD (5%) n=6	*6.29*				*n=30*	***2.81***

Values are the mean of 6 replicates with 3 treatment units in each replicate

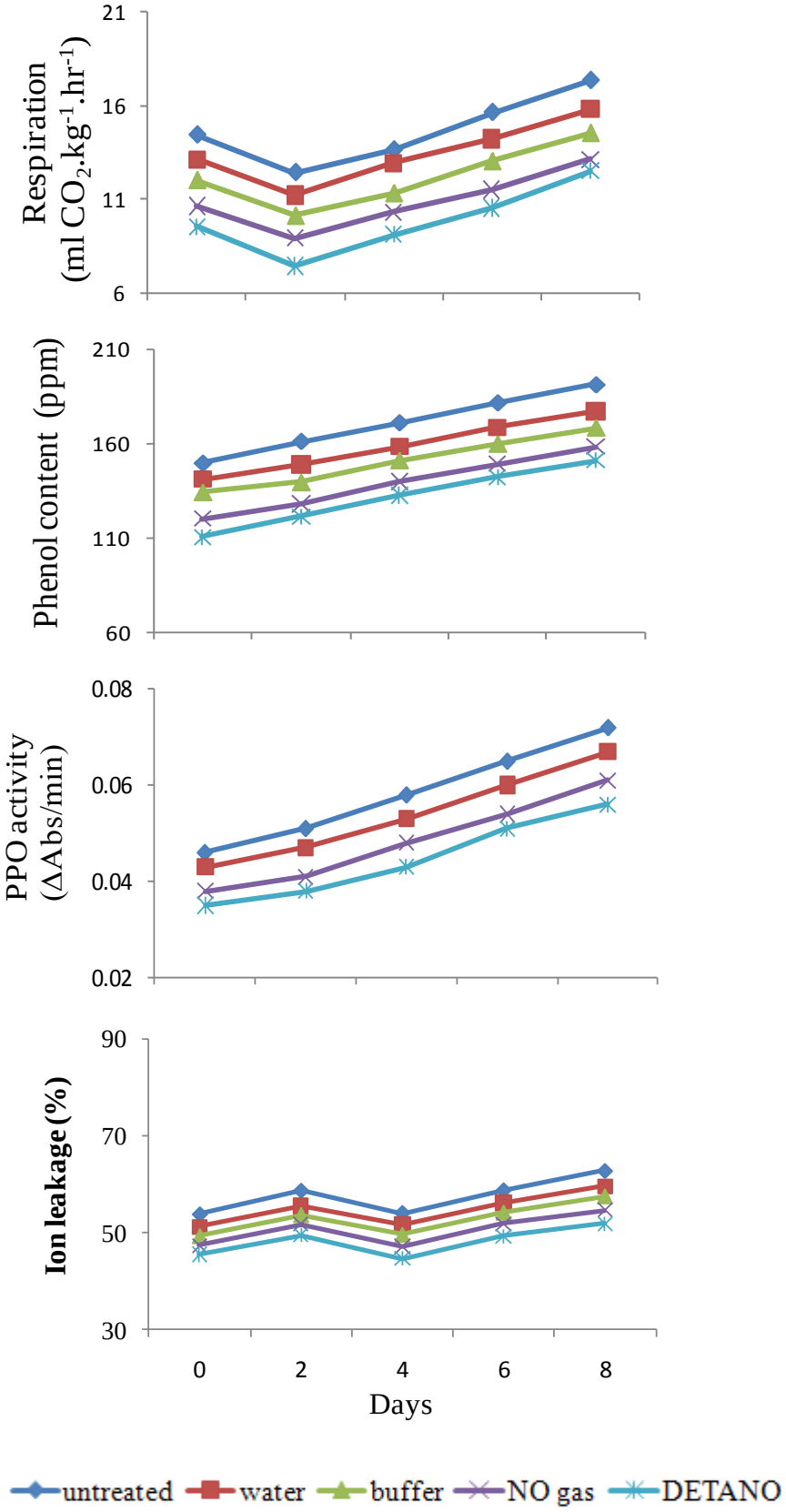

Fig 4.2. Respiration, total phenol, PPO activity and ion leakage of Granny Smith apple slices on season-1 and 2.

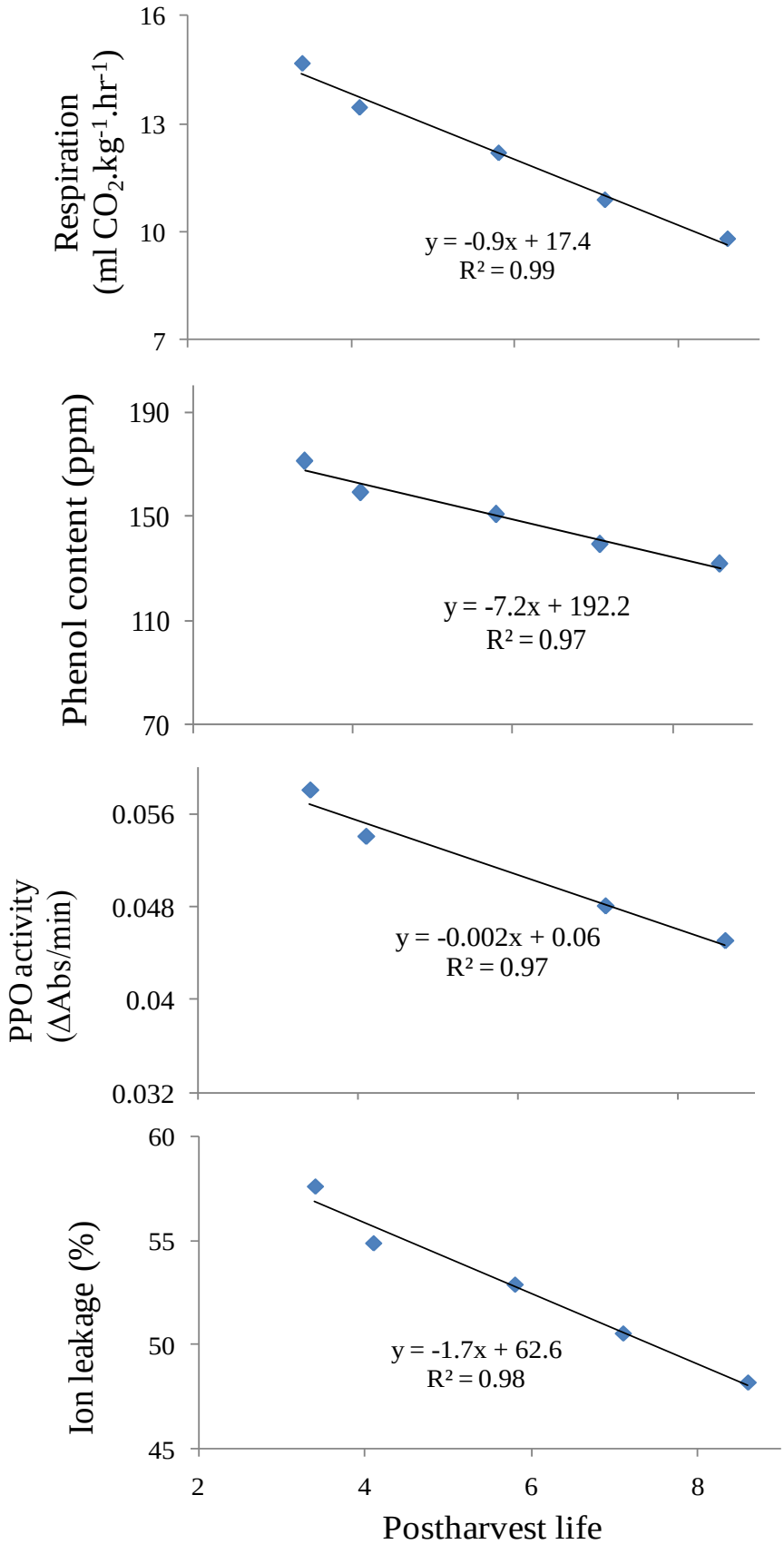

Fig 4.3 Relationship between postharvest life and mean respiration, phenol content, PPO activity and ion leakage of Granny Smith apple slices during storage at 5ºC.

Table 4.11 Relationship between storage time and mean value of total phenol content, PPO activity, ion leakage and respiration of Granny Smith apple slices of season -1 and 2 during storage at 5ºC

Treatment	Regression equation	Level of significance	
		Slope	Intercept
	Respiration		
Untreated	y= 0.45x + 12.7	0.14	0.001
Water	y= 0.42x + 11.8	0.20	0.001
Buffer	y= 0.40x + 10.6	0.15	0.002
NO gas	y= 0.28x + 9.4	0.13	0.002
DETANO	y= 0.46x + 7.9	0.13	0.005
	Total phenol		
Untreated	y= 5.2x + 150.2	0.001	0.001
Water	y= 4.6 x + 140.5	0.001	0.001
Buffer	y= 4.4x + 132.9	0.001	0.001
NO gas	y= 4.9x + 119.7	0.001	0.001
DETANO	y= 5.1x + 111.2	0.001	0.001
	PPO activity		
Untreated	y= 0.003x + 0.045	0.001	0.001
Water	y= 0.003x + 0.418	0.001	0.001
NO gas	y= 0.003x + 0.037	0.001	0.001
DETANO	y= 0.003x + 0.034	0.001	0.001
	Ion leakage		
Untreated	y = 1.40x + 49.9	0.1	0.001
Water	y = 0.87x + 51.4	0.11	0.001
Buffer	y = 0.84x + 49.5	0.12	0.001
NO gas	y = 0.73x + 47.6	0.18	0.001
DETANO	y = 0.62x + 45.7	0.23	0.001

Values are the mean of 6 replicates with 3 treatments units in each replication

Table 4.12 Relationship between postharvest life at 5ºC and the intercept of a regression equation of total phenol content, PPO activity, ion leakage and respiration of individual treatments applied to Granny Smith apple slices in season -1 and 2

Treatment	Regression equation	Level of significance
Respiration	y = -0.90x + 15.7	0.001
Total phenol content	y = -7.28 x +173.1	0.001
PPO activity	y = -0.0021x + 0.0514	0.012
Ion Leakage	y = - 0.96x + 54.4	0.024

Values are the mean of 6 replicates with 3 treatment units in each replicate

4.1.4. Effect of chlorogenic acid

4.1.4.1. Effect on postharvest life

From previous experiments it was found that DETANO and NO reduced total phenol and PPO activity. Chlorogenic acid is a major phenolic compound in apple and is considered to be a likely natural substrate for PPO and hence it may have an effect on the browning process.

The effect of different concentrations 0.1, 0.01 and 0.001% (w/v) of chlorogenic acid dissolved in water on the development of browning of fresh-cut apple slices was examined in conjunction with dipping in DETANO in phosphate buffer and fumigation with NO gas.

Two sets of experiments were conducted with 0.1% chlorogenic acid. In first experiment, apple slices were dipped into DETANO, phosphate buffer and water and after 5 min were then dipped into 0.1% chlorogenic acid. In the second study, the order was reversed with apple slices dipped into chlorogenic acid then into DETANO, buffer or water. Data in Table 4.13 show that in both experiments, browning developed within 1 hr in slices dipped into chlorogenic acid compared to about 4 days for water-dipped or untreated slices. The addition of a water dip, either before or after the chlorogenic acid dip, also resulted in the rapid development of browning. However, dipping in DETANO solution and the buffer largely negated the effect of chlorogenic acid whether applied before or after dipping in chlorogenic acid solution. Statistical analysis was carried out without the inclusion chlorogenic acid and chlorogenic acid with water since the postharvest life was essentially zero.

A lower value of 0.01% chlorogenic acid was applied to try and obtain a longer postharvest life for chlorogenic acid-treated slices. However, the data in Table 4.14 show that the

postharvest life of slices dipped into chlorogenic acid was still almost zero while the addition of DETANO-dip negated the effect of chlorogenic acid as evidenced by a greater postharvest life compare to control slices. Dipping slices in buffer also negated most of the effect of chlorogenic acid but was less effectives than DETANO.

Table 4.13 Postharvest life of Granny Smith apple slices dipped into 10 mg.l^{-1} DETANO, phosphate buffer and water for 5 min before and after dipping in 0.1% chlorogenic acid solution for 10 sec followed by storage at 5°C

Treatment	Postharvest life (days)
Experiment 1	
Untreated	3.8
Water	4.5
Chlorogenic acid	< 1 hr *
DETANO + Chlorogenic acid	2. 8
Phosphate buffer + Chlorogenic acid	2.3
Water + Chlorogenic acid	<1 hr *
LSD (5%) n=3	*2.31*
Experiment 2	
Untreated	4.4
Water	4.5
Chlorogenic acid	<1 hr *
Chlorogenic acid + DETANO	3.9
Chlorogenic acid + phosphate buffer	2.8
Chlorogenic acid + Water	<1 hr *
LSD (5%) n= 3	*1.89*

Values are the mean of 3 replicates with 3 treatments units in each replicate
*Not included in statistical analysis

Three experiments were conducted with a further reduced concentration of 0.001% chlorogenic acid. In first experiment, the data in Table 4.14 that show the postharvest life of slices dipped into chlorogenic acid was about 1 day. Similar to results obtained with 0.01% chlorogenic acid, the addition of a DETANO-dip and buffer-dip negated the effect of chlorogenic acid by extending postharvest life with DETANO more effective than the buffer. There was no significant difference in postharvest life between slices with chlorogenic acid plus water-dip than those with only chlorogenic acid-dip.

In the second experiment, the appropriate control non-cholorogenic acid dip treatments were also included. The data in Table 4.15 show that addition of a DETANO or buffer-dip negated the action of chlorogenic acid but they were not significantly different in postharvest life from their respective control. The addition of water did not reduce the effect of chlorogenic acid.

In the third experiment, apple slices were fumigated with NO gas before and after dipping in chlorogenic acid. The data in Table 4.16 show that the addition of NO gas at least partially negated the effect of chlorogenic acid whether fumigated before or after dipping in chlorogenic acid with both treatments showing no significant difference in postharvest life to untreated slices although they were significantly less than slices treated with NO gas only.

4.1.4.2. UV-spectral change of chlorogenic acid in solution

To observe whether any chemical change of chlorogenic acid was occurring over time, the UV-spectra of 0.001% chlorogenic acid dissolved in water was monitored. The effect of 10 $mg.l^{-1}$ DETANO in phosphate buffer, phosphate buffer solution and fumigation with 10 $\mu l.l^{-1}$ NO gas on a chlorogenic acid solution was also determined. The spectral change was

Table 4.14 Postharvest life of Granny Smith apple slices dipped into 10 $mg.l^{-1}$ DETANO, phosphate buffer and water for 5 min before dipping in 0.01% and 0.001% chlorogenic acid solution for 10 sec followed by storage at 5°C

Treatment	Postharvest Life (days)
Experiment 1- with 0.01% chlorogenic acid	
Untreated	4.2
Water	4.9
Chlorogenic acid	= 2 hr *
DETANO + Chlorogenic acid	4.4
Phosphate buffer + Chlorogenic acid	3.3
Water + Chlorogenic acid	= 2 hr *
LSD (5%) n=3	*1.19*
Experiment 2 - with 0.001% chlorogenic acid	
Chlorogenic acid	1.1
Water+ Chlorogenic acid	1.3
DETANO + Chlorogenic acid	7.7
Phosphate buffer + Chlorogenic acid	6.6
LSD (5%) n=3	*0.73*

Values are the mean of 3 replicates with 3 treatments units in each replicate
* Not included in statistical analysis

Table 4.15 Postharvest life of Granny Smith apple slices dipped into 10 mg.l^{-1} DETANO, phosphate buffer and water for 5 min before dipping in 0.001% chlorogenic acid solution for 10 sec followed by storage at 5^{o}C

Treatment	Postharvest life (days)
Chlorogenic acid	1.4
Water+ Chlorogenic acid	1.7
DETANO	8.7
DETANO + Chlorogenic acid	7.3
Phosphate buffer	7.0
Phosphate buffer + Chlorogenic acid	6.5
LSD (5%) n=3	*1.38*

Values are the mean of 3 replicates with 3 treatments units in each replicate

Table 4.16 Postharvest life of Granny Smith apple slices fumigated with 10 µl.l^{-1} NO gas for 1 hr before and after dipping in 0.001% chlorogenic acid solution for 10 sec followed by storage at 5^{o}C

Treatment	Postharvest life (days)
Untreated	3.8
Chlorogenic acid	1.8
NO gas	7.0
Chlorogenic acid+ NO gas	3.9
NO gas + Chlorogenic acid	4.5
LSD (5%) n=3	*0.68*

Values are the mean of 3 replicates with 3 treatments units in each replicate

assessed immediately after preparation of the solutions but for NO gas the spectral change was first checked after 1 hr. For all solutions spectral changes were assessed up to 8 days of storage. The spectral scans are presented in Fig 4.4.

The scan of chlorogenic acid solution showed a peak absorbance at about 324 nm, but there was a marked decrease in absorbance from 0.82 to 0.41 over the storage period.

The scan of the chlorogenic acid solution that had been fumigated with NO gas also showed a reduction in absorbance at 324 nm but the effect was much stronger with no discernible peak present after 6 and 8 days

The scan of buffer added to the chlorogenic acid solution showed a similar pattern of absorbance change at about 324 nm as chlorogenic acid alone.

The scan of cholorogenic acid with DETANO showed a lower initial absorbance at 324 of 0.66 than other treatments and it decreased further on storage with no discernible peak at 6 and 8 days, similar to the NO gas treatment.

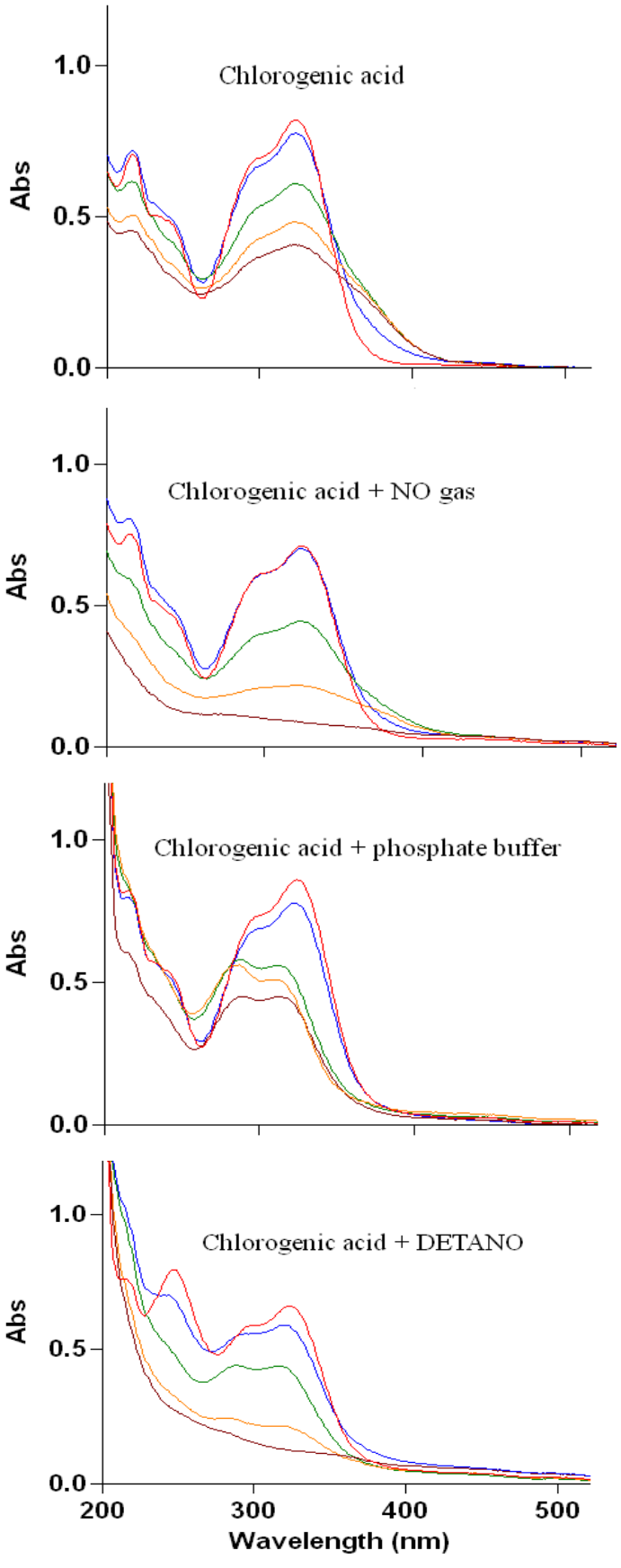

Fig 4.4 UV- spectral changes of chlorogenic acid in solution during 0-8 days at 20ºC

4.1.5. Effect of SNP and Piloty's acid on postharvest life

Granny Smith apple slices dipped in 10 - 750 mg.l^{-1} SNP dissolved in water for 5 min then stored at 5ºC were examined for development of browning. The data in Table 4.17 show a significant increase in postharvest life through delayed onset of surface browning between the apples dipped with 10-500 mg.l^{-1} SNP over control slices. Dipping into 500 mg.l^{-1} SNP resulted in the longest postharvest life.

In a separate experiment, apple slices were dipped into 10 - 1000 mg.l^{-1} Piloty's acid dissolved in water for 5 min stored at 5ºC. The data in Table 4.17 show that the postharvest life of apples dipped in 100 mg.l^{-1} Piloty's acid had a significantly longer postharvest life than all other treatments except for the 10 mg.l^{-1} treatment. The postharvest life of apples dipped in 750 mg.l^{-1} Piloty's acid was not significantly greater than the water control.

In both experiments, a higher postharvest life was found in water-dipped slices than untreated slices. Apple slices treated with 750 mg.l^{-1} SNP and 1000 mg.l^{-1} Piloty's acid treatment caused damage to slices by rapidly softening the flesh to an unacceptable level.

Comparison was made of the effect of the optimum concentration of SNP and Piloty's acid dissolved in water with DETANO dissolved in phosphate buffer and fumigation with NO gas to inhibit browning. The results in Table 4.18 show that all forms of NO extended the postharvest life of apple slices over water but 10 mg.l^{-1} DETANO and 500 mg.l^{-1} SNP solutions were the most effective treatments with DETANO significantly more effective than SNP. The postharvest life of apple slices fumigated with 10 µl.l^{-1} NO gas and dipped in 100 mg.1^{-1} Piloty's acid solution were not significantly different.

Table 4.17 Postharvest life of Granny Smith apple slices dipped in different concentration of SNP and Piloty's acid for 5 min at 20°C followed by storage at 5°C in air

Dip conc. ($mg.l^{-1}$)	Postharvest life (days)
Experiment 1- Dipped in SNP solution	
Untreated	3.5
Water	4.4
10	5.5
50	6.3
100	7.1
500	7.9
750	*
LSD (5%) n=3	*0.75*
Experiment 2- Dipped in Piloty's acid solution	
Untreated	3.9
Water	5.0
10	6.8
50	5.2
100	7.5
500	6.0
750	5.3
1000	*
LSD (5%) n=3	*0.95*

Values are the mean of 3 replicates with 3 treatments units in each replicate
*Not assessed due to flesh softening

4.1.6. Effect of cutting slices with a metal or ceramic knife on browning

A study examined the development of browning of apples cut with a metal or a ceramic knife then dipped or not dipped in water at 20^{o}C with all slices stored at 5^{o}C. The data in Table 4.19 show that apple slices cut by the metal and ceramic knives then dipped in water had a significantly longer postharvest life than those not dipped in water. There was, however, no significant difference in postharvest life between metal and ceramic knife-cut apple slices whether dipped or not dipped in water.

Table 4.18 Postharvest life of Granny Smith apple slices treated at 20^{o}C with the optimum concentration of different forms of NO followed by storage at 5^{o}C in air

Treatment	Postharvest life (Days)
Water for 5 min	4.3
10 µl.l^{-1} NO gas for 1 hr	6.1
100 mg.l^{-1} Piloty's acid for 5 min	6.3
500 mg.l^{-1} SNP for 5 min	7.6
10 mg.l^{-1} DETANO for 5 min	9.1
LSD (5%) n=3	*1.23*

Values are the mean of 3 replicates with 3 treatments units in each replicate

Table 4.19 Postharvest life of Granny Smith apple slices cut with ceramic and metal knife and stored at 5ºC

Treatment		Postharvest life (days)
Knife	Dipped in water	
Ceramic	no	3.4
Metal	no	3.3
Ceramic	yes	5.0
Metal	yes	4.8
LSD (5%) n=6		*1.10*

Values are the mean of 6 replicates with 3 treatments units in each replicate

4.1.7. Effect of DETANO in conjunction with a controlled atmosphere

4.1.7.1. Effect of carbon dioxide

Fresh-cut apple slices were dipped in distilled water or 10 $mg.l^{-1}$ DETANO dissolved in phosphate buffer for 60 sec and stored in a sealed jar with a controlled atmosphere of 2, 4 and 6% CO_2 in air at 20°C. Data for the postharvest life, ethylene production and water loss are shown in Table 4.20.

The postharvest life of DETANO-dipped slices was significantly ($P<0.001$) longer than those dipped in water. There was also a significant effect of atmosphere ($P<0.001$) but there was also a significant interaction ($P<0.05$) between treatment and atmosphere. This interaction can be seen in Fig 4.5 with the postharvest life of DETANO-dipped slices increased linearly as the percentage of CO_2 increased ($y = 0.16x + 1.24$) but there was no significant effect for water-dipped slices.

ANOVA analysis for ethylene production showed there was no significant difference between DETANO and water-dipped slices similar to the previous studies (section 4.1.1.). There was, however, a significant effect of CO_2 on ethylene production ($P<0.05$). Regression analysis showed ethylene production decreased linearly as the percentage of CO_2 increased ($y = -0.23x + 1.46$); ($P>0.001$). There was no significant interaction between treatment solution and controlled atmosphere.

Water loss showed no significant difference between water and DETANO-dipped slices. The level of CO_2 had no significant effect on water loss and there was no significant interaction between treatment and atmosphere.

Table 4.20 Postharvest life, ethylene production and water loss of Granny Smith apple slices dipped into DETANO and water for 60 sec and stored in a controlled atmosphere of 0, 2, 4 and 6% CO_2 in air at 20°C

Atmosphere (% CO_2 in air)	DETANO	Water
	Postharvest Life (Days)	
0 (Air)	1.48	1.00
2 %	1.46	0.95
4 %	1.70	0.97
6 %	1.94	1.11
LSD (5%) n=3	*0.20*	
	Ethylene production (ml.kg^{-1}.hr^{-1})	
0	1.33	1.30
2 %	0.89	0.86
4 %	0.75	0.87
6 %	0.55	0.53
LSD (5%) n=3	*0.67*	
	Water loss (%)	
0	0.94	1.07
2 %	0.86	0.80
4 %	0.53	0.74
6 %	0.42	0.65
LSD (5%) n=3	*0.58*	

Values are the mean of 3 replicates with 3 treatment units in each replicate

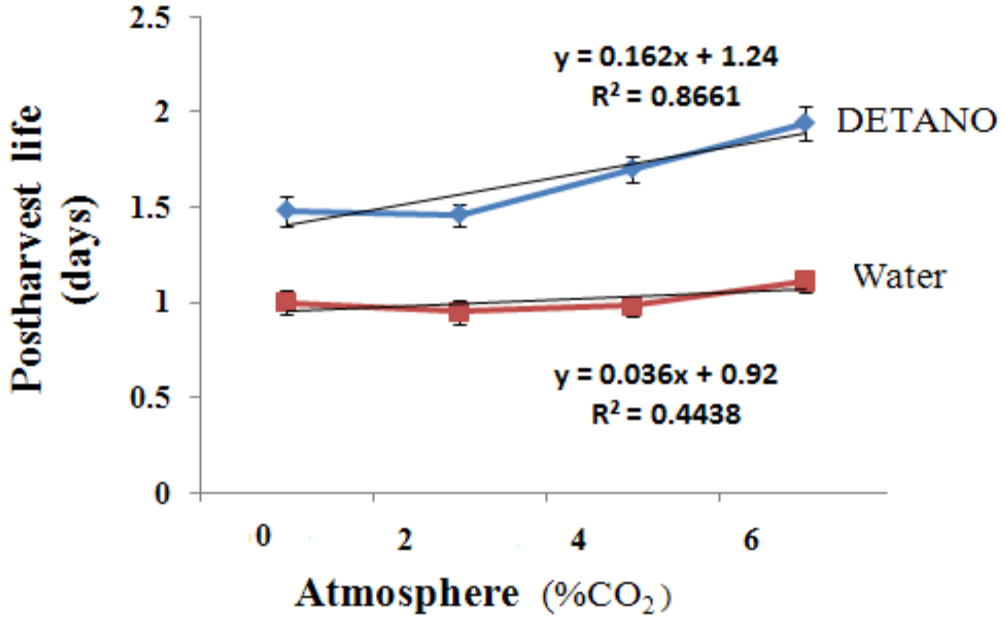

Fig 4.5 Postharvest life of Granny Smith apple slices treated with DETANO and water and stored in a controlled atmosphere at 20ºC.

4.1.7.2. Effect of oxygen

The effect of controlled atmospheres of 1, 2, 5 % O_2 and air (21% O_2) in nitrogen on the postharvest life, ethylene production and water loss of DETANO and water-treated apple slices is shown in Table 4.21.

There was a significantly longer postharvest life in DETANO-dipped slices than water-treated slices at 2 and 5% oxygen levels ($P<0.001$). Storage atmosphere, however, had no significant effect on postharvest life and there was no significant interaction between treatment and atmosphere.

ANOVA analysis for ethylene production showed no significant difference between DETANO and water-treated slices but there was a significant effect of atmosphere ($P<0.001$) with ethylene production higher in air compared to all low oxygen atmospheres. There was no significant interaction between treatment and atmosphere.

There was no significant effect of treatment or oxygen level on water loss.

Table 4.21 Postharvest life, ethylene production and water loss of Granny Smith apple slices dipped into DETANO and water for 60 sec and stored in a controlled atmosphere of 0, 1, and 5 % O_2 in nitrogen at 20^oC

Atmosphere (% O_2 in nitrogen)	DETANO	Water
	Postharvest Life (Days)	
21%	1.24	0.84
1 %	1.19	0.82
2 %	1.40	0.93
5 %	1.56	0.98
LSD (5%) n=2	*0.42*	
	Ethylene production (ml.kg^{-1}.hr^{-1})	
21%	0.30	0.27
1 %	0.06	0.06
2 %	0.08	0.04
5 %	0.11	0.12
LSD (5%) n=2	*0.10*	
	Water loss (%)	
21%	0.26	0.35
1 %	0.40	0.52
2 %	0.48	0.24
5 %	0.30	0.46
LSD (5%) n= 2	*0.37*	

Values are the mean of 2 replicates with 3 treatment units in each replicate

4.2. Fancy lettuce

4.2.1. Effect of NO gas, DETANO and SNP on Green oak lettuce

In the initial studies, Green oak lettuce slices were fumigated with 0 (air), 10, 50, 100 and 500 $\mu l.l^{-1}$ NO gas in air for 2 hr at 20˚C followed by storage at 5˚C. The results in Table 4.22 show that 100 $\mu l.l^{-1}$ NO was the most effective concentration to extend the postharvest life of lettuce with 10 and 50 $\mu l.l^{-1}$ also having a greater postharvest life than untreated slices. The effect of NO gas on browning of lettuce slices is shown in Fig 4.6. Lettuce fumigated with 500 $\mu l.l^{-1}$ of NO gas caused damage to leaves soon after treatment (Fig 4.7). Green oak lettuce was less tolerant of NO gas than iceberg lettuce, for which Wills et al. (2008) reported that leaf damage occurred only when fumigated with 1000 $\mu l.l^{-1}$ NO gas.

In the second experiment, lettuces slices were dipped into water, pH 6.5 phosphate buffer (control) and different concentrations (10, 50, 100 and 500 $mg.l^{-1}$) of DETANO dissolved in the pH 6.5 phosphate buffer for 5 min at 20˚C then stored at 5˚C. The results in Table 4.22 show that the postharvest life increased with increasing concentration of DETANO with the optimum concentration being 500 $mg.l^{-1}$. There was no significant difference in the postharvest life of lettuce between phosphate buffer and 10 $mg.1^{-1}$ DETANO solutions.

To examine the effect of SNP, Green oak lettuce slices were dipped in water (control), 10, 50, 100 and 500 $mg.l^{-1}$ SNP dissolved in water at 20˚C for 5 min and then stored at 5˚C. Treatment with 500 $mg.1^{-1}$ SNP generated the maximum postharvest life while other SNP concentrations were greater than the water treatment (Table 4.22).

Table 4.22 Postharvest life of Green oak lettuce slices fumigated with NO gas for 2 hr, dipped in DETANO dissolved in phosphate buffer and SNP dissolved in water for 5 min at 20˚C, followed by storage in air at 5˚C.

Concentration	Postharvest life (days)	$LSD_{(5\%)}$ n= 2
Experiment 1 - Fumigated with NO gas		
Control (no gas)	5.5	**0.40**
10 $\mu l.l^{-1}$	7.1	
50	7.6	
100	8.6	
500	*	
Experiment 2 – Dipped in DETANO solution		
Water	6.0	
Control (buffer)	6.2	**0.22**
10 $mg.l^{-1}$	6.4	
50	7.3	
100	8.7	
500	11.5	
Experiment 3 – Dipped in SNP solution		
Control (water)	5.9	**0.31**
10 $mg.l^{-1}$	6.7	
50	7.1	
100	8.0	
500	9.1	

Values are the mean of 2 replicates with 3 treatment units in each replicate
* Not assessed due to chemical injury

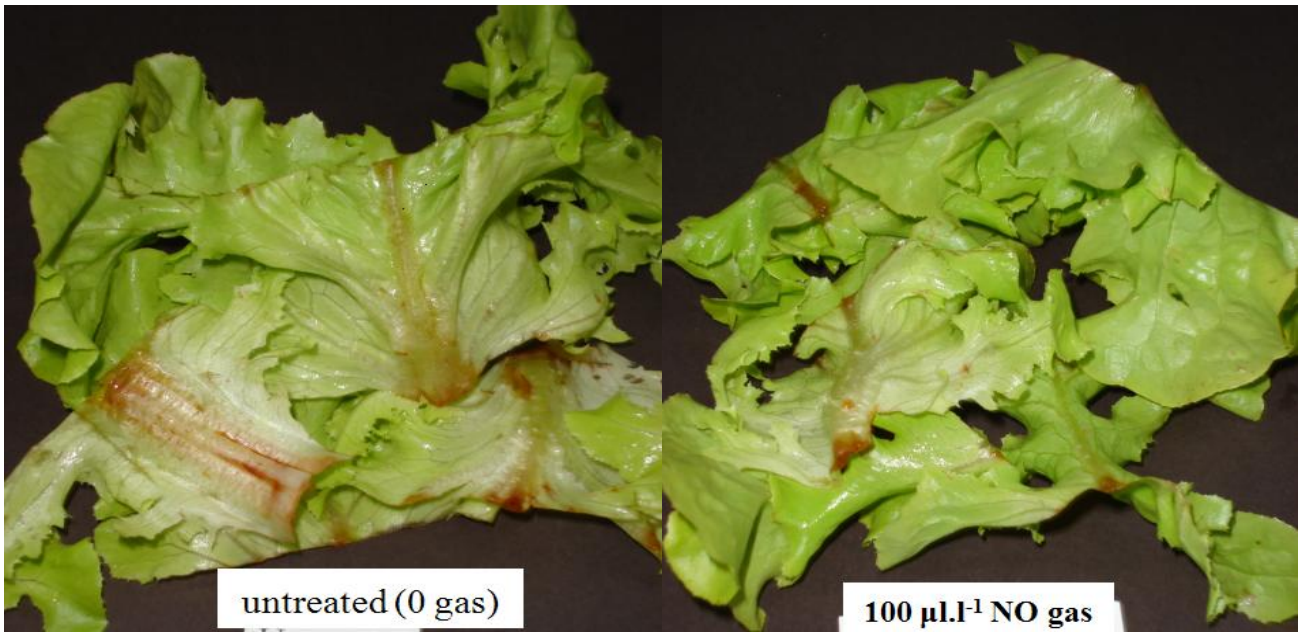

Fig 4.6 Green oak lettuce slices fumigated with 100 µl.l^{-1} NO for 2 hr and stored for 8 days at 5^{o}C in air

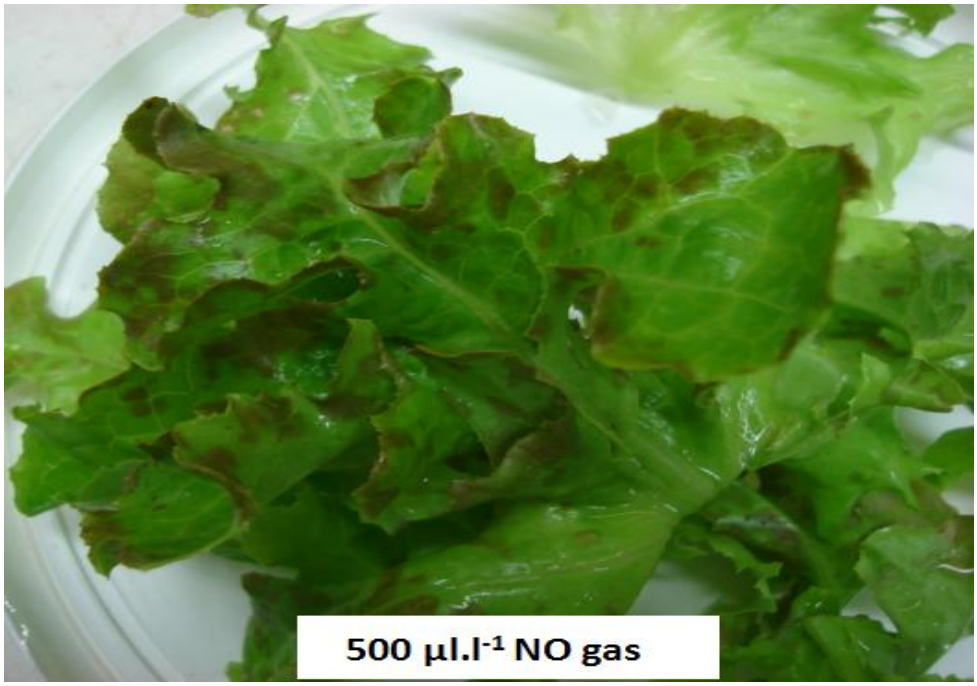

Fig 4.7 Injury on Green oak lettuce slices fumigated with 500 µl.l^{-1} NO gas 2 hr after treatment.

The relative effect of the optimum concentration of NO gas, DETANO and SNP to inhibit browning was investigated with Green oak lettuces with all treatments applied in the same experiment. DETANO and SNP were also added at a concentration of 100 mg.l^{-1} to allow some comparison with 100 µl.l^{-1} NO gas. The results in Table 4.23 show that dipping in DETANO at 500 mg.l^{-1} was the most effective treatment in extending postharvest life with 500 mg.l^{-1} SNP the next most effective treatment. Lettuce slices fumigated with 100 µl^{-1} NO gas had an extended postharvest life over the relevant control treatment (untreated) and there was no significant difference to those dipped in 100 mg.1^{-1} DETANO or SNP solution.

4.2.2. Effect of DETANO, SNP and NO gas on postharvest life of Green coral, Baby cos and Butter lettuces

The treatments applied to Green oak lettuce slices were also examined on Green coral, Baby cos and Butter lettuce slices (Table 4.23). For Green coral lettuce, the longest postharvest life was found for slices treated with 500 mg.l^{-1} SNP but 500 mg.l^{-1} DETANO was also quite effective. There was no significant difference in postharvest life between 100 mg.l^{-1} SNP and 100 mg.1^{-1} DETANO treatment which were significantly greater than for 100 µl^{-1} NO gas.

For Baby cos lettuce slices, 500 mg.l^{-1} SNP and DETANO solution were equally effective and significantly increased postharvest life over all other treatments. There was no significant difference in the postharvest life of lettuces treated with 100 µl.l^{-1} NO gas and 100 mg.l^{-1} of SNP and DETANO.

For Butter lettuce slices, the greatest increase in postharvest life was achieved with 500 mg.l^{-1} of SNP. Lettuce slices dipped into 100 mg.l^{-1} SNP was significantly greater than when dipped in 100 mg.l^{-1} DETANO or 100 μl.l^{-1} NO gas.

4.2.3. Comparison of DETANO, SNP and NO gas on postharvest life of four fancy lettuces

Table 4.23 also shows the mean values of postharvest life of the four fancy lettuce slices for each treatment. Analysis of the data show that there was a significant difference between treatments ($P<0.001$) but there was also a significant interaction between lettuce type and treatment at $P >0.001$. Lettuce slices treated with DETANO and SNP at 500 mg.l^{-1} showed the longest postharvest life compared to all other treatments but there was no significant difference between DETANO and SNP. Treatments with 100 mg.l^{-1} SNP and DETANO resulted in a longer postharvest life than 100 μl.l^{-1} NO gas with all three being longer than all the control treatments. There was a significant difference in postharvest life between control treatments with phosphate buffer > water > untreated lettuce slices.

The relative effectiveness of the optimal concentrations of NO gas, DETANO or SNP on browning development in Green oak, Green coral, Baby Cos and Butter lettuce slices was investigated. DETANO or SNP were also added at a concentration of 100 mg l^{-1}. The data in Table 4.23 show that all NO treatments significantly increased the post-harvest life of the four lettuces over the respective controls. DETANO or SNP solutions at 500 mg l^{-1} were the most effective treatments in extending post-harvest life. While DETANO was significantly more effective than SNP on Green oak lettuce, SNP was more effective on Green coral and Butter lettuces and there was no significant difference in effectiveness on Baby cos lettuce.

The mean values across all lettuces show that 500 mg l^{-1} DETANO and SNP were equally effective.

The increase in postharvest life between NO gas, DETANO and SNP over the respective controls for the mean values of all lettuces at the 100 mg.l^{-1} concentration, while small from postharvest technology perspective, were all significantly different (P<0.05) at 2.2, 1.8 and 2.5 days, respectively. The increase in postharvest life of DETANO and SNP at 500 mg.l^{-1} over the respective control was also significantly different (P<0.05) at 3.1 and 3.6 days, respectively. Thus, it would seem that the relative effectiveness of the three NO treatments in extending postharvest life of lettuce slices is SNP > NO > DETANO.

Table 4.23 Postharvest life of slices of four fancy lettuce slices treated with NO gas for 2 hr, DETANO and SNP for 5 min at 20^{o}C followed by storage at 5^{o}C

Treatment	Postharvest life (days)				
	Green oak	Green coral	Baby cos	Butter	**Mean**
Untreated	5.1	7.7	5.0	7.7	**6.3**
Water	6.1	7.7	5.2	7.8	**6.7**
Phosphate buffer	6.5	8.2	5.7	8.7	**7.3**
100 $\mu l.l^{-1}$ NO gas	8.2	9.5	5.87	10.5	**8.5**
100 $mg.l^{-1}$ DETANO	8.6	10.6	6.1	11.0	**9.1**
500 $mg.l^{-1}$ DETANO	11.6	11.5	7.3	11.4	**10.4**
100 $mg.l^{-1}$ SNP	8.4	10.9	5.8	11.7	**9.2**
500 $mg.l^{-1}$ SNP	9.3	12.7	7.0	12.2	**10.3**
LSD $_{(5\%)}$ n= 2	*0.31*	*0.28*	*0.33*	*0.33*	***0.28***
n= 16					

Values are the mean of 2 replicates with 3 treatment units in each replicate

4.2.4. Effect of NO gas and DETANO on respiration and ethylene of Green oak lettuce

The respiration rate and ethylene production of Green oak lettuce was determined in slices fumigated with 10 $\mu l.l^{-1}$ NO gas for 2 hr and dipped in 10 $mg.l^{-1}$ DETANO and phosphate buffer solution for 5 min followed by storage 5°C. The data in Table 4.24 show that there was a significant reduction in respiration rate of lettuce treated with NO gas and DETANO soon after treatment compared to the other treatments and was sustained over the storage period. There was no significant difference in respiration rate between lettuces treated with water and phosphate buffer but both were significantly less than untreated slices.

There was no significant difference in ethylene production in any treatment at any time during storage (Table 4.24).

Table 4.24 Respiration and ethylene production of Green oak lettuce slices fumigated with 10 µl.l^{-1} NO gas for 2 hr and dipped in 10 mg.l^{-1} DETANO and phosphate buffer for 5 min at 20^{o}C, followed by storage at 5^{o}C in air

Treatment	day 0	2	4	6	8	**Mean**
			Respiration (ml CO_2.kg^{-1}.hr^{-1})			
Untreated	20.9	19.5	19.1	22.7	24.4	**21.3**
Water	19.3	17.9	17.5	20.6	22.7	**19.6**
Phosphate buffer	18.4	16.6	16.5	19.5	20.9	**18.4**
NO gas	16.2	14.9	14.7	17.7	19.8	**16.5**
DETANO	15.0	13.5	12.8	16.2	17.6	**15.0**
LSD (5%) n=3	*2.83*			*n=15*		***1.27***
			Ethylene production (ml.kg^{-1}.hr^{-1})			
Untreated	0.16	0.18	0.18	0.2	0.2	**0.18**
Water	0.14	0.17	0.15	0.2	0.19	**0.17**
Phosphate buffer	0.15	0.13	0.14	0.18	0.14	**0.15**
NO gas	0.14	0.15	0.13	0.17	0.18	**0.15**
DETANO	0.15	0.13	0.12	0.17	0.16	**0.15**
LSD (5%) n=3	*0.09*			*n=15*		***0.04***

Values are the mean of 3 replicates with 3 treatment units in each replicate

5. DISCUSSION

The extension of postharvest life of Granny Smith apple slices with NO gas (10 $\mu l.l^{-1}$) and DETANO (10 $mg.l^{-1}$) dissolved in phosphate buffer (pH 6.5) solution by inhibiting the development of surface browning confirmed the findings of Pristijono et al. (2006) and Pristijono et al. (2008), respectively. While DETANO was more effective than NO gas in absolute terms, DETANO and NO gas resulted in approx. 50% and 100 % increase in postharvest life over their respective control treatments of phosphate buffer and untreated. Thus, NO gas had a proportionately greater effect over its control than did DETANO. Phosphate buffer itself extended the postharvest life of apple slices. If the action of phosphate buffer and the NO released from DETANO on apple slices were different, an additive effect could be expected when DETANO in phosphate buffer was applied that is, a 100% increased in postharvest life similar to NO gas. This suggests that DETANO and phosphate buffer shared some common effects that were not additive when both were applied to the fruit.

A significant increase in postharvest life was found when apple slices were dipped in aqueous SNP solution compared to the water control slices. This is consistent with the findings that SNP inhibited internal flesh browning in intact plum fruits (Zhang et al., 2008) and longan fruit (Duan et al., 2007). An extension in postharvest life was also found when apple slices were dipped in aqueous solution of Piloty's acid, an effect that has not been previously reported.

The relative effectiveness of treatment of apple slices with the optimum concentration of the four NO forms to inhibit browning is considered to be DETANO > SNP > NO gas = Piloty's

acid. DETANO was dissolved in phosphate buffer, and SNP and Piloty's acid were dissolved in water and since both phosphate buffer and water-dip inhibited browning development, the relative effectiveness NO gas and DETANO could be respectively higher and lower than that found for the actual treatments applied. DETANO and NO gas release the $NO^{\bullet}$ free radical while SNP releases the NO^{+} cation and Piloty's acid releases the nitroxyl anion (NO^{-}) (Hou et al., 1999; Saavedra and Keefer, 2002; Hughes and Cammack,1999). It might be expected that each NO moiety has different reactivity on browning inhibition of apple slices with the moiety more directly linked to the metabolic action that inhibits browning being more effective. Since all NO donors produced some inhibition of browning, the released NO moieties it is possible they are inter-convertible. Direct comparison of SNP and Piloty's acid is possible since both were dissolved in water. The greater inhibition of browning by SNP might suggest that NO^{+} was more reactive against browning than NO^{-}. However, it is not possible to determine whether $NO^{\bullet}$ or NO^{+} were more directly linked with browning inhibition.

NO gas and DETANO solution reduced the rate of respiration in apple slices during storage at 5ºC compared to the other treatments. Since an increased rate of respiration is associated with a more rapid onset of senescence (Fernández-Trujillo et al.,1998), the present study suggests that NO has an anti-senescent action which could be a general reduction in the rate of cellular metabolism. Millar and Day (1996) and Zottini et al. (2002) reported that NO affects the function of mitochondria in plant cells and reduces cell respiration by inhibiting the cytochrome pathway while the alternative oxidase pathway was found to be resistant to NO. A reduced rate of respiration in intact strawberry treated with SNP (Zhu and Zhou (2007) and in Japanese plums (Singh et al., 2009) and peaches (Flores et al., 2008) fumigated with NO gas have been reported. The significant reduction in respiration from 0 day to 2

days found in all treatments could be due to an elevated respiration induced by cutting which returns more normal level at 2 days. Elevation of respiration may be due to increased surface area exposed to the atmosphere that causes a more rapid diffusion of oxygen to the internal cell and increased metabolic activity of injured cells (Zagory, 1999).

The mode of action of NO is often attributed to an antagonistic effect against ethylene. For example, NO was reported to have an inverse relationship with ethylene during plant development and stress regulation (Leshem et al., 1998; Leshem and Pinchasov, 2000) with the mode of action claimed to be by inactivating ACC oxidase (ACO) through oxidation of ascorbate or Fe^{2+} (Leshem, 2000). Also, NO was found to reduce ethylene production in mature green banana slices which was associated with reduction in the activity of ACO and the expression of the *MA-ACO1* gene (Cheng et al., 2009). In the present study, NO gas and DETANO solution showed no marked effects on ethylene production in apple slices during storage. This lack of effect of NO is probably due to the apples being post-climacteric and thus having a substantial production of ethylene (Wills et al., 2007). Nevertheless, the data tend to suggest that ethylene is either not a direct causative factor in surface browning or NO can inhibit browning through other modes of action.

A role for PPO activity in enzymatic browning has been extensively reported for intact and fresh-cut fruits and vegetables (Art´es et al., 1998). NO and DETANO were found to inhibit PPO activity which could be through NO interacting with a transition metal and/or thiol-containing enzymes (Bogdan, 2001). The PPO enzyme complex has two copper ions in its active centre and Zhu et al. (2009) speculated that NO could react with the copper to produce a copper-nitrosyl complex which could change the normal structure of the active site in PPO and result in reduced PPO activity. A similar inhibitory effect of NO on PPO activity

was found in intact longan fruit (Duan et al., 2007), in peach slices (Zhu et al., 2009) and in intact Chinese winter jujube (Zhu et al., 2009). However, Zhang et al. (2008) reported that SNP had no inhibitory effect on PPO activity in intact plum fruits stored at low temperature.

PPO catalyses the oxidation of phenolic compounds to quinones which then condense to form brown polymers (Milani and Hamedi, 2005). In the present study, treatment with DETANO and NO gas reduced the total phenol content which suggests that inhibition of browning could be due to a lower level of phenol available to be oxidised possibly in conjunction with a reduced PPO activity. Zhang et al. (2008) similarly found SNP delayed the increase in total phenolic content of intact plum fruits. On the other hand, Zhu et al. (2009) reported that NO gas fumigation induced a higher total phenol content in intact Chinese winter jujube along with an inhibition of PPO activity. Other studies have also shown that treatment with NO increased total phenol content of peach slices (Zhu et al., 2009) and intact longan fruits (Duan et al., 2007).

Reactive oxygen species (ROS), are directly or indirectly involved in lipid peroxidation and result in the formation of several by-products such as MDA which causes membrane deteriotion in plant tissue (Lesham, 2000). Beligni et al. (2002) confirmed a functional interaction of ROS and NO during the growth and development of plants and Zhu et al. (2008) found a marked effect of NO was reduced accumulation of MDA in kiwi fruit. A reduced MDA content was reported for SNP applied to dune reed callus (Song et al., 2006) and longan fruits (Duan et al., 2007) and NO gas applied to kiwi fruit (Zhang et al., 2007). The current study surprisingly showed that NO had no significant effect on MDA content in apple slices. Second measure of ROS, hydrogen peroxide also showed no significant change due to NO treatment. Zhang et al. (2007) had found that NO gas reduced the hydrogen

peroxide level in intact kiwi fruits. A lack of significant change in MDA and hydrogen peroxide levels implies firstly that NO has no effect in mitigating any oxidative damage caused by ROS during storage. It may also imply that if lipid peroxidation is involved in the browning of apple slices, browning is inhibited by NO affecting other some aspect of apple metabolism.

Increased leakage of ions, an indicator of membrane permeability (Bajji et al., 2002) has been correlated with increased membrane viscosity in intact apples (Lurie and Ben-Arie, 1983). In the present study, a reduced rate of ion leakage was found in NO-treated slices indicating that NO could assist in maintaining membrane integrity in apple slices. Zhu et al. (2009) also showed that NO solution inhibited the increased rate of ion leakage of fresh cut peach slices during storage. Similarly, Song et al. (2006) reported that SNP and SNAP alleviated the increase of ion leakage in calluses from two ecotypes of reed under heat stress and Beligni and Lamattina (1999) found they mediated decreased ion leakage in various growing plant tissues. The current finding on apple slices suggests that a lower percentage of electrolyte leakage in both DETANO and NO gas treatments may be inhibiting browning by delaying loss of cellular integrity. It is possible that delayed loss of cellular integrity is reducing the release of browning precursors from cells to the surface of the cut fruit. However, it is recognized that unchanged levels of MDA and H_2O_2 would suggest that membrane integrity was not affected by NO treatments. Any loss of cellular integrity would then come about by some other mechanism.

Considering the effect of all the treatments used in the study including the three control treatments of phosphate buffer, water and untreated, as well as DETANO and NO gas, the study showed an inverse linear relationship between respiration, total phenol content, PPO

activity and ion leakage with postharvest life. This could suggest firstly, that, whatever the metabolic sequence induced by cutting that leads to browning, all treatments including a water-dip, were having a similar affect on inhibiting that pathway but with different levels of effectiveness. Since the difference between treatments were all evident within a few hours of application, the action of cutting would seem to have triggered induction of the browning sequence and that this induction could be inhibited to some extent by various dipping treatments and NO gas fumigation.

An obvious consideration is that the metal knife used to cut the slices could have left traces of metal which catalysed some enzyme sequence, but the lack of effect of the ceramic knife compared to the metal knife suggests that this does not occur.

Considering the four affected factors, respiration, phenol content, PPO activity and ion leakage, a possible suggestion is that surface browning is due to leakage of some substrates from cells to the cut surface that reacts to produce browning. Reducing respiration would reduce general metabolism and hence result in a reduced rate of ion leakage hence a slower rate of release of metabolites involved in browning.

Phenol content and PPO activity are both implicated in the browning sequence reaction. An obvious question is - are either or both the key metabolites involved in the slower development of browning caused by the treatments? To assist in answering this question, the major phenolic compound in apple, chlorogenic acid (Alvarez-Parrilla et al., 2005) dissolved in water was applied to apple slices in conjunction with dipping in DETANO in phosphate buffer and fumigation with NO gas. Dipping in apple slices in 0.1 and 0.01% chlorogenic acid showed instant surface browning while a 0.001% solution resulted in browning after 2

days. This could suggest that sufficient PPO activity is present on the cut surface at harvest to cause browning and that phenol is the rate limiting compound. The addition of NO gas and DETANO negated the action of chlorogenic acid suggesting that NO was able to inhibit the oxidation of cholorogenic acid. Use of buffer also negated much of the effect of chlorogenic acid but was less effective than DETANO and NO gas.

The UV- spectral scan of chlorogenic acid dissolved in water showed loss of cholorogenic acid in a few days although it is not possible to state what type of oxidation occurred. Since no enzyme was present, the reaction must be non-enzymatic presumably oxidation by atmospheric oxygen dissolved in the water. Grodzinska-Zachwieja et al. (1973) also found chlorogenic acid was unstable in acidified methanol solution from a study on UV-spectral changes over time. The UV-scan also showed that DETANO and NO gas accelerated the loss of chlorogenic acid with no absorbance found at day 4 suggesting that NO gas and DETANO had converted the chlorogenic acid to some form which is not involved in browning. The results also suggest that browning of apple slices could be due to non-enzymatic as well as enzymatic reactions and the amount of phenols on surface could have a key role in development of browning. It is possible that an increase in phenols occurs on the apple surface soon after cutting and NO could effectively reduce the level of phenol on the surface as well as slow its leakage from cells. The buffer could wash phenol off the surface and delay its release from cells by stabilizing surface cells. Water also could wash off surface phenol.

NO gas, DETANO and SNP treatments, all inhibited the development of browning of fresh-cut Green oak, Green coral, Baby cos and Butter lettuces during storage at 5°C. DETANO and SNP resulted in a 60% increase in postharvest life while the NO gas resulted in a 30%

increase. The findings thus support those of Wills et al. (2008) for NO gas and DETANO inhibiting the development of browning in cut iceberg lettuce and extend it to SNP. However, the effectiveness in inhibiting browning of the three NO compounds relative to the respective controls was SNP > NO gas > DETANO. This suggests that the NO^+ cation released by SNP was more efficiently utilized by lettuce tissues than the $NO^\bullet$ free radical. The greater effectiveness of NO gas over DETANO may be due to the gas being able to more easily reach the sites of active absorption in lettuce.

Dipping lettuce slices in SNP was considered to be the most feasible option for commercial processors due to the stability of SNP in unbuffered water, the universality of its effectiveness on a range of lettuce types, and the lack of any logistical difficulties in incorporating an additional water-dipping step into existing commercial line operations. The need for DETANO to be used in a buffered solution (Pristijono et al., 2008) adds an additional layer of complexity and cost to the system for no further gain in postharvest life. Future studies should examine dipping and fumigation concentrations of DETANO and SNP between 500 and 1000 mg. l^{-1} to determine if further gains in browning inhibition can be achieved without inducing damage to leaves.

For commercial usage with apples, however, DETANO offers the greatest gain in postharvest life. The low pH of apple juices (Wills et al., 2007) that would leach from cut slices into any dipping solution necessitate having a solution buffered at around pH 6.5 to prevent premature degradation of DETANO in acid solution (Hrabie et al., 1993). The quality of water used in commercial operations is variable and would require the use of a buffer solution to ensure a near neutral pH is maintained throughout dipping. Elevated CO_2 enhanced the postharvest life of DETANO-dipped apple slices to a greater extent than for water-dipped slices. There thus

appears to be a synergistic effect of CO_2 and DETANO. Low oxygen has no effect on extension of postharvest life of apple slices while DETANO-dipped slices show greater postharvest life than water-dipped slices at all oxygen levels.

6. REFERENCES

Abe, K., and Watada, A.E. (1991). Ethylene absorbent to maintain quality of lightly processed fruits and vegetables. *Journal of Food Science* 56, 1589-1592.

Abeles, F.B., Morgan, P.W., and Saltveit, M.E. (1992). "Ethylene in plant biology". (2nd ed.) Academic Press, San Diego. p 414.

Abbott, J.A., Saftner, R.A., Gross, K.C., Vinyard, B.T., and Janick, J. (2004). Consumer evaluation and quality measurement of fresh-cut slices of 'Fuji,' 'Golden Delicious,' 'GoldRush,' and 'Granny Smith' apples. *Postharvest Biology and Technology* 33. 127–140.

Agar, I. T., Massantini, R., Hess-Pierce, B., and Kader, A. A. (1999). Postharvest CO_2 and ethylene production and quality maintenance of fresh-cut kiwi fruit slices. *Journal of Food Science* 64, 433-440.

Al-Ati, T., and Hotchkiss, J.H. (2002). Application of packaging and modified atmosphere to fresh-cut fruits and vegetables. *In* "Fresh-cut fuits and vegetables science, technology and market" (O. Lamikanra, ed.), CRC Press., Boca Raton, Florida. 305-338.

Alonso-Salces, R.M., Korta, E., Barranco, A., Berrueta, L.A., Gallo, B., and Vicente, F. (2001). Determination of polyphenolic profiles of Basque Cider apple varieties using accelerated solvent extraction. *Journal of Agricultural and Food Chemistry* 49, 3761-3767.

Altunkaya, A., and Gökmen, V. (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chemistry* 107, 1173-1179.

Altunkaya, A., and Gökmen, V. (2009). Effect of various anti-browning agents on phenolic compounds profile of fresh lettuce (*L. sativa*). *Food Chemistry* 117, 122–126.

Alvarez-Parrilla, E., Dela Rosa, L.A., Torresrivas, F., Rodrigo-Garcia, J., and GONZA´ Lez-Aguilar, G.A. (2005). Complexation of apple antioxidants: chlorogenic acid, quercetin and rutin by b-cyclodextrin (b-CD). *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 53,121–129.

Amiot, M.J., Tacchini, M., Aubert, S., and Nicholas, J. (1992). Phenolic composition and browining susceptibility of various apple cultivars at maturity. *Journal of Food Science* 57, 958-962.

Anderson, L.S., and Mansfield, T.A. (1979). The effects of nitric oxide pollution on the growth of tomato. *Environmental Pollution* 20, 113-121.

Arasimowicz, M., and Floryszak-Wieczorek, J. (2007). Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Science* 172, 876-887.

Arnold, E.V., Citro, M.L., Saavedra, E.A., Davies, K.M., Keefer, L.K., and Hrabie, J.A. (2002). Mechanistic insight into exclusive nitric oxide recovery from a carbonbound diazeniumdiolate. *Nitric Oxide* 7, 103-108.

Art´es, F., Casta˜ner, M., and Gil, M.I. (1998). Enzymatic browning in minimally processed fruit and vegetables. *Food Science and Technology International* 4, 377–389.

Atrooz, O.M. (2008). The effects of Maillard reaction products on apple and potato polyphenoloxidase and their antioxidant activity. *International Journal of Food Science and Technology* 43, 490–494.

Awad, M.A., Wagenmakers, P.S., and Jager, A.D. (2001). Effects of light on flavonoid and chlorogenic acid levels in the skin of `Jonagold' apples. *Scientia Horticulturae* 88, 289-298.

Badiyan, D., Wills, R.B.H., and Bowyer, M.C. (2004). Use of a nitric oxide donor compound to extend the vase life of cut flowers. *HortScience* 39, 1371–1372.

Bai, J., Baldwin, E.A., Fortuny, R.C.S., Mattheis, J.P., Stanley, R., Perera, C., and Brecht, J.K. (2004). Effect of pretreatment of intact `Gala' apple with ethanol vapor, heat, or 1-methylcyclopropene on quality and shelf life of fresh-cut slices. *Journal of the American Society for Horticultural Science* 129, 583-593.

Bajji, M., Kinet, L-M., and Lutts, S. (2002). The use of electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in darum wheat. *Plant Growth Regulation* 36, 61-70.

Bartz, J.A. and Brecht, J.K. (2003). "Postharvest physiology and pathology of vegetables". (2nd ed.), Marcel Dekker, Inc., New York, p 733.

Beaudry, R.M. (1999). Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biology and Technology* 15, 293-303.

Beligni, M.V., Fath, A., Bethke, P.C., Lamattina, L., and Jones, R.L. (2002). Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers1. *Plant Physiology* 129, 1642–1650.

Beligni, M.V., and Lamattina, L. (1999). Nitric oxide protects against cellular damage produced by methylviologen herbicides in potato plants. *Nitric Oxide* 3, 199–208.

Beligni, M.V., and Lamattina, L. (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta* 210, 215-221.

Beligni, M.V., and Lamattina, L. (2002). Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. *Plant Cell and Environment* 25, 737-748.

Beltran, D., Selma, M.V., Marin, A., and Gil, M.I. (2005). Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agricultural and Food Chemistry* 53, 5654-5663.

Beveridge, T. (1997). Haze and cloud in apple juices. *Critical Reviews in Food Science and Nutrition* 37, 75-91.

Bogdan, C. (2001). Nitric oxide and the immune response. *Nature Immunology* 2, 907-916.

Bonner, W.D. (1957). Soluble oxidases and their functions. *Annual Review of Plant Physiology* 8, 427-452.

Bowyer, M.C., Wills, R.B.H., Badiyan, D., and Ku, V.V.V. (2003). Extending the postharvest life of carnations with nitric oxide--comparison of fumigation and in vivo delivery. *Postharvest Biology and Technology* 30, 281-286.

Brecht, J.K. (1995). Physiology of lightly processed fruits and vegetables. *Hortscience* 30, 18–22.

Brecht, J.K., Saltveit, M.E., Talcott, S.T., Schneider, K.R., Felkey, K., and Bartz, J.A. (2004). Fresh-cut vegetables and fruits. *Horticultural Reviews* 30, 185-251.

Burdurlu, H.S., and Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry* 80, 91-97.

Buta, J.G., Moline, H.E., Spaulding, D.W., and Wang, C.Y. (1999). Extending storage life of fresh-cut apples using natural products and their derivatives. *Journal of Agricultural and Food Chemistry* 47, 1-6.

Campos-Vargas, R., and Saltveit, M.E. (2002). Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum* 114, 73-84.

Cantos, E., Espin, J.C., and Tomas-Barberan, F.A. (2001). Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. *Journal of Agricultural and Food Chemistry* 49, 322-330.

Castañer, M., Gil, M.I., Ruíz, M.V., and Artés, F. (1999). Browning susceptibility of minimally processed baby and romaine lettuces. *European Food Research and Technology* 209, 52-56.

Castaner, M., Gil, M. I., Artes, F., and Tomas-Barberan, F. A. (1996). Inhibition of browning of harvested head lettuce. *Journal of Food Science.* 61, 314-316.

Cevahir, G., Aytamka, E., and Erol, Ç. (2007). The role nitric oxide in plants. *Biotechnology and Biotechnological Equipment* 21, 13-17.

Cheng, G., Yang, E., Lu, W., Jia, Y., Jiang, Y., and Duan, X. (2009). Effect of nitric oxide on ethylene synthesis and softening of banana fruit slice during ripening. *Journal of Agricultural and Food Chemistry* 57, 5799–5804.

Chen, C., Trezza, T.A., Wong, D.W.S., Camir, W.M., and Pavlath, A.E. (1999). Methods for preserving fresh fruit and products there of *US Patent 5939117. Mantrose-Haeuser Co., Inc.*

Chen, Z., Zhu, C., Zhang, Y., Debao Niu, D., and Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology* 58, 232–238.

Cheng, G. W. W., and Crisosto, C. H. (1997). Iron-polyphenol complex formation and skin discoloration in peaches and nectarines. *Journal of the American Society for Horticultural Science* 122, 95-99.

Chinnici, F., Gaiani, A., Natali, N., Riponi, C., and Galassi, S. (2004). Improved HPLC determination of phenolic compounds in cv. Golden delicious apples using a monolithic column. *Journal of Agricultural and Food Chemistry* 52, 3-7.

Clifford, M.N., Johnston, K.L., Knight, S., and Kuhnert, N. (2003). Hierarchical scheme for LC-MSn identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry* 51, 2900-2911.

Coseteng, M.Y., and Lee, C.Y. (1987). Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science* 52, 985–989.

Couture, R., Cantwell, M.I., Ke, D., and Saltveit, M.E. (1993). Physiological attributes related to quality attributes and storage life of minimally processed lettuce, *HortScience* 58, 609–610.

Crawford, N.M. (2006). Mechanisms for nitric oxide synthesis in plants. *Journal of Experimental Botany* 57, 471–478.

Das, E., Gurakan, G. C., and Bayendirli, A. (2006). Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella Enteritidis* on cherry tomatoes. *Food Microbiology.* 23, 430-438.

Davies, K.M., Wink, D.A., Saavedra, J.E., and Keefer, L.K. (2001). Chemistry of the diazeniumdiolates. 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution. *Journal of the American Chemical Society.* 123, 5473-5481.

Degl'Innocenti, E., Pardossi, A., Tognoni, F., and Guidi, L. (2007). Physiological basis of sensitivity to enzymatic browning in `lettuce', `escarole' and `rocket salad' when stored as fresh-cut products. *Food Chemistry* 104, 209-215.

del Rio, L.A., Javier Corpas, F., and Barroso, J.B. (2004). Nitric oxide and nitric oxide synthase activity in plants. *Phytochemistry* 65, 783-792.

Delledonne, M. (2005). NO news is good news for plants. *Current Opinion in Plant Biology* 8, 390-396.

Delledonne, M., Xia, Y., Dixon, R.A., and Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance *Nature* 394, 585-588.

Delledonne, M., Zeier, J., Marocco A., and Lamb, C. (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Sciences USA* 98, 13454–13459.

Duan, X., Su, X., You, Y., Qu, H., Li, Y., and Jiang, Y. (2007). Effect of nitric oxide on pericarp browning of harvested longan fruit in relation to phenolic metabolism. *Food Chemistry* 104, 571-576.

Du, Z., and Bramlage, W.J. (1995). Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* 30, 205–209.

DuPont, M.S., Mondin, Z., Williamson, G., and Price, K.R. (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of Agricultural and Food Chemistry* 48, 3957-3964.

Durner, J., Wendehenne, D., and Klessig, D.F. (1998). Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Plant Biology* 95, 10328-10333.

Escalona, V.H., Verlinden, B.E., Geysen, S., and Nicolai, B.M. (2006). Changes in respiration of fresh-cut butterhead lettuce under controlled atmospheres using low and superatmospheric oxygen conditions with different carbon dioxide levels. *Postharvest Biology and Technology* 39, 48-55.

Eum, H.L., Hwang, D.K., and Lee, S.K. (2009)**.** Nitric oxide reduced chlorophyll degradation in broccoli (*Brassica oleracea* L. var. italica) florets during senescence. *Food Science and Technology International* 15, 223-228.

Euromonitor International. (2009). Fresh fruit and vegetable sales hit by recession. *http://www.thefoodsummit.com/pdf/Fresh fruit vegetable sales hit recession.pdf*. 29-06-2010.

Fan, X., and Mattheis, J.P. (2000). Reduction of ethylene-induced physiological disorders of carrots and iceberg lettuce by 1-methylcyclopropene. *HortScience* 35, 1312–1314.

Fan, X., Niemera, B.A., Mattheis, J.P., Zhuang, H., and Olson, D.W. (2005a). Quality of fresh-cut apple slices as affected by low-dose ionizing radiation and calcium ascorbate treatment. *Journal of Food Science* 70, S143-S148.

Fan, X., Sokorai, K.J.B., Sommers, C.H., Niemira, B.A., and Mattheis, J.P. (2005b). Effects of calcium ascorbate and ionizing radiation on the survival of *Listeria monocytogenes* and product quality of fresh-cut 'Gala' apples. *Journal of Food Science* 70, M352-M358.

Farber, J.M. (1991). Microbiological aspects of modified atmosphere packaging- a review. *Journal of Food Protection*. 54, 58–70.

Feldman, P.L., Griffith, O.W., and Stuehr, D.J. (1993). The surprising life of nitric oxide. *Chemical and Engineering News* 20, 26-38.

Fernandez-Trujillo, J.P., Cano, A., and Art´es, F. (1998). Physiological changes in peaches related to chilling injury and ripening. *Postharvest Biology and Technology* 13, 109–119.

Ferreres, F., Gil, M.I., Castaner, M., and Tomas-Barberan, F.A. (1997). Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *Journal of Agriccultural and Food Chemistry* 45, 4249- 4254.

Fitzhugh, A.L., and Keefer, L.K. (2000). Diazeniumdiolates:: Pro- and antioxidant applications of the "NONOates". *Free Radical Biology and Medicine* 28, 1463-1469.

Flores, F.B., Sánchez-Bel, P., Valdenegro, M., Romojaro,F., Martínez-Madrid, M.C., and Egea, M.I. (2008). Effects of a pretreatment with nitric oxide on peach (*Prunus persica* L.) storage at room temperature, *European Food Research and Technology* 227, 1599–1611.

Food and Drug Administration (FDA). (1989). Chemical preservatives. 21CFR1823798. Washington, DC, USA.

Fox, R. (1995). Fresh-cut produce. Practical hydroponics and greenhouses 23. *http://www.hydroponics.com.au/php/viewtopic.php.* 25-03-2009.

Friedman, M., (1996). Food browning and its prevention: an overview. *Journal of Agricultural and Food Chemistry* 44, 631-653.

Friedman, M., and Jurgens, H.S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry* 48, 2101-2110.

Fulcrand, H., Cheminat, A., Brouillard, R., and Cheynier, V. (1994). Characterization of compounds obtained by chemical oxidation of caffeic acid in acidic conditions. *Phytochemistry* 35, 499-505.

Gajhede, M. (2001). Plant peroxidases: substrate complexes with mechanistic implications. *Biochemical Society Transactions* 29, 91–99.

Garces, H., Durzan, D., and Pedroso, M.C. (2001). Mechanical stress elicits nitric oxide formation and DNA fragmentation in *Arabidopsis thaliana*. *Annals of Botany* 87, 567-574.

Garcia, E., and Barrett, D.M. (2002). Preservative treatments for fresh-cut fruits and vegetables. *In* "Fresh-cut fruits and vegetables science, technology and market" (O. Lamikanra, ed.), CRC Press., Boca Raton, Florida, 267–303.

Garcia, A., Wichers, J.H., and Wichers, H.J. (2007). Decrease of the IgE-binding by Mal d 1, the major apple allergen, by means of polyphenol oxidase and peroxidise treatments. *Food Chemistry* 103, 94-100.

Garthwaite, J., and Boulton, C.L. (1995). Nitric oxide signaling in the central nervous system. *Annual Review of Physiology* 57, 683-706.

Gil, M.I., Castañer, M., Ferreres, F., Artés, F., and Tomás-B, F.A., (1998). Modified-atmosphere packaging of minimally processed "Lollo Rosso" (*Lactuca sativa*) phenolic metabolites and quality changes. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* 206, 350–354.

Gonza´lez-Aguilar, G.A., Ayala-Zavala, J.F., Olivas, G.I., de la Rosa, L.A., and Alvarez-Parrilla, E. (2010). Preserving quality of fresh-cut products using safe technologies. *Journal fur Verbraucherschutz und Lebensmittelsicherheit* 5, 65–72.

Gorin, N., and Heidema, F.T. (1976). Peroxidase activity in Golden Delicious apples as a possible parameter of ripening and senescence. *Journal of Agricultural and Food Chemistry* 24, 200-201.

Gorny, J.R. (1997). Summary of CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. *In*: "CA'97 Proceedings: Fresh-cut Fruits and Vegetables and MAP" University California, Davis, CA, 30–66.

Gorny, J.R., Cifuentes, R.A., Hess-Pierce, B., and Kader, A.A. (2000). Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. *Journal of Food Science* 65, 541-544.

Goupy, P., Amiot, M.J., Richard-Forget, F., Duprat, F., Aubert, S., and Nicolas, J. (1995). Enzymatic browning of model soutions and apple phenolic substrates by apple polyphenoloxidase. *Journal of Food Science* 60, 497-505.

Grodzinska-Zachweija, Z , Kahl,W., and Klimczak, M. (1973). Spectrometric investigation of changes caffeic, chlorogenic and isochlorogenic acids under the influence of some physiochemical factors. *Polish Journal of Pharmacology and Pharmacy* 25, 299-303.

Gunes, G., Watkins, C.B., and Hotchkiss, J.H. (2001). Physiological responses of fresh-cut apple slices under high CO_2 and low O_2 partial pressures. *Postharvest Biology and Technology* 22, 197-204.

Guo, F.Q., Okamoto, M., and Crawford, N.M. (2003). Identification of a plant nitric oxide synthase gene involved in hormonal signaling. *Science* 302, 100-103.

Halliwell, B. (1994). Free radicals, antioxidants and human disease: curiosity, cause or consequence, *The Lancet* 344, 721–724.

Harte, J. B., Siddiq, M., Dolan, K.D., Yen, K., Beaudry, R.M., and Novak, D.K. (2003). Effect of storage on the physical, chemical and sensory characteristics of fresh-cut apple slices from different apple varieties. *Session 104B, Fruit and Vegetable Products: Fruits (Processed), 2003 IFT Annual Meeting - Chicago*.

Heath, R.L., and Packer, L. (1968*)*. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives in Biochemistry and Biophysics* 125, 189-198.

Heimdal, H., Kuhn, B.F., Poll, L., and Larsen, L.M. (1995). Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. *Journal of Food Science* 60, 1265-1276.

Hendricks, M.T., and Hotchkiss, J.H. (1997). Effect of carbon dioxide on the growth of *Pseudomonas fluorescens* and *Listeria monocytogenes* in aerobic atmospheres *Journal of Food Protection* 60, 1548-1552.

Hisaminato, H., Murata, H., and Homma, S. (2001). Relationship between the enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. *Bioscience, Biotechnology and Biochemistry* 65, 1016-1021.

Hiscock S., Bright J., McInnis S.M., Desikan R., and Hancock J.T. (2007). Signaling on the stigma: potential new roles for ROS and NO in plant cell signaling. *Plant Signaling and Behavior* 2, 23-24.

Hodge, J.E. (1953). Browning reactions in model systems. *Journal of Agricultural and Food Chemistry* 1, 928-943.

Hoffman, N.E., and Yang, S.F. (1982). Enhancement of wound-induced ethylene synthesis by ethylene in preclimacteric cantaloupe. *Plant Physiology* 69, 317-322.

Hou, Y.C., Janczuk, A., and Wang, P.G. (1999). Current trends in the development of nitric oxide donors. *Current Pharmaceutical Design* 5, 417-441.

Howard,C.J. (1980). Stratospheric chemistry and the ozone problem. *In "*Oxygen and Oxy-radicals in Chemistry and Biology". (M. Rodgers, and E. Powers, ed.). Academic Press, New York, 535-589.

Hrabie, J.A., Klose, J.R., Wink, D.A., and Keefer, L.K. (1993). New nitric oxide releasing zwitterions derived from polyamines. *The Journal of Organic Chemistry* 58, 1472-1476.

Hu, W.Z., Pang, K., Jiang, A.L., and Tian, M.X. (2007). Changes in ethylene, respiration and polyphenol oxidase of fresh-cut apple. *Acta Horticulturae.*746, 369-373.

Huang, Z ., Wang, B., Eaves, D.H., Shikany, J.M., and Pace, R.D.(2006). Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast United States. *Food Chemistry* 103, 1395-1402.

Hughes, M.N., and Cammack, R. (1999). Synthesis, chemistry, and applications of nitroxyle ion releaser sodium trioxodinitrate or Angeli's salt and Piloty's acid. *Methods in Enzymology* 301, 279-287.

Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., and Smith-Warner, S.A. (2004). Fruit and vegetable intake and risk of major chronic disease, *Journal of National Cancer Institute* 96, 1577–1584.

Hung, K.T., and Kao, C.H. (2007). Hydrogen peroxide, calcium, and leaf senescence in rice. *Crop, Environment and Bioinformatics* 4, 145-150.

IFPA (2005). USDA, Agricultural Research Service. International Fresh-cut Produce Association (IFPA). *http://www.ars.usda.gov/is/AR/archive/aug05/fruit0805.htm*.25-02-2009.

IFPA and PMA. (1999). "Handling guidelines for the fresh-cut produce industry" (3rd ed.) IFPA, Alexandria, VA. p 5.

Ignarro, L. J. (2000). "Nitric oxide: biology and pathobiology" (L. J. Ignarro, ed.), Academic Press, San Diego, CA, p 141.

Ignarro, L.J., Byrns, R.E., and Woods, K.S. (1988). Biochemical and pharmacological properties of endothelium-derived relaxing factor and its similarity to nitric oxide radical. *In*

"Vasodilatation : vascular smooth muscle, peptides, autonomic nerves, and endothelium " (P.M. Vanhoutte, ed.), Raven Press, New York, 427-435.

Iyengar, R., and McEvily, A.J. (1992). Antibrowning agents, alternatives to use of sulfites in food. *Trends in Food Science and Technology* 3, 60-64.

Jaffrey, S.R., and Snyder, S.H. (1995). Nitric oxide: a neural messenger. *Annual Review of Cell and Developmental Biology* 11, 417-440.

Jang, J.H., and Moon, K.D. (2011). Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. *Food Chemistry* 124, 444-449.

Jiang, Y.M., and Joyce, D.C. (2002). 1-methylcyclopropene treatment effects on intact and fresh-cut apple. *Journal of Horticultural Science and Biotechnology* 77, 19-21.

Jih P.J., Chen Y.C., and Jeng S.T. (2003). Involvement of hydrogen peroxide and nitric oxide in expression of the ipomoelin gene from sweet potato. *Plant Physiology* 132, 381-389.

Kader, R., Irmoulj, M., Nicolas, J.P., and Metche, M. (2002). Involvement of blueberry peroxidases in the mechanism of anthocyanin degradation in blueberry juice. *Journal of Food Science* 67, 911–915.

Kader, A.A., and Saltveit, M.E. (2003). Atmosphere modification. *In* "Postharvest physiology and pathology of vegetables" (J.A. Bartz and J.K. Brecht, ed.), Marcel Dekker, Inc., New York, 229-246.

Kang, H.M., and Saltveit, M.E. (2003). Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions. *Postharvest Biology and Technology* 29, 271-277.

Karaibrahimoglu, Y., Fan, X., Sapers, G.M., and Sokorai, K. (2004). Effect of pH on the survival of *Listeria innocua* in calcium ascorbate solutions and on quality of fresh-cut apples. *Journal of Food Protection* 67, 751-757.

Karpuzoglu, E., and Ahmed, S.A. (2006). Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: implications for immunity, autoimmune diseases, and apoptosis. *Nitric Oxide* 15, 177-186.

Ke, D., and Saltveit, M.E. (1989). Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiologia Plantarum* 76, 412-418.

Keefer, L.K. (2003). Progress toward clinical application of the nitric oxide-releasing diazeniumdiolates. *Annual Review of Pharmacology and Toxicology* 43, 585-609.

Keefer, L.K., Nims, R.W., Davies, K.M., and Wink, D.A. (1996). "NONOates" (1-substituted diazen-1-ium-1,2-diolates) as nitric oxide donors: convenient nitric oxide dosage forms methods in enzymology. *In* "Nitric oxide part A: sources and detection of NO; NO synthase" (L. Packer, ed.), Academic Press, San Diego, CA, 281-293.

Kim, D.H., Smith, N.L., and Lee, C.Y. (1993). Quality of minimally processed apple slices from selected cultivars. *Journal of Food Science* 58, 1115–1117.

Klepper, L.A. (1978). Nitric oxide (NO) evolution from herbicide-treated soybean plants. *Plant Physiology* 61, S65.

Klepper, L. (1979). Nitric oxide (NO) and nitrogen dioxide (NO2) emissions from herbicide-treated soybean plants. *Atmospheric Environment* 13, 537-542.

Koshland, DE Jr. (1992). The Molecule of the Year. *Science*. 258, p 1861.

Koukounaras, A., Diamantidis, G., and Sfakiotakis, E. (2008). The effect of heat treatment on quality retention of fresh-cut peach, *Postharvest Biology and Technology* 48, 30–36.

Ku, V.V.V., Wills, R.B.H., and Leshem, Y.Y. (2000). Use of nitric oxide to reduce postharvest water loss from horticultural produce. *The Journal of Horticultural Science and Biotechnology* 75, 268-270.

Lamattina, L., Beligni, M.V., Garcia-Mata, C., and Laxalt, A.M.(2001). Method of enhancing the metabolic function and the growing conditions of plants and seeds. *US Patent. US 6242384 B1*.

Lamattina, L., García-Mata, C., Graziano, M., and Pagnussat, G. (2003). Nitric oxide : the versatility of an extensive signal molecule. *Annual Review of Plant Biology* 54, 109-136.

Lamikanra, O. (2002). Enzymatic effects on flavor and texture of fresh-cut fruits and vegetables. *In* "Fresh-cut fruits and vegetables science, technology and market" (O. Lamikanra, ed.), CRC Press., Boca Raton, Florida, 125-185.

Lamikanra, O., and Watson, M.A. (2001) .Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon, *Journal of Food Science* 66, 1283–1286.

Lamotte, L., Courtois, C., Barnavon, L., Pugin, A., and Wendehenne D. (2005). Nitric oxide in plants: The biosynthesis and cell signalling properties of fascinating molecule. *Planta*. 221, 1-4.

Landrigan, M., Morris, S.C., Eamus, D., and McGlasson, W.B. (1996). Postharvest water relationship and tissue browning of rambutan fruit. *Scientia Horticulturae* 66, 201-208.

Lee, K.W., Kim, Y. J., Kim, D.O., Lee, H. J., and Lee, C.Y. (2003). Major phenolics in apple and their contribution to the total antioxidant capacity. *Journal of Agricultural and Food Chemistry* 51, 6516-6520.

Lemaire, G., Alvarez-Pachon, F.J., Beuneu, C., Lepoivre, M., and Petit, J.F. (1999). Differential cytostatic effects of NO donors and NO producing cells. *Free Radical Biology and Medicine* 26, 1274-1283.

Leshem, Y.Y. (2000). "Nitric oxide in plants: occurrence, function and use," Kluwer, Dordrecht, The Netherlands, p 154 .

Leshem, Y.Y., and Haramaty, E. (1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage. *Journal of Plant Physiology* 148, 258-263.

Leshem, Y.Y., and Pinchasov, Y. (2000). Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripeneing of strawberries *Fragaria anannasa* (Dutch.) and avocados *Persea americana* (Mill.), *Journal of Experimental Botany* 51, 1471-1473.

Leshem, Y.Y., Wills, R.B.H., and Ku, V.V.V. (1998). Evidence for the function of the free radical gas -- nitric oxide (NO-) -- as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry* 36, 825-833.

Li, L. and Steffens, J. C. (2002). Over expression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* 215, 239-247.

Lipton, S.A., Choi, Y.B., Pan, Z.H., Lei, S.A., Chen, H.S., Sucher, N.J, Loscalzo, J., Singel, D.J., and Stamler, J.S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 364, 626−632.

Lloyd-Jones, M.D.D.M., and Bloch, M.D.K.D. (1996). The vascular biology of nitric oxide and its role in atherogenesis. *Annual Review of Medicine* 47, 365-375.

Loaiza-Velarde, J.G., and Saltveit, M.E. (2001). Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *Journal of the American Society for Horticultural Science* 126, 227–234.

Loaiza-Velarde, J.G., Tomas-Barbera, F.A., and Saltveit, M.E. (1997). Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *Journal of the American Society for Horticultural Science* 122, 873-877.

Lopez-Galvez, G., Saltveit, M., and Cantwell, M. (1996). The visual quality of minimally processed lettuces stored in air or controlled atmosphere with emphasis on romaine and iceberg types. *Postharvest Biology and Technology* 8, 179–190.

Lu, C., and Toivonen, P.M.A. (2000). Effect of 1 and 100 kPa O_2 atmospheric pretreatments of whole `Spartan' apples on subsequent quality and shelf life of slices stored in modified atmosphere packages. *Postharvest Biology and Technology* 18, 99-107.

Lu, S., Song, J., and Campbell-Palmer, L. (2009). A modified chemiluminescence method for hydrogen peroxide determination in apple fruit tissues. *Scientia Horticulturae* 120, 336–341.

Luna-Guzman, I., Cantwell, M., and Barrett, D.M. (1999). Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology* 17, 201-213.

Lurie, S., and Ben-Arie, R. (1983). Microsomal membrane changes during the ripening of apple fruit. *Plant Physiology* 73, 636-638.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., and Lerici, C.R. (2001). Review of nonenzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology* 11, 340–346.

Mao, L., Wang, G., and Que, F. (2007). Application of 1-methylcyclopropene prior to cutting reduces wound responses and maintains quality in cut kiwi fruit. *Journal of Food Engineering* 78, 361-365.

Markowski, J., and Plocharski, W. (2006). Determination of phenolic compounds in apples and processed apple products. *Journal of Fruit and Ornamental Plant Research* 14, 133-142.

Marks, G.S., Mclaughlin, B.E., Brown, L.B., Beaton, D.E., Booth, B.P., Nakatsu, K., and Brien, J.F. (1991). Interaction of glyceryl trinitrate and sodium nitroprusside with bovine pulmonary vein homogenate and 10000 x g supernatant: biotransformation and nitric oxide formation. *Canadian Journal of Physiology and Pharmacology* 69, 889-892.

Martinez, I., Ares, G., and Lema, P. (2008). Influence of cut and packaging film on sensory quality of fresh-cut butterhead lettuce (*Lactuca sativa* L., cv Wang). *Journal of Food Quality* 31, 48-66.

Martinez, M.V., and Whitaker, J.R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology* 6, 195-200.

Martin-Belloso, O., Soliva-Fortuny, R., and Oms-Oliu, G. (2006). Fresh-cut fruits. *In* "Handbook of fruits and food processing" (Y.H. Hui, J. Barta, M.P. Cano, T.W. Gusek, J.S. Sidhu and N. Sinha, ed.), Blackwell Publishing, Oxford, UK. 129-144.

Martin-Diana, A.B., Rico, D., Barry-Ryan, C., Frias, J.M., Mulcahy, J., and Henehan, G.T.M. (2005a). Calcium lactate washing treatments for salad-cut iceberg lettuce: effect of temperature and concentration on quality retention parameters. *Food Research International* 38, 729-740.

Martin-Diana, A.B., Rico, D., Barry-Ryan, C., Mulcahy, J., Frias, J., and Henehan, G.T.M. (2005b). Effect of heat shock on browning-related enzymes in minimally processed iceberg lettuce and crude extracts. *Bioscience Biotechnology and Biochemistry* 69, 1677-1685.

Mathew, A. G., and Parpia, H. A. B. (1971). Browning as a polyphenol reaction. *Advances in Food Research* 19, 75-132.

Mayer, A.M. (1987). Polyphenol oxidases in plants-recent progress. *Phytochemistry* 26, 11-20.

Mayer, B., and Hemmens, B. (1997). Biosynthesis and action of nitric oxide in mammalian cells. *Trends in Biochemical Sciences* 22, 477-481.

Mayr, U., Michalek, S., Treutter, D., and Feucht, W. (1997). Phenolic compounds of apple and their relationship to scab resistance *Journal of Phytopathology* 145, 69-75.

Megson, I.L., and Webb, D.J. (2002) Nitric oxide donor drugs: current status and future trends. *Expert Opinion on Investigational Drugs* 11, 587-601.

Milani, J., and Hamedi, M. (2005). Susceptability of five apple cultivars to enzymatic browning. *Acta Horticulturae 682*, 2221-2226.

Millar, A.H., and Day, D.A. (1996). Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. *FEBS Letters* 398, 155-158.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Science* **7**, 405–410.

Moreira, M.R., Ponce, A.G., del Valle, C.E., Ansorena, R., and Roura, S.I. (2006). Effects of abusive temperatures on the postharvest quality of lettuce leaves: ascorbic acid loss and microbial growth. *Journal of Applied Horticulture* 8, 103–113.

Moridani, M.Y., Scobie, H., Jamshidzadeh, A., Salehi, P., and O'Brien, P.J. (2001). Caffeic acid, chlorogenic acid, and dihydrocaffeic acid metabolism: glutathione conjugate formation. *Drug Metabolism and Disposition* 29, 1432-1439.

Moulding, P.H., Grant, H.F., McLellan, K.M., and Robinson, D.S. (1987). Heat stability of soluble and ionically bound peroxidases extracted from apples. *International Journal of Food Science and Technology* 22, 391-397.

Muharrem, B., Jiwon, J., Donald, H.J., and Daniel, C.J. (2007). Physiology of fresh-cut 'Galia' (*Cucumis melo* var. reticulatus) from ripe fruit treated with 1-methylcyclopropene. *Postharvest Biology and Technology* 44, 286-292.

Murata, M., Kurokami, C., Homma, S., and Matsuhashi, C. (1993). Immunochemical and immunohistochemical study of apple chlorogenic acid oxidase. *Journal of Agricultural and Food Chemistry* 41, 1385-1390.

Murgia, I., Delledonne, M., and Soave, C. (2002). Nitric oxide mediates iron-induced ferritin accumulation in *Arabidopsis*. *The Plant Journal* 30, 521-528.

Nakayama, T., Yonekura-Sakakibara, K., Sato, T., Kikuchi, S., Fukui, Y., Fukuchi-Mizutani, M., Ueda, T., Nakao, M., Tanaka, Y., Kusumi, T., and Nishino, T. (2000). Aureusidin synthase: a polyphenol oxidase homolog responsible for flower coloration. *Science* 290, 1163–1166.

Namiki, M. (1988). Chemistry of Maillard reactions: recent studies of browning reaction mechanism and the development of antioxidant and mutagens. *Advances in Food Research* 32, 116-170.

Neighbour, E.A., Pearson, M., and Mehlhorn, H. (1990). Purafil-filtration prevents the development of ozone-induced frost injury: a potential role for nitric oxide. *Atmospheric Environment* 24, 711-715.

Neill, S.J., Desikan, R., Clarke, A., and Hancock, J.T. (2002). Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiology* 128, 13-16.

Neill, S.J., Desikan, R., Clarke, A., and Hancock, J.T. (2003). Nitric oxide signalling in plants. *New Phytologist*. 159, 11-35.

Nicolas, J.J., Richard-Forget, F., Goupy, M.P., Amiot, M.J., and Aubert, Y.S. (1994). Enzymatic browning reactions in apple and apples product. *Critical Review in Food Science and Nutrition* 34, 109-157.

Nicoli, M.C., Calligaris, S., and Lara Manzocco, L. (2000). Effect of enzymatic and chemical oxidation on the antioxidant capacity of catechin model systems and apple derivatives. *Journal of Agricultural and Food Chemistry* 48, 4576-4580.

Noritake, T., Kawakita, K., and Doke, N. (1996). Nitric oxide induces phytoalexin accumulation in potato tuber tissues. *Plant and Cell Physiology* 37, 113 - 116.

Oktay, M., Ku¨ frevioglu, I., and Sakiroglu, H. (1995). Polyphenol oxidase from Amasya apple. *Journal of Food Science* 60, 494–496.

Olivas, G.I., Mattinson, D.S., and Barbosa-Canovas, G.V. (2007). Alginate coatings for preservation of minimally processed `Gala' apples. *Postharvest Biology and Technology* 45, 89-96.

Oszmianski, J., Cheynier, V., and Moutounet, M. (1996). Iron-catalyzed oxidation of (+) catechin in model systems. *Journal of Agricultural and Food Chemistry* 44, 1712-1715.

Pacelli, R., Wink, D.A., Cook, J.A., Krishna, M.C., DeGraff, W., Friedman, N., Tsokos, M., Samuni, A., and Mitchell, J. B. (1995). Nitric oxide potentiates hydrogen peroxide-induced killing of *Escherichia coli*. *Journal of Experimental Medicine* 182, 1469-1479.

Pagnussat, G.C., Lanteri, M.L., and Lamattina, L. (2003). Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiology* 132, 1241-1248.

Peiser, G., Lopez-Galvez, G., Cantwell, M., and Saltveit, M.E. (1998). Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. *Postharvest Biology and Technology* 14, 171-177.

Pe´rez, K., Mercado, J., and Soto-Valdez, H. (2004). Note. Effect of storage temperature on the shelf life of hass avocado (*Persea americana*). *Food Science and Technology International* 10, 73-77.

Piretti, M.V., Gallerani, G., and Brodnik, U. (1996). Polyphenol polymerisation involvement in apple superficial scald. *Postharvest Biology and Technology* 8, 11- 18.

Pitkanen, O.P., Laine, H., Kemppainen, J., Eronen, E., Alanen, A., Raitakari, M., Kirvela, O., Ruotsalainen, U., Knuuti, J., Koivisto, V.A., and Nuutila, P. (1999). Sodium nitroprusside increases human skeletal muscle blood flow, but does not change flow distribution or glucose uptake. *The Journal of Physiology* 521, 729-737.

Pleiffer, S., Mayer, B., and Hemmens, B. (1999). Nitric oxide: chemical puzzles posed by a biological messenger. *Angewandte Chemie International Edition* 38, 1714-1731.

Ponting, J.D., Jackson, R., and Watters, G. (1972). Refrigerated apple slices : preservative effects of ascorbic acid, calcium and sulfites. *Journal of Food Science* 37, 434-436.

Pristijono, P. (2007). Use of nitric oxide to extend the postharvest life of fresh-cut and intact fruits and vegetables. Ph.D. Thesis. Univesity of Newcastle, NSW, Australia.

Pristijono, P., Wills, R.B.H., and Golding, J.B. (2006). Inhibition of browning on the surface of apple slices by short term exposure to nitric oxide (NO) gas. *Postharvest Biology and Technology* 42, 256–259.

Pristijono, P., Wills, R.B.H., and Golding, J.B. (2008). Use of nitric oxide donor compound, Diethylenetriamine-nitric oxide (DETANO), as an inhibitor of browning in apple slices. *The Journal of Horticultural Science and Biotechnology* 83, 555-558.

Rand, M.J., and Li, C.G. (1995). Nitric oxide as a neurotransmitter in peripheral nerves: nature of transmitter and mechanism of transmission. *Annual Review of Physiology* 57, 659-682.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J., Sobrino-Lopez, A., Soliva-Fortuny, R., and Martin-Belloso, O. (2007). Shelf-life extension of fresh-cut 'Fuji' apples at different ripeness stages using natural substances. *Postharvest Biology and Technology* 45, 265-275.

Raybaudi-Massilia, R.M., Rojas-Grau, M.A., Mosqueda-Melgar, J., and Martin-Belloso, O. (2008). Comparative study on essential oils incorporated into an alginate-based edible coating to assure the safety and quality of fresh-cut Fuji apples. *Journal of Food Protection* 71, 1150–1161.

Ray, H., and Hammerschmidt, R. (1998). Responses of potato tuber to infection by *Fusarium sambucinum*. *Physiological and Molecular Plant Pathology* 53, 81-92.

Rice-Evans, C.A., Miller, N.J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* 20, 933-956.

Rice-Evans, C.A, Miller, N.J., and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science* 2, 152-159.

Richard-Forget, F., Amiot, M.J., Goupy, P., and Nicolas, J. (1995). Evaluation of chlorogenic acid O-quinones in model. *In* "Enzymatic browning and its prevention" (C.Y. Lee and J.R. Whitaker, ed.). ACS Symposium Series 600, American Chemical Society, Washington, DC. 144-158.

Richard-Forget, F.C., and Gauillard, F.A. (1997). Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. *Journal of Agricultural and Food Chemistry* 45, 2472-2476.

Rico, D., Martín-Diana, A.B., Barat, J.M., and Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science and Technology* 18, 373-386.

Riener, J., Noci, F., Cronin, D.A., Morgan,D.J., and Lyng, J.G. (2008). Combined effect of temperature and pulsed electric fields on apple juice peroxidase and polyphenoloxidase. inactivation. *Food Chemistry* 109, 402–407.

Rizzi, G.P. (1994). The Maillard reaction in food. *In*: "Maillard reaction in chemistry food and health*"* (Labuza, T.P., Reineccius, G.A., Morrier, V.M., O'Brien, J. and Baynes, J.W. ed.), The Royal Society of Chemistry Publisher, Cambridge, 11–19.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., and Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* 66, 401-436.

Rocha, A.M.C.N., Brochado, C.M., and Morais, A.M.M.B. (1998). Influence of chemical treatment on quality of cut apple, *Journal of Food Quality* 21**,** 13–28.

Rocha, A.M.C.N., and Morais, A.M.B. (2000). Effects of controlled atmosphere on quality of minimally processed apple (cv. Jonagored). *Journal of Food Processing Preservation* 24, 435-451.

Rocha, M.C.N.A., and Morais, M.M.B.A. (2001). Polyphenoloxidase activity and total phenolic content related to browning of minimally processed 'Jonagored' apple. *Journal of the Science of Food and Agriculture* 82, 120-126.

Rogers, G. (2006). Agronomic and postharvest improvement in iceberg and cos lettuce to extend shelf-life for fresh-cuts salads. Horticultural Australia Ltd. 1-134.

Roginsky, V., and Lissi, E.A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry* 92, 235-254.

Rojas-Grau, M.A., Grasa-Guillem, R., and Martin- Belloso, O. (2007). Quality changes in fresh-cut Fuji apple as affected by ripeness stage, antibrowning agents and storage condition. *Journal of Food Science* 72. 5036-5043.

Rojas-Grau, M.A., Oms-Oliu, G., Soliva-Fortuny, R., and Martın-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: a review. *International Journal of Food Science and Technology* 44, 875–889

Rojas-Graü, M.A., Tapia, M.S., and Martín-Belloso, O. (2008). Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples, *LWT-Food Science and Technology* 41, 139–147.

Rolle, R., and Chism, G.W. (1987). Physiological consequences of minimally processed fruits and vegetables. *Journal of Food Quality* 10, 157–165.

Romero-Puertas, M.C., Perazzolli, M., Zago, E.D., and Delledonne, M. (2004). Nitric oxide signalling functions in plant-pathogen interactions. *Cellular Microbiology* 6, 795-803.

Rouet-Mayer, M.A., Ralambosoa, J., and Pilippon, J. (1990). Roles of o-quinones and their polymers in the enzymatic browning of apples. *Phytochemistry* 29, 435- 440.

Roura, S.I., Pereyra, L., and del Valle, C.E. (2008). Phenylalanine ammonia lyase activity in fresh cut lettuce subjected to the combined action of heat mild shocks and chemical additives. LWT - *Food Science and Technology*. 41, 919–924.

Rupasinghe, H.P.V., Murr, D.P., DeEll, J.R., and Odumeru, J. (2005). Influence of 1-methylcyclopropene and natureseal on the quality of fresh-cut "Empire" and "Crispin" apples. *Journal of Food Quality* 28, 289-307.

Ryall, A.L., and Lipton,W.J. (1972). “Handling, transportation and storage of fruits and vegetables”. AVI Pub. Co., Westport, CN. p 587.

Saavedra, J. E., and Keefer, L. K. (2002). Nitrogen-based diazeniumdiolates: versatile nitric oxide-releasing compounds in biomedical research and potential clinical applications. *Journal of Chemical Education* 79, 1427–1434.

Salcini, M.C., and Massantini, R. (2005). Minimally processed fruits: an update on browning control. *Stewart Postharvest Reviews* 3, 1-7.

Saltveit, M.E. (2000). Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biology and Technology* 21, 61-69.

Saltveit, M.E., (2003). Fresh-cut vegetables. *In*: “Postharvest physiology and pathology of vegetables” (Bartz, J.A., Brecht, J.K. ed.), Marcel Dekker, New York, 691–712.

Saltveit, M.E., and Loaiza-Velarde, J.G. (2000). Method of preventing wound induced browning in produce. *United States Patent 6113958*.

Santerre, C. R., Cash, J. N., and Vannorman, D. J. (1988). Ascorbic acid/citric acid combination in the processing of frozen apple slices. *Journal of Food Science* 53, 1713–1716.

Sapers, G.M., and Hicks, K.B. (1989). Inhibition of enzymatic browning of fruits and vegetable. *In* "Quality factors of fruits and vegetables : chemistry and technology" (J.J. Jen, ed.). ACS Symposium Series 405, Washington DC. 29-43.

Sapers, G., Hicks, K.B., Phillips, J.G., Garzarella, L., Pondish, D.L., Matulaitis, R.M., McCormack, T.J., Sondey, S.M., Seib, P.A., and El-Atawy, Y.S. (1989). Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors and complexing agents. *Journal of Food Science* 54, 997-1002.

Scherer, G.F.E., and Holk, A. (2000). NO donors mimic and NO inhibitors inhibit cytokinin action in betalaine accumulation in *Amaranthus caudatus*. *Plant Growth Regulation* 32, 345-350.

Shahidi, F., and Naczk, M. (2004). “Phenolics in food and nutraceuticals”. CRC Press, Boca Raton, Florida. 489–490.

Sherman, T.D., Gardeur, T.l., and Lax, A.R. (1995). Implications of the phytogenic distribution of polyphenol oxidase in plants. *In* "Enzymatic browning and its prevention" (C.Y. Lee and J.R. Whitaker, eds.), ACS Symposium Series 600, American Chemical Society, Washington, DC, 103-119.

Singleton, V.L., and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enolology and Viticulture* 16, 144–158.

Siddiq, M., Cash, J.N., Sinha, N.K., and Akhter, P. (1994). Characterization and inhibition of polyphenol oxidase from pears (cv 'Bosc' and 'Red'). *Journal of Food Biochemistry* 17, 327– 337.

Singh, S.P., Singh, Z., and Swinny, E.E. (2009). Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). *Postharvest Biology and Technology* 53, 101–108.

Smyth, A.B., Song, J., and Cameron, A.C. (1998). Modified atmosphere packaged cut iceberg lettuce: effect of temperature and O_2 partial pressure on respiration and quality. *Journal of Agricultural and Food Chemistry* 46, 4556-4562.

Soegiarto, L., and Wills, R.B.H. (2004). Short term fumigation with nitric oxide gas in air to extend the postharvest life of broccoli, green bean and bok choy. *Horticulture Technology* 14, 538-540.

Soegiarto, L., and Wills, R.B.H. (2006). Effect of nitric oxide, reduced oxygen and elevated carbon dioxide levels on the postharvest life of strawberries and lettuce. *Australian Journal of Experimental Agriculture* 46, 1097-1100.

Soegiarto, L., Wills, R.B.H., Seberry, J A., and Leshem, Y.Y. (2003). Nitric oxide degradation in oxygen atmospheres and rate of uptake by horticultural produce. *Postharvest Biology and Technology* 28, 327-331.

Soliva-Fortuny, R.C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., and Martin-Belloso, O. (2001). Browning evaluation of ready-to-eat apples as affected by modified atmosphere packaging. *Journal of Agricultural and Food Chemistry* 49, 3685-3690.

Soliva-Fortuny, R.C., Oms-Oliu, G., and Martin-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. *Journal of Food Science* 67, 1958-1963.

Son, S.M., Moon, K.D., and Lee, C.Y. (2000). Kinetic study of oxalic acid inhibition on enzymatic browning. *Journal of Agricultural and Food Chemistry* 48, 2071-2074.

Son, S.M., Moon, K.D., and Lee, C.Y. (2001). Inhibitory effects of various antibrowning agents on apple slices. *Food Chemistry* 73, 23-30.

Song, L.L., Ding., W., Zhao, M.G., Sun, B.T., and Zhang, L.X. (2006). Nitric oxide protects against oxidative stress under heat stress in the calluses from two ecotypes of reed. *Plant Science* 171, 449-458.

Sozzi, G.O., Trinchero, G.D., and Fraschina, A.A., (2003). Delayed ripening of 'Bartlett' pears treated with nitric oxide. *The Journal of Horticultural Science and Biotechnology* 78, 899-903.

Srinivasan, A., Kebede, N., Saavedra, J.E., Nikolaitchik, A.V., Brady, D.A., Yourd, E., Davies, K.M., Keefer, L.K., and Toscano, J.P. (2001). Chemistry of the diazeniumdiolates. 3. photoreactivity. *Journal of the American Chemical Society.* 123, 5465-5472.

Stamler, J.S., and Feelisch, M. (1996). "Biochemistry of nitric oxide and redox-related species, methods in nitric oxide research". John Wiley and Sons, New York, p 19.

Stamler, J.S., Singel, D.J., and Loscalzo, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. *Science* 258, 1898-1902.

Stamp, J.A., and Labuza, T.P. (1983). Kinetics of the Maillard reaction between aspartame and glucose in solution at high temperatures. *Journal of Food Science* 48, 543-544.

Stuehr, D.J. (1999). Mammalian nitric oxide synthases. *Biochimica et Biophysica Acta* 1411, 217-230.

Subramanian, N., Venkatesh, P., Ganguli, S., and Sinkar, V.P. (1999). Role of polyphenol oxidase and peroxidase in the generation of black tea the aflavins. *Journal of Agricultural and Food Chemistry* 47, 2571 -2578.

Sun, S.Q., He, M., Cao, T., Yusuyin, Y., Han, W., and Li, J.L**.** (2010). Antioxidative responses related to H_2O_2 depletion in *Hypnum plumaeforme* under the combined stress induced by Pb and Ni. *Environmental Monitoring and Assessment* 163, 303–312.

Suttirak, W., and Manurakchinakorn, S. (2010). Potential application of ascorbic acid, citric acid and oxalic acid for browning inhibition in fresh-cut fruits and vegetables. *Walailak Journal of Science and Technology* 7, 5-14.

Tan, B.K., and Harris, N. D. (1995). Maillard reaction products inhibit apple polyphenoloxidase. *Food Chemistry* 53, 267-273.

Tapia, M.S., Rodríguez, F.J., Rojas-Graü, M.A., and Martín-Belloso, O. (2005). Formulation of alginate and gellan based edible coatings with antioxidants for fresh-cut apple and papaya. *IFT Annual Meeting. Paper 36E-43, New Orleans, USA.*

Teisson, C. (1972). Internal bruising of pineapple. *Fruit* 27, 603–607.

Thomas, D.D., Miranda, K.M., Espey, M.G., Citrin, D., Jourd'heuil, D., Paolocci, N., Hewett, S.J., Colton, C.A., Grisham, M.B., Feelisch, M., and Wink, D.A. (2002). Guide for the use of nitric oxide (NO) donors as probes of the chemistry of NO and related redox species in biological systems. *Methods Enzymology* 359, 84-105.

Toivonen, P.M.A., and Brummel, D.A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology* 48, 1-14.

Toivonen, P.M.A. and DeEll, J.R. (2002), Physiology of fresh-cut fruits and vegetables. *In*: "Physiology of fresh-cut fruits and vegetables: science, technology, and market" (O. Lamikanra, ed.), CRC Press, Boca Raton, Florida, 91–123.

Toivonen, P.M.A., and Hampson, C.R. (2009). Apple cultivar and temperature at cutting affect quality of fresh slices. *HortTechnology* 19, 108-112.

Toivonen, P.M.A., Hampson, C., and Stan S. (2005). Apoplastic levels of hydroxyle radicals in four different apple cultivars are associated with severity of cut-edge browning. *Acta Horticulturae* 682, 1819-1822.

Tomas-Barberan, F.A., Gil, M.I., Castaner, M., Artes, F., and Saltveit, M.E. (1997). Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. *Journal of Agricultural and Food Chemistry* 45, 583-589.

Tsukahara, H., Ishida, T., and Mayumi, M. (1999). Gas-phase oxidation of nitric oxide: chemical kinetics and rate constant. *Nitric Oxide* 3, 191-198.

Tuteja, N., Chandra, M., Tuteja, R., and Misra, M.K. (2004). Nitric oxide as a unique bioactive signalling messenger in physiology and pathophysiology. *Journal of Biomedicine and Biotechnology* 4, 227-237.

Umans, J.G., and Levi, R. (1995). Nitric oxide in the regulation of blood flow and arterial pressure. *Annual Review of Physiology* 57, 771-790.

Vamos-Vigyazo, L. (1995). Prevention of enzymatic browning in fruits and vegetables : a review of principles and practice. *In* "Enzymatic browning and its prevention" (C.Y. Lee and J.R. Whitaker, ed.), ACS Symposium Series 600, American Chemical Society, Washington, DC. 49-62.

Vámos-Vigyázó, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *Critical Review Food Science and Nutrition* 15, 49-127.

Van der Sluis, A.A., Dekker, M., de Jager, A., and Jongen, W.M.F. (2001). Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry* 49, 3606- 3613.

Verner, I.R. (1974). Sodium nitroprusside: theory and practice. *Postgraduate Medical Journal* 50, 576-581.

Vilas-Boas, E.V.B., and Kader, A.A. (2007). Effect of 1-methylcyclopropene (1-MCP) on softening of fresh-cut kiwi fruit, mango and persimmon slices. *Postharvest Biology and Technology* 43, 238–244.

Wakayama, T. (1995). Polyphenol oxidase activity in Japanese apples : differences among cultivar and prevention by heat, ascorbic acid and reduced oxygen. *In* "Enzymatic browning and its prevention" (C.Y. Lee and J.R. Whitaker, ed.) ACS Symposium Series 600, American Chemical Society, Washington, DC, 251- 266.

Walker, J.R.L., and Wilson, E.L. (1975). Studies on the enzymic browning of apples: inhibition of apple o-diphenoloxidase by phenol acids. *Journal of the Science of Food and Agriculture* 26, 1825-1831.

Wang, H., Feng, H., and Luo, Y. (2007). Control of browning and microbial growth on fresh-cut apples by sequential treatment of sanitizers and calcium ascorbate. *Journal of Food Science* 72, M001-M007.

Wang, P.G., Xian, M., Tang, X., Wu, X., Wen, Z., Chai, T., and Janczuk, A. (2002). Nitric oxide donors: chemical activities and biological applications. *Chemical Reviews* 102, 1091-1134.

Watada, A.E., Abe, K., and Yamauchi, N. (1990). Physiological activities of partially processed fruits and vegetables. *Food Technology* 44, 116-122.

Watada, A.E., Ko, N.P., and Minott, D.A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology* 9, 115- I25.

Wedzicha, B.L. (1984). A kinetic model for the sulfite inhibited Maillard reaction. *Food Chemistry* 14, 173- 187.

Weenen, H., and Tjan, S.B. (1994). 3-Deoxyhexosone as flavour precursor. *In* "Trends in flavour research : proceedings of the 7th Weurman Flavour Research Symposium, Noordwijkerhout, The Netherlands, 15-18 June, 1993 " (Maarse. and D.G. Van der Heij, eds.), Elsevier, New York, 303-327.

Wendehenne, D., Pugin, A., Klessig, D.F., and Durner, J. (2001). Nitric oxide: comparative synthesis and signaling in animal and plant cells. *Trends in Plant Science* 6, 177-183.

Wiley, R.C. (1994). Preservation methods for minimally processed refrigerated fruits and vegetables. *In* "Minimally processed refrigerated fruits and vegetables" (R.C. Wiley, ed.), Chapman and Hall Inc., New York, 66-134.

Wills, R.B.H., Ku, V.V.V., and Leshem, Y.Y. (2000). Fumigation with nitric oxide to extend the postharvest life of strawberries. *Postharvest Biology and Technology* 18, 75-79.

Wills, R.B.H., Ku, V.V.V., and Warton, M.A. (2002). Use of 1-methylcyclopropene to extend the postharvest life of lettuce. *Journal of the Science of Food and Agriculture* 82, 1253–1255.

Wills, R.B.H., McGlasson, B., Graham, D. and Joyce, D. (2007). "Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals" (5th ed.). UNSW Press, Sydney.

Wills, R. B. H., Pristijono, P., and Golding, J. B. (2008). Browning on the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO). *Food Chemistry* 107, 1387–1392.

Wills, R.B.H., Soegiarto, L., and Bowyer, M.C. (2007). Use of a solid mixture containing diethylenetriamine/nitric oxide (DETANO) to liberate nitric oxide gas in the presence of horticultural produce to extend postharvest life. *Nitric Oxide* 17, 44–49.

Wilson, I.D., Neill, S.J., and Hancock, J.T.(2008). Nitric oxide synthesis and signalling in plants. *Plant Cell and Environment* 31, 622-631.

Wink, D.A., and Mitchell, J.B. (1998). Chemical biology of nitric oxide: insight into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide. *Free Radical Biology and Medicine* 25, 434-456.

Wojtaszek, P. (2000). Nitric oxide in plants: to NO or not to. *Phytochemistry* 54, 1-4.

Yaylayan, V.A. (1997). Classification of the Maillard reaction: a conceptual approach. *Trends in Food Science and Technology* 8, 13–18.

Yingsanga, P., Srilaong, V., Kanlayanarat, S., Noichinda, S., and McGlasson, W.B. (2008). Relationship between browning and related enzymes (PAL, PPO and POD) in rambutan fruit (*Nephelium lappaceum* Linn.) cvs. Rongrien and See-chompoo. *Postharvest Biology and Technology* 50, 164-168.

Zagory, D.(1999). Controlled and modified atmosphere. I- General aspect of film technology and selection. *In* : "5th Annual workshop on fresh-cut products: manufacturing quality and safety". University of California. Davis p 1-3. (section 7a).

Zamora, R., Grzesiok, A., Webert, H., and Feelisch, M. (1995). Oxidative release of nitric oxide accounts for guanylyl cyclase stimulating, vasodilator and anti-platelet activity of Piloty's acid: a comparison with Angeli's salt. *Biochemical Journal* 312, 333-339.

Zawistowski, J., Biliaderis, C.G., and Eskin, N.A.M. (1991). Polyphenol oxidase. *In* "Oxidative enzymes in food" (D.S. Robinson and N.A.M. Eskin, ed.), Elsevier, London, 217-274.

Zhang, O.D., Cheng, G.P., Li, J., Yi, C., Yang, E., Qu, H.X., Jiang, Y.M. and Duan X.W. (2008). Effect of nitric oxide on disorder development and quality maintenance of plum fruit stored at low temperature. *Acta Horticulturae* 804, 549-554.

Zhang, X.P., Ren, X.L., Ren, Y.M., Wang, X.H., Sun, F.J., and Bai, J.X. (2007) Effects of nitric oxide treatment on storage ability and chlorophyll content of postharvest kiwi fruit. *Food Research and Development* 28, 145–148.

Zhang, L., Zhou , S., Xuan , Y., Miao Sun, M., and Zhao, L. (2009). Protective effect of nitric oxide against oxidative damage in *Arabidopsis* leaves under ultraviolet-B irradiation. *Journal of Plant Biology* 52, 135–140.

Zheng, X., Tian, S., Meng, X., and Li, B. (2007). Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature. *Food Chemistry* 104, 156–162.

Zhu, L.Q., Zhou, J., Zhu, S.H., and Guo, L.H. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chemistry,* 114, 174-179.

Zhu, S., Liu, M., and Zhou, J. (2006). Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. *Postharvest Biology and Technology* 42, 41-48.

Zhu, S., Sun, L., Liu, M., and Zhou, J (2008). Effect of nitric oxide on reactive oxygen species and antioxidant enzymes in kiwi fruit during storage. *Journal of the Science of Food and Agriculture* 88, 2324–2331.

Zhu, S., Sun, L., and Jie Zhou, J. (2009) Effects of nitric oxide fumigation on phenolic metabolism of postharvest Chinese winter jujube (*Zizyphus jujuba* Mill. cv. Dongzao) in relation to fruit quality. *LWT - Food Science and Technology* 42, 1009–1014.

Zhu, S., and Zhou, J. (2007). Effect of nitric oxide on ethylene production in strawberry fruit during storage. *Food Chemistry* 100, 1517-1522.

Zottini, M., Formentin, E., Scattolin, M., Carimi, F., Schiavo, F. L., and Terzi, M. (2002). Nitric oxide affects plant mitochondrial functionality in vivo. *FEBS Letters* 515, 75-78.

Zuo, L., Seog, E.J., and Lee, J.H. (2008). Effects of ascorbic acid and citric acid on CIE color values of fresh-cut apple cubes. *Journal of Food Technology* 6, 20-24.

APPENDIX

Related publications

Huque, R., Wills, R. B. H. and Golding, J. B. (2011). Nitric oxide inhibits cut-surface browning in four lettuce types. *Journal of Horticultural Science & Biotechnology* 86 (2) , 97 -100

HOU, Y. C., JANCZUK, A. and WANG, P. G. (1999). Current trends in the development of nitric oxide donors. *Current Pharmacological Design*, **5,** 417–441.

HRABIE, J. A., KLOSE, J. R., WINK, D. A. and KEEFER, L. K. (1993). New nitric oxide-releasing zwitterions derived from polyamines. *Journal of Organic Chemistry*, **58,** 1472–1476.

KEEFER, L. K. (1998). Nitric oxide-releasing compounds: from basic research to promising drugs. *Chemtech*, **28,** 30–35.

LAMATTINA, L., GARCÍA-MATA, C., GRAZIANO, M. and PAGNUSSAT, G. (2003). Nitric oxide: the versatility of an extensive signal molecule. *Annual Review of Plant Biology*, **54,** 109–136.

LANGDON, T. T. (1987). Preventing of browning in fresh prepared potatoes without the use of sulfiting agents. *Food Technology*, **41,** 64–67.

LESHEM, Y. Y., WILLS, R. B. H. and KU, V. V. V. (1998). Evidence for the function of the free radical gas – nitric oxide (NO˙) as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry*, **36,** 825–833.

PRISTIJONO, P., WILLS, R. B. H. and GOLDING, J. B (2006). Inhibition of browning on the surface of apple slices by short-term exposure to nitric oxide (NO) gas. *Postharvest Biology and Technology*, **42,** 256–259.

PRISTIJONO, P., WILLS, R. B. H. and GOLDING, J. B (2008). Use of the nitric oxide donor compound, diethylenetriamine-nitric oxide (DETANO), as an inhibitor of browning in apple slices. *Journal of Horticultural Science & Biotechnology*, **83,** 555–558.

VERNER, I. R. (1974). Sodium nitroprusside: theory and practice. *Postgraduate Medical Journal*, **50,** 576–581.

WILLS, R. B. H., PRISTIJONO, P. and GOLDING, J. B. (2008). Browning of the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO). *Food Chemistry*, **107,** 1387–1392.

ZHANG, D. D., CHENG, G. P., LI, J., YI, C., YANG, E., QU, H. X., JIANG, Y. M. and DUAN, X. W. (2008). Proceedings of the Europe-Asia Symposium on Quality Management in Postharvest Systems (EURASIA 2007). *Acta Horticulturae*, **804,** 549–554.

ZHU, L. Q., ZHOU, J., ZHU, S. H. and GUO, L. H. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chemistry*, **114,** 174–179.