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SUMÁRIO

O kiwi (*Actinidia deliciosa* A. Chev.) tem sido considerado um fruto climatérico. No entanto, este fruto comporta-se atipicamente em resposta à aplicação externa de etileno a várias temperaturas. A incapacidade do kiwi para produzir etileno a baixas temperaturas, a produção de etileno à temperatura ambiente após um período de frio, o limite máximo de temperatura para produção de etileno e o seu comportamento pós-armazenamento não estão suficientemente estudados. Nesta tese investigaram-se algumas características particulares do percurso da biossíntese do etileno e o amadurecimento do kiwi induzidos por propileno a temperaturas de 10 a 45°C, após armazenamento a baixas temperaturas, assim como após armazenamento em atmosfera controlada (CA) e nível de oxigénio ultra baixo (ULO).

À temperatura de 20°C, o kiwi comportou-se como um fruto climatérico típico enquanto a 10°C o seu comportamento foi não-climatérico (capítulo 3).

As razões pelas quais os frutos tratados com propileno não produziram etileno a 10°C foram a falta de transcrição dos genes da 1-aminociclopropano-1-carboxilase sintase (ACC sintase) induzidos por propileno e a possível modificação da 1-aminociclopropano-1-carboxilase oxidase (ACC oxidase) pós-transcrição (capítulo 4).

À temperatura ambiente, o kiwi apresentou autocatálise de etileno e aumento da taxa respiratória acompanhados pelo amadurecimento simultâneo do fruto em aproximadamente 19 dias após a colheita. O kiwi respondeu à aplicação de propileno a 20-34°C antecipando o amadurecimento e a respiração climatérica, enquanto a autocatálise de etileno ocorreu apenas no final do processo de maturação (capítulo 4 e 5). A principal razão para a produção tardia de etileno foi o atraso na indução da actividade da ACC sintase. No entanto, o período de tempo necessário para o início da autocatálise de etileno foi menor nas temperaturas mais elevadas.

Kiwis expostos a concentrações de 130µl/l de propileno apresentaram uma produção reduzida de etileno a 38°C e quase nula a 40°C (capítulo 5). A ACC oxidase foi a primeira enzima a ser afectada a altas temperaturas seguindo-se a ACC sintase.

As temperaturas baixas atrasaram o amadurecimento, enquanto que as temperaturas elevadas o bloquearam ou causaram amadurecimento anormal. A respiração do kiwi aumentou com a temperatura até 45°C. A esta temperatura deu-se a decomposição dos tecidos e por conseguinte a produção de CO_2 desceu para níveis basais (capítulo 5).

Cinco dias de exposição a baixas temperaturas não foram suficientes para induzir a autocatálise de etileno após re-aquecimento. Kiwis expostos a temperaturas de 0 a 15°C durante 12 dias começaram a produzir etileno imediatamente após re-aquecimento a 20°C, com um aumento simultâneo das actividades da ACC sintase e da ACC oxidase (Capítulo 6). A produção de etileno foi acompanhada pelo aumento da respiração e amadurecimento. Verificou-se um aumento na insaturação dos ácidos gordos e na permeabilidade da membrana durante a exposição a baixas temperaturas. No entanto, não foi encontrada nenhuma correlação evidente entre estes aumentos e a produção de etileno ou o amadurecimento.

A CA e ULO prolongaram o periodo de armazenamento do kiwi em relação ao armazenamento convencional (CS) em atmosfera normal a 0°C (capítulo 7). A CA ($2\%O_2+5\%CO_2$) e ULO ($1\%O_2+1\%CO_2$) foram os mais eficientes a atrasar a perda de dureza dos frutos. Kiwis de CS foram os que amadureceram mais rapidamente e os frutos do tratamento ULO ($0,7\%O_2+0,7\%CO_2$) acumularam níveis significativos de etanol e acetaldeído. Os kiwis não produziram etileno durante 180 dias de armazenamento a 0°C em nenhum dos tratamentos. Num armazenamento prolongado de 60 dias a 0°C, a actividade da ACC sintase foi induzida e verificou-se acumulação do ácido 1-aminociclopropano-1-carboxilico (ACC). A actividade da ACC sintase após 60 dias de armazenamento a 0°C deveu-se, provavelmente, à activação dos genes da ACC sintase induzidos pelas baixas temperaturas. No entanto, não se observou actividade da ACC oxidase, sendo esta a principal razão para a não produção de etileno durante o armazenamento. Após o reaquecimento dos frutos depois de 60, 120 e 180 dias de armazenamento a 0°C, só os kiwis de CS e CA produziram etileno. Porém, a capacidade do kiwi para produzir etileno mostrou-se progressivamente mais baixa devido ao decréscimo gradual das actividades da ACC sintase e da ACC oxidase.

Frutos de ULO perderam a sua capacidade para produzir etileno, mesmo quando tratados com propileno à temperatura ambiente, devido sobretudo à reduzida actividade da ACC oxidase.

ABSTRACT

Kiwifruit (*Actinidia deliciosa* A. Chev.) is considered a climacteric fruit. However, it behaves atypically in response to external ethylene application at different temperatures. The lack of ability of the kiwifruit to produce ethylene at low temperatures, its chilling requirements and maximum temperature limit for ethylene production and its behaviour post-storage are poorly understood. In this thesis were studied some particular features of the ethylene biosynthesis pathway and ripening of kiwifruit induced by propylene at temperatures from 10 to 45°C, during shelf-life after chilling, as well as after storage in controlled atmosphere (CA) and ultra low oxygen (ULO) conditions.

At temperatures of 20°C kiwifruit behaved as a typical climacteric fruit, while at 10°C it behaved as a non-climacteric fruit (chapter 3).

The main reasons for the inhibition of the propylene-induced autocatalytic ethylene production in kiwifruit at 10°C were primarily the suppression of the propylene-induced ACC synthase gene expression and the possible post-transcriptional modification of ACC oxidase (chapter 4).

Kiwifruit at room temperature showed simultaneous autocatalysis of ethylene, climacteric respiration rise and ripening at about 19 days after harvest (chapter 3). Kiwifruit sensed propylene at 20-34°C by advancing the onset of ripening and respiration, while the ethylene burst occurred late in the ripening process (chapter 4 and 5). The main reason for the late ethylene production was the tardy increase of ACC synthase activity. However, the lag period for ethylene production was decreased with temperature increase.

Propylene-treated kiwifruit had a reduced ethylene production at 38°C and almost null at 40°C (chapter 5). The 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) was the first to be affected at high temperatures followed by ACC synthase.

Low temperatures slowed down ripening, while high stress temperatures blocked or caused abnormal ripening. Fruit respiration was increased with temperature up to

45°C. At this temperature there was irreversible breakdown of the tissues and consequently respiration came to basal levels (chapter 5).

Five days storage of kiwifruit at low temperature were not enough to induce autocatalytic ethylene production and ripening upon transference to 20°C. Kiwifruit exposed to temperatures from 0 to 15°C for 12 days started to produce ethylene immediately upon rewarming to 20°C, with concomitant increase in ACC synthase and ACC oxidase activities (Chapter 6). Ethylene production was accompanied by the increase in respiration and ripening. There was an increase in fatty acid unsaturation and membrane permeability during cold treatment, but we found no clear correlation between them and ethylene production or ripening.

The CA and ULO increased storage life in relation to conventional storage (CS) in air at 0°C. The CA ($2\%O_2+5\%CO_2$) and ULO ($1\%O_2+1\%CO_2$) were the most effective in keeping fruit firmness for longer time. The CS fruit ripened faster and the ULO ($0.7\%O_2+0.7\%CO_2$) accumulated significant levels of ethanol and acetaldehyde. Kiwifruit did not produce ethylene during storage up to 180 days at 0°C in any treatment (chapter 7). Prolonged storage of 60 days at 0°C induced ACC synthase activity and 1-aminocyclopropane-1-carboxylic acid (ACC) accumulation, but not ACC oxidase activity which was the main reason for the lack of ethylene production during storage. The increase in ACC synthase activity was probably due to the activation of the chilling-induced ACC synthase genes. Upon rewarming of the fruit after 60, 120 and 180 days storage, only CS and CA fruit produced ethylene with no lag period. However, the capacity of kiwifruit to produce ethylene was progressively lower due to gradually lower ACC synthase and ACC oxidase activities. The ULO treated fruit lost the ability to produce ethylene upon rewarming, even when treated with propylene, mostly due to the reduced ACC oxidase activity.

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To my family

ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylic acid
ACC synthase	1-aminocyclopropane-1-carboxylate synthase
ACC oxidase	1-aminocyclopropane-1-carboxylate oxidase
AEC	1-amino-2-ethylcyclopropane-1-carboxilic acid
AOA	aminooxyacetic acid
AVG	aminoethoxyvinilglycine
CA	controlled atmosphere
CS	conventional storage
CTAB	cetyltrimethylammonium bromide
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
EDTA	ethylenediaminetetraacetic acid
ETR1	*Arabidopsis thaliana* gene essential for ethylene signaling
HPLC	high performance liquid chromatography
KWACC1	311bp ACC synthase cDNA from ethylene treated kiwifruit mesocarp
KWACC2	305bp ACC synthase cDNA from wounded kiwifruit mesocarp
IAA	indole-acetic acid
MACC	1-(malonylamino) cyclopropane-1-carboxylic acid
MTA	5'-methylthioadenosine
PLP	pyridoxal 5'-phosphate
pMEL1	cDNA clone of ACC oxidase from a climacteric melon
PMSF	phenylmethylsulphonyl fluoride
pTOM13	cDNA clone of ACC oxidase from ripe tomato
SAM	S-adenosil-L-methionine
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SSC	soluble solids content
TNS	Tri-isopropyl Naphthalene Sulphonic acid - sodium salt
ULO	ultra low oxygen

CHAPTER 1. INTRODUCTION

The kiwifruit plant (*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson var. *deliciosa* 'Hayward') is one of the most important commercially (Ferguson, 1990). The success of the kiwifruit as an export crop depends on the ability to store the fruit for extended periods and to transport them over long distances. Much of the research done until now is related to the development of procedures for harvesting, handling and storing the fruit (Given, 1993). Because much of the investigation on kiwifruit ripening has been driven from a commercial perspective (Given, 1993), there is a lack in the physiology and biochemistry of fruit ripening.

The simple gas ethylene is an endogenous regulator of a variety of stress responses and developmental processes (Abeles et al., 1992). Tucker (1993) reported that the conversion of methionine to S-adenosil-L-methionine (SAM), which is used in other biochemical pathways, is considered to be constant throughout the development and ripening of the fruit. Thus, the two key control enzymes for the biosynthesis of ethylene are 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) and 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase). Hoffman and Yang (1980) found low levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in green fruits and its rapid accumulation coinciding with ethylene biosynthesis. This implies that ACC synthase may be a key enzyme in the control of ethylene synthesis (Tucker, 1993). Though ACC oxidase is expressed constitutively in most tissues, its synthesis increases during ripening in some fruit (Picton et al., 1993).

Kiwifruit was believed to be a climacteric fruit whose ripening was mediated by ethylene (McDonald and Harman, 1982; Arpaia et al., 1994a). Ethylene production rate by mature kiwifruit at harvest is very low (0.1 to 1.0 µl/kg/h) and increases markedly with ripening to 50 to 100 µl/kg/h after 17±7 days (Arpaia et al., 1994a). However, Yano and Hasegawa (1993a) reported that the ripening related ethylene production in kiwifruit rarely occurred after harvest without the help of exogenous ethylene. The application of exogenous ethylene enhanced ripening of kiwifruit but, contrarily to most climacteric fruit, the burst in ethylene production occurred very late in the ripening process (Stavroulakis and Sfakiotakis, 1993; Wittaker et al., 1997), suggesting that ripening and ethylene biosynthesis in kiwifruit may be regulated by two

independent mechanisms (Stavroulakis and Sfakiotakis, 1993). Stavroulakis and Sfakiotakis (1993) treated kiwifruit with 130μl/l propylene, which is equivalent to 1μl/l ethylene (Burg and Burg, 1967), at temperatures from 0 to 35°C and found that below a critical range temperature of 11 to 14.8°C ethylene biosynthesis of the propylene-treated kiwifruit did not occur. Above the critical range, autocatalysis of ethylene proceeded normally. The same authors reported that kiwifruit is an unique climacteric fruit which at low temperature lacks the ability for autocatalysis of ethylene production, being the ACC availability the limiting factor rather than ACC oxidase activity.

Ethylene plays a crucial role in ripening of kiwifruit (Arpaia et al., 1994a), and the elucidation of the controlling factors in ethylene biosynthesis is important in prolonging the storage life and keeping the fruit quality during the handling operations. Many factors can initiate autocatalysis of ethylene production in the harvested fruit and the control of this factors can be of important significance in prolonging the storage life and keeping the quality of the fruit.

Although much effort is devoted to avoid ethylene exposure of kiwifruit in prolonged storage, ethylene treatment may be desirable during harvest and early storage to accelerate kiwifruit ripening in order to capitalize on marketing opportunities (Arpaia et al., 1994b).

Chilling stress can advance the onset of ripening and ethylene production in kiwifruit after rewarming by stimulating the formation of ACC as for other fruit (Hyodo and Fukasawa, 1985). Yang and Hoffman (1984) suggested that chilling treatment unmasked or stimulated production of mRNA coding for ACC synthase, but translation did not occur until the tissue was transferred to warm temperatures.

Storage of kiwifruit in controlled atmosphere (CA) of $2\%O_2+5\%CO_2$ is known to extend storage life and the fruit ripe normally during shelf-life (Arpaia et al., 1994b). Thomai and Sfakiotakis (1997) found that ultra low oxygen (ULO) stored kiwifruit ($O_2<1\%$) did not ripe normally during shelf-life.

The mechanisms of initiation, regulation and the co-ordination of the diverse biochemical changes during ripening are not yet well understood. However, they must rely on an interplay between the regulation of gene expression and enzyme activity (Tucker, 1993). The atypical behaviour of kiwifruit in relation to ethylene sensitivity and ethylene production and the lack of ability to produce ethylene at low temperatures makes it a good system for analyzing the role of ethylene in fruit ripening.

The objectives of this thesis are to examine and describe the effects of temperature and ambient gas composition on 'Hayward' kiwifruit ethylene biosynthesis pathway induced or not by propylene, the pattern of CO_2 production and fruit ripening, after harvest. Those are important points for the manipulation of harvesting time, storage capacity and induction of ripening. The aim is to keep high quality products at low cost and to be able to put them in the market at the appropriate time, so that it is more profitable for producers and consumers.

Chapter 2 develops the literature background pertinent to the thesis problem.

The first manuscript (Chapter 3) describes the thermoregulation of autocatalytic ethylene production and respiration climacteric induced by propylene in ripening 'Hayward' kiwifruit. This chapter gives evidence for the non-climacteric behaviour of kiwifruit at low temperatures.

The second manuscript (chapter 4) depicts the effect of low temperature on the propylene-induced autocatalytic ethylene biosynthesis, respiration and ripening of 'Hayward' kiwifruit. In this chapter it is elucidated why propylene treated kiwifruit does not produce ethylene at low temperatures.

The third manuscript (chapter 5) characterizes the effect of high temperature stress on ethylene biosynthesis, respiration and ripening of 'Hayward' kiwifruit. In this chapter it is defined the upper temperature limits for ethylene and CO_2 production and ripening.

The fourth manuscript (chapter 6) investigates the effect of chilling on the induction of ethylene biosynthesis and associated changes of respiration, ripening parameters and fatty acids composition of 'Hayward' kiwifruit. This chapter clarifies the chilling requirements for ethylene production in terms of temperature range and time required.

The fifth manuscript (chapter 7) researches the ethylene biosynthesis and ripening behaviour of 'Hayward' kiwifruit subjected to conventional storage (CS) in air at 0°C, CA and ULO conditions. In this chapter it is elucidated the ethylene production pattern and ripening behaviour of kiwifruit during storage in the referred conditions, and shelf-life post-storage.

The chapter 8 makes a general discussion where the different chapters are related to grant some more information about the post-harvest behaviour of kiwifruit and ways to manipulate it according to our objectives of faster ripening or extended storage life.

Finally, the chapter 9 outlines the most important conclusions of the present thesis.

CHAPTER 2. LITERATURE REVIEW

1. The pathway of ethylene biosynthesis

The plant hormone ethylene plays an important role in the ripening process of climacteric fruits and the post-harvest life of many horticultural products (Pech et al., 1994). Ethylene is produced by plant tissues in amounts ranging from almost none up to 500 nl/g/h and is biologically active in trace amounts (as little as 10-100 nl/l of air) (Burg, 1962). Ethylene production is induced during several developmental stages, including fruit ripening, seed germination, leaf and flower senescence and abscission. It is also induced by external factors, such as wounding, anaerobiosis, viral infection, auxin treatment, chilling injury, drought and Cd^{2+} and Li^{+} ions (Yang and Hoffman, 1984).

Ethylene is formed from methionine via SAM and the cyclic, nonprotein amino acid ACC. The enzymes catalysing the individual steps of this pathway are SAM synthetase, ACC synthase and ACC oxidase (Kende, 1993).

SAM synthetase which catalyses the conversion of methionine into SAM, has been extensively studied (Chou et al., 1977). Inasmuch as SAM is constantly synthesised and also utilised by some other reactions, such as methylation and poliamine synthesis, additional utilisation of SAM for ACC synthesis may not significantly alter the steady state level of SAM. As long as SAM is maintained at a normal level even when ethylene synthesis is active, it is unlike that SAM synthetase becomes a rate-limiting enzyme in ethylene biosynthesis (Yang and Hoffman, 1984). The ethylene pathway is designed to allow high rates of ethylene production without high intracellular concentrations of methionine. This is achieved by recycling 5'-methylthioadenosine (MTA), produced as well as ACC from SAM in the reaction catalysed by ACC synthase, to methionine (Theologis, 1992).

Besides being converted to ethylene, ACC is also metabolised to 1-(malonylamino) cyclopropane-1-carboxylic acid (MACC). Malonylation of ACC may contribute to the regulation of ACC levels and the rate of ethylene formation (Kende, 1993). The final

step of ethylene biosynthesis (conversion of ACC to ethylene, HCN and CO_2) is catalysed by ACC oxidase (Theologis, 1992).

1.1. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase)

ACC synthase is a pyridoxal enzyme which catalyses the conversion of SAM to ACC (Yang and Hoffman, 1984). ACC synthase activity was first identified in homogenates of ripening tomato pericarp tissue by Boller et al. (1979) who found that ACC synthase was soluble, had a Km of 13μM for SAM and its activity was inhibited by aminoethoxyvinilglycine (AVG) with a Km of 0.2μM, and optimum pH 8.5. Yu et al. (1979) confirmed these findings and determined the pyridoxal phosphate requirement of ACC synthase and showed that the enzymatic reaction yielded, besides ACC, MTA.

ACC synthase activity was thereafter shown in mung been hypocotyls (Yoshii et al., 1980), been leaf tissue (Fuhrer, 1982), cantaloupe fruit (Hoffman and Yang, 1982), cucumber skin (Wang and Adams, 1982), orange peel (Riov and Yang, 1982a), winter squash mesocarp (Hyodo et al., 1983) and apples (Bufler, 1984; Mansour et al., 1986; Yip et al., 1991). Its activity was shown to limit ethylene synthesis in many instances and to be enhanced by factors that promote ethylene formation, e.g. by indole-acetic acid (IAA) and by stress such as wounding (Yang and Hoffman, 1984).

Bufler and Bangerth (1983) reported that ACC synthase could be extracted from apple fruits in the presence of the detergent Triton X-100. Yip et al. (1991) demonstrated that ACC synthase in homogenates of apples is associated with a particulate fraction. Because the enzyme activity was found in all organelle fractions, it is likely that the binding of the enzyme to particulate cell components is artificial and that occurs during cell disruption (Kende, 1993).

ACC synthase is a key regulatory enzyme in the pathway of ethylene biosynthesis (Yang and Hoffman, 1984). Many internal factors that induce ethylene production in plant tissues were found to exert their effect by inducing the *de novo* synthesis of ACC synthase. These factors include flooding (Bradford and Yang, 1980), flower senescence (Bufler et al., 1980; Suttle and Kende, 1980), auxin (Yu et al., 1980; Yoshii and Imaseki, 1981; Yoshii and Imaseki, 1982), physical wounding (Boller and Kende, 1980; Yu and Yang, 1980; Hyodo and Nishino, 1981; Kende and Boller 1981;

Konze and Kwiatkowski, 1981; Hoffman and Yang, 1982; Riov and Yang, 1982b), chemical wounding (Hogsett et al., 1981; Fuhrer, 1982), fruit ripening (Hoffman and Yang, 1980; Kende and Boller, 1981), chilling (Wang and Adams, 1980; Field, 1981b; Wang and Adams, 1982), wilting (Apelbaum and Yang, 1981), and ethylene (Bufler, 1984).

The progress in the purification of ACC synthase has been slow because of its low abundance and lability (Yang and Hoffman, 1984; Kende, 1993). Blecker et al. (1986) estimated that the level of ACC synthase in ripening tomato pericarp tissue was<0.0001% of the total soluble protein. This low level can be boosted about 10-fold by wounding the tissue (Kende, 1989).

ACC synthase activity can be inhibited by AVG (Adams and Yang, 1979) and aminooxyacetic acid (AOA) (Yu et al., 1979) known inhibitors of pyridoxal enzymes (Rando, 1974). ACC synthase can be also inactivated by its own substrate SAM. Boller et al. (1979) found progressive inhibition of ACC synthase at SAM concentrations above 50 μM. These observations were confirmed by Satoh and Esashi (1986). Furthermore, Satoh and Yang (1988; 1989a; 1989b) discovered that substrate inactivation of ACC synthase was accompanied by covalent attachment of at least a fragment of SAM, probably 2-aminobutyric acid, to the active site of the enzyme. Yip et al. (1990) reported that the same active-site lysine binds the pyridoxal 5'-phosphate (PLP) and covalently links to the 2-aminobutyrate portion of SAM during inactivation.

Most efforts have been concentrated on characterising and purifying ACC synthase from tomato pericarp tissue. Conventional and high performance liquid chromatography (HPLC) gel filtration indicated that ACC synthase in homogenates of wounded pericarp tissue had a molecular mass of 55 to 57 kDa (Yang, 1980; Acaster and Kende, 1983; Bleecker et al., 1986). The enzyme was purified >6500-fold by a series of chromatographic methods and identified as a protein of 50 kDa by two-dimensional gel electrophoresis (Bleecker et al., 1986). The calculated specific activity of purified ACC synthase was $4x10^5$ units per mg protein (one unit = one nmol ACC produced per hour at 30°C).

A partially purified ACC synthase was used to induce antibody production in mice, and monoclonal antibodies were obtained from murine hybridoma cell lines (Bleecker et al., 1986). Immunopurified protein was shown to have a molecular mass of 50 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bleecker et al., 1986; Bleecker et al., 1988). Immunoassays and radioactive labelling showed that ACC synthase was *de novo* synthesised in wound-induced tissue (Bleecker et al., 1986; Bleecker et al., 1988). Van Der Staeten et al. (1989) also purified ACC synthase 5000-fold from wound-induced pericarp tissue of ripening tomatoes and determined by two-dimensional gel electrophoresis and substrate labelling that the molecular mass of the enzyme was about 45 kDa.

Purification of ACC synthase in wound-induced mesocarp of winter squash led to the isolation of a 50 kDa protein (Nakajima and Imaseki, 1986; Nakajima et al., 1988). In mung bean hypocotyls, it was found a 65 kDa protein by SDS-PAGE (Tsai et al., 1988) and immunoaffinity purification gave a protein of 48 kDa in apple fruit (Dong et al., 1991b; Yip et al., 1991).

Antibodies against ACC synthase from wound-induced mesocarp tissue of winter squash recognised ACC synthase from wounded tomato pericarp tissue and wounded winter squash hypocotils but not from auxin-treated winter squash, tomato or mung bean hypocotils (Nakagawa et al., 1988). This result indicate that there are two isoforms of ACC synthase, one wound-induced and one auxin-induced and that the two forms are sufficiently different to be distinguished immunologically (Kende, 1993).

ACC synthase requires pyridoxal phosphate, and most such enzymes have a lysine residue in their active site (Theologis, 1992). Lysine-278 of a tomato izoenzyme, which is conserved in all ACC synthases so far, has been shown to be the site of pyridoxal phosphate attachment (Yip et al., 1990). The active-site of ACC synthase was found from apple or from ripe and wounded tomato fruit, to have a peptide sequence of Ser-Leu-Ser-Lys-Asp-Met-Gly-Leu-Pro-Phe-Arg (Yip et al., 1990). Purification of ACC synthase by gel electrophoresis or imunoprecipitation in tomato (Bleeker et al., 1986; 1988), winter squash (Nakajima and Imaseki, 1986; Nakajima et al., 1988), zucchini (Sato and Theologis, 1989; Sato et al., 1991) and apples (Dong et al., 1991b) showed a molecular weight of 50-60kDa.

Increases in ACC synthase activity during wounding (Sato and Theologis, 1989; Nakajima et al., 1990; Huang et al., 1991; Olson et al., 1991; Yip et al., 1992), auxin and cytokinin (Sato and Theologis, 1989; Van Der Straeten et al., 1990; Huang et al., 1991; Nakagawa et al., 1991; Kim et al., 1992; Yip et al., 1992), fruit ripening (Van Der Straeten et al., 1990; Dong et al., 1991a; Olson et al., 1991; Rottmann et al., 1991; Yip et al., 1992), flower fading (Rottmann et al., 1991; Park et al., 1992; Woodson et al., 1992) and ethylene (Rottmann et al., 1991; Woodson et al., 1992) appear to be based on increased levels of ACC synthase mRNA as shown by RNA (northern) blotting.

A cDNA encoding ACC synthase has been cloned from zucchini using immunochemical approaches, and its authenticity has been confirmed by expression in E. coli and yeast (Sato and Theologis, 1989). Thereafter, cDNA clones encoding ACC synthase have been reported from winter squash (Nakajima et al., 1990; Nakagawa et al., 1991), tomato (Van Der Straeten et al., 1990; Olson et al., 1991; Rottmann et al., 1991; Yip et al., 1992; Mattoo et al. 1993; Yip, 1993), zucchini (Huang et al., 1991; Sato et al., 1991), apple (Dong et al., 1991a; Kim et al., 1992), carnation (Park et al., 1992; Henskens et al., 1994), *Arabidopsis* (Van Der Staten et al., 1992; Rodrigues-Pousada et al., 1993) and mung bean (Botella et al., 1992; kim et al., 1992; Botella et al., 1995).

There is an emerging picture that ACC synthase is encoded by a highly divergent multigene family differentially expressed during ripening, wounding, hormonal and environmental stimuli (Nakajima et al., 1990; Van Der Staten et al., 1990; Huang et al., 1991; Olson et al., 1991; Rottman et al., 1991; Sato et al., 1991; Liang et al., 1992; Yip et al., 1992; Botella et al., 1995).

In kiwifruit, two cDNAs for ACC synthase were cloned and the expression of these genes was studied (Ikoma et al., 1995). The two cDNAs were named KWACC1 (311bp, from ethylene treated kiwifruit) and KWACC2 (305bp, from wounded mesocarp). The KWACC2 was transcribed only in wounded tissue, while KWACC1 was transcribed in ethylene-treated ripe fruit and wounded tissue. Both cDNAs showed about 50 to 80% amino acid sequence homology to the cDNA of various origins (Ikoma et al., 1995).

Differential expression of two ACC synthase genes in ripening and wound-induced tomato pericarp tissue have been reported (Olson et al., 1991). Similarly, tomato fruit, cell cultures and hypocotyls express four ACC synthase genes that are differentially regulated during ripening, by wounding and by auxin (Yip et al., 1992). Expression of two ACC synthase genes in winter squash is also differentially regulated by auxin and wounding (Nakagawa et al., 1991). Two ACC synthase cDNA fragments from apple and mung been hypocotyls showed by northern blot analyses that their expression is regulated by auxin treatment (Kim et al., 1992). These authors reported that auxin induces ethylene production transcriptionally by increasing the ACC synthase transcripts and that ripening and auxin regulated ACC synthases are encoded by different genes.

RNA (northern) blotting indicates that the size of the mRNA encoding different forms of ACC synthase varies from 1.8-2.1kb (Kende, 1993). The highest level of homology between different ACC synthases is in the interior portion of the polypeptide, while the carboxyl termini is more divergent. There are seven regions of high amino acid sequence homology among ACC synthases most notable around the active site of the enzyme (Kende, 1993). All known ACC synthases contain, at comparable positions, 11 of 12 invariant amino acids that are involved in the binding of pyridoxal phosphate and substrate in aminotransferases (Huang et al., 1991; Rottman et al., 1991). The amino acid sequence identities of the various ACC synthases vary from 48%-97% (Kende, 1993). It appears that the polymorphism of ACC synthases arose prior to the divergence of monocotyledonous and dicotyledonous plants (Theologis, 1992).

ACC synthase may also be regulated at the pos-transcriptional level (Chappell et al., 1984; Felix et al., 1991). They found that fungal elicitors added to cultured parsley and tomato cells, respectively, induced ethylene synthesis and ACC synthase activity in the presence of the RNA synthesis inhibitors actinomicin D and cordicepin.

1.2. 1-aminocyclopropane-1-carboxylic acid (ACC)

Adams and Yang (1979) observed that methionine was efficiently converted to ethylene in air while in nitrogen was converted to MTA and ACC. In the presence of

air ACC was rapidly converted to ethylene, indicating that ACC is an intermediate and that the conversion of ACC to ethylene is oxygen dependent.

Lizada and Yang (1979) developed an assay for ACC based on the conversion of ACC to ethylene with NaOCl (a commercial bleach solution) in the presence of Hg^{2+}. Boller et al. (1979) also reported a chemical assay of ACC based on the liberation of ethylene in a two-step reaction with pyridoxal phosphate, $MnCl_2$ and H_2O_2.

1.3. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)

ACC oxidase previously named ethylene-forming enzyme, the enzyme that catalyses the conversion of ACC to ethylene is readily assayed *in vivo* by supplying tissues with its substract (ACC), but its study *in vitro* was delayed because its activity completely disappeared when tissues were homogenised (Yang and Hoffman, 1984; Kende, 1989). ACC oxidase catalyses directly the oxidation of ACC yielding ethylene (Yang and Hoffman, 1984). The internal concentration of ACC which brings about half-maximal ethylene production rate in pea epicotyls was estimated to be 66nmol/g fresh weight (McKeon and Yang, 1982). From the results obtained *in vivo*, it is clear that the reaction is oxygen dependent (Adams and Yang, 1979). The concentration of oxygen which results in half-maximal ethylene production by apple fruit (Burg, 1973) and flower tissue (Konze et al., 1980) was estimated to be about 0.2% and 1% (v/v) respectively.

It has been recognised that various lipophylic compounds which could modify membrane structure, greatly reduced the rate of ethylene synthesis in plant tissues (Odawara et al., 1977; Imaseki and Watanabe, 1978). These observations together with the loss of ACC oxidase activity after homogenisation of tissues leaded to the suggestion that the enzyme required membrane integrity (Lieberman, 1979). This suggestion was supported by the observation that avocado fruits undergo ultrastructural changes in plasma membrane structure (Platt-Aloia and Thomson, 1981) and a loss in ability to produce ethylene at post-climacteric stages (Hoffman and Yang, 1980), and the observation that there was a rapid decline in ethylene production accompanied by a marked increase in electrolyte leakage when bean leaf discs were incubated at high temperature (Field, 1981a). Apelbaun et al. (1981) showed that

TritonX-100 and osmotic shock inhibited ethylene production at the conversion of ACC to ethylene step.

Guy and Kende (1984) found that vacuoles isolated from pea leaves produced 80% of the ethylene evolved by protoplasts. The ACC oxidase activity of isolated vacuoles exhibited the same stereospecificity as did the *in vivo* enzyme. Just as ACC oxidase activity of intact tissues was destroyed by homogenisation, so was the activity of the vacuolar enzyme by lysis of the vacuole (Guy and Kende, 1984; Mayne and Kende, 1986).

ACC oxidase activity has been observed in membrane vesicles in the juice squeezed from the pericarp of ripe kiwifruit (Mitchell et al., 1988). The ACC oxidase activity of kiwifruit membranes showed the characteristics of the enzyme *in vivo* in its stereoselectivity towards isomers of 1-amino-2-ethylcyclopropane-1-carboxilic acid (AEC), its high affinity for ACC, its pattern of development during post-harvest ripening and its sensitivity towards inhibitors. However, only 0.5% of the *in vivo* activity could be recovered *in vitro*. The requirement for vesicle integrity of the kiwifruit enzyme resembles that present in vacuoles isolated from pea mesophyl protoplasts (Guy and Kende, 1984; Mayne and Kende, 1986).

Further work using isolated vacuoles of *Vicia faba* provided evidence that ACC oxidase was associated with the inside face of the tonoplast and that the activity of this enzyme depended on membrane integrity, probably because of the requirement for a transmembrane ion gradient (Mayne and Kende, 1986). Later work showed that ACC oxidase activity in *Vicia faba* was independent of membrane potential, as neither depolarisation nor hyperpolarisation affected ethylene formation (Guy, 1990).

Localisation of ACC oxidase in vacuoles derived from mesophyl protoplasts of *Petunia hybrida* was also reported by Erdmann et al. (1989). These authors also showed that evacuolation of protoplasts eliminated ACC oxidase activity and that the re-formation of the central vacuole restored it. Porter et al. (1986) found that isolation of leaf cells and protoplasts of *Vicia faba* led to a 95% loss of ACC oxidase activity compared with *in vivo* measurements. The same authors reported that vacuoles retained much of the ACC oxidase activity of the protoplasts. They conclude that in

addition to membrane integrity ACC oxidase also required tissue integrity. Bouzayen et al. (1990) studied the subcellular localisation of ACC oxidase and concluded that cells of some plants have an external and internal site of ACC oxidase activity, while cells of other plants have only an internal site. The external site was very sensitive to plasmolysis and appeared to be associated with the plasma membrane. The internal site was not sensitive to plasmolysis, and its localisation was not determined. The above studies indicated that ACC oxidase activity was in some way dependent on membrane and tissue integrity, and that the tonoplast and plasma membrane were likely sites for the localisation of ACC oxidase (Kende, 1993).

On the basis of *in vivo* conversion of ACC to ethylene, ACC oxidase was judged to be constitutive in most instances (Yang and Hoffman, 1984). Increases in ACC oxidase activity were observed in wounded cantaloup fruit tissue (Hoffman and Yang, 1982), citrus leaf discs (Riov and Yang, 1982b), tobacco leaf discs (Chalutz et al., 1984), preclimacteric tomato and cantaloupe fruits in response to applied ethylene (Liu et al., 1985) and avocado pericarp discs (Sitrit et al., 1986). Induction of ACC oxidase was also observed in tomato leaves infected with *Phytophthora infestans* (Spanu and Boller, 1989) and in cultured tomato cells treated with an elicitor isolated from yeast extract (Bouzayen et al., 1991; Felix et al., 1991). On this basis, it was concluded that induction of ethylene biosynthesis is based, in many instances, on enhancement of ACC synthase and ACC oxidase is often constitutive, at least at low level of activity. However, the transcription and activity of ACC oxidase also increases in some plants in response to factors that induce ethylene production (Woodson et al., 1992; Kende, 1993).

ACC oxidase genes were identified before the enzyme could be isolated. The cDNA clone of ACC oxidase pTOM13 had been isolated from a ripe tomato fruit library and coded for a protein of 35 kDa (Slater et al., 1985). Expression studies showed that levels of pTOM13-homologous RNA correlated positively with ethylene evolution in wounded leaves and ripening fruits (Holdsworth et al., 1987) and wounded and senescing leaves (Davies and Grierson, 1989). Transgenic tomato plants transformed with the pTOM13 antisence gene produced very low levels of ethylene supporting the hypothesis that pTOM protein was involved in ethylene biosynthesis (Hamilton et al.,

1990). The ultimate proof that pTOM13 encoded ACC oxidase was given by functional expression of pTOM13 cDNA in *Saccharomyces cerevisiae* (Hamilton et al., 1991) and *Xenopus laevis* oocytes (Spanu et al., 1991).

Several cDNA clones encoding ACC oxidase have been isolated from avocado (McGarvey et al., 1990), carnation petals (Wang and Woodson, 1991), apple (Dong et al., 1992; Ross et al., 1992), *Pseudomonas syringae* (Fukuda et al., 1992), melon (Balague et al., 1993), kiwifruit (MacDiarmid and Gardner, 1993) and pea (Peck et al., 1993). All of them showed a high level of homology to pTOM13.

It was found that the deduced amino acid sequence of pTOM13 exhibited similarity to that of flavanone 3-hydroxylase (Hamilton et al., 1990). Based on this founding, Ververidis and Jonh (1991) extracted and assayed ACC oxidase activity in homogenates of melon fruits under conditions that had been shown to preserve flavanone 3-hydroxylase activity of *Petunia hybrida* petals. The ACC oxidase activity extracted anoxically and assayed in the presence of Fe^{2+} and ascorbate showed similar activity to that observed *in vivo* throughout the course of ripening. Similar ACC oxidase activity *in vivo* and *in vitro* was also reported by Dilley et al. (1993). ACC oxidase activity *in vitro* has also been recovered from apple (Fernandez-Maculet and Yang, 1992; Kuai and Dilley, 1992) and avocado fruits (McGarvey and Christoffersen, 1992). Ververidis (1991) reported that although ACC oxidase can be fully recovered *in vitro* as a soluble enzyme, the involvement of a cell membrane could still be considered as a possible site for ACC oxidase, if the enzyme was to be loosely associated with that membrane, rather than being an integral protein.

Further partial purification and characterisation of ACC oxidase gave a molecular weight of 41kDa by gel filtration, an apparent Km of 60μM, and a pH optimum of about 7.5 (Smith et al., 1992). The region between amino acids 113 to 134 of tomato ACC oxidase shows strong probability for the formation of an amphipatic α-helix containing multiple leucine residues on the hydrophobic site. The presence of this putative leucine zipper is conserved in all known ACC oxidases and may cause binding of ACC oxidase to the particulate fraction in yeast and may also explain earlier results indicating an association of ACC oxidase with plant membranes (Kende, 1993).

Accumulation of mRNA encoding ACC oxidase has been reported during carnation flowers senescence (Woodson et al., 1992), during ripening of apple (Dilley et al., 1993) and avocado (Dopico et al., 1993) and after wounding of melon (Balague et al., 1993).

2. Factors affecting ethylene biosynthesis

A variety of environmental factors such as temperature, drought, salinity, flooding, light, carbon dioxide and oxygen are known to influence ethylene production in plant tissues (Yang and Hoffman, 1984).

It has been recently reported that the plant senses ethylene by a protein kinase cascade and that ethylene sensors are encoded by multigene families with members that are differentially expressed during plant growth and development (Theologis, 1995). Mutations in the ETR1 gene of *Arabidopsis thaliana* confer insensitivity to ethylene, which indicates a role for the gene product in ethylene signal transduction (Schaller and Bleecker, 1995). These authors found that the ETR1 protein acts as an ethylene receptor in *Arabidopsis*.

2.1. Temperature

2.1.1. *Low temperature*

Low temperatures are generally applied for extending the storage life of fruit. Some chilling-sensitive species show marked reduction in ethylene production when they are incubated below a growing temperature of 20° to 25°C (Field, 1981b; Wang and Adams, 1982). In some cases, a cold treatment is absolutely required or advances the induction of ethylene production as it has been observed in pears (Sfakiotakis and Dilley, 1974), cucumbers (Wang and Adams, 1980; 1982), bean leaf (Field, 1981b; 1984), apples (Blankenship and Richardson, 1985; Jobling et al., 1991; Gaudierre and Vendrell, 1993) and tomato (Chen and Shewfelt, 1988). Hyodo and Fukazawa, 1985; Hyodo et al. (1987) and Arpaia et al. (1994a) reported that the time required for kiwifruit to produce ethylene at room temperature becomes much shorter and uniform as the storage period at chilling temperatures (1°-2°C) is extended. The acceleration of

ethylene production can occur during chilling in some fruit (Sfakiotakis and Dilley, 1974; Wang et al., 1985), or only upon transference of the fruit to warm temperatures (Wang and Adams, 1982; Andersen and Kent, 1983; Mencarelli et al., 1983). In some plant tissues ACC synthase is reduced at low temperatures increasing only upon rewarming (Wang and Adams, 1982; Knee, 1987). In others ACC accumulates being the increase in ethylene production observed upon rewarming mainly a consequence of the activation of ACC oxidase activity (Jobling et al., 1991; Gaudierre and Vendrell, 1993). Kiwifruit increased their ACC content in parallel with MACC, ACC oxidase activity and ethylene production only upon rewarming of the fruit (Hyodo et al. 1987). Prolonged chilling can reduce ethylene production upon rewarming by damaging ACC oxidase (Wang and Adams, 1980; 1982; Andersen, 1986).

Gaudiere and Vendrell (1993) reported the accumulation of two polypeptides of about 50 and 55 kD in apples in cold storage. In contrast, a protein of 35kD abundantly synthesised by unchilled fruits, declined significantly in chilled fruits. The same authors referred a possible relationship between the 50 and 55 kD protein with ACC synthase and the 35kD with ACC oxidase.

2.1.2. High temperature

High temperatures are generally applied as heat shock in order to extend storage life. However, this technology if not applied correctly may cause the failure of the fruit to ripe normally when returned to lower temperatures and lead to some physiological disorders (Mitchell, 1986; Pech et al., 1994).

High temperatures inhibit some physiological disorders (Porrit and Lidster, 1978; Lee and Young, 1984; Yakir et al., 1984), respiration and ethylene production (Yu et al., 1980; Lee and Young, 1984; Yakir et al., 1984; Lurie and Klein, 1990), ripening (Mitchell, 1986; Lurie and Klein, 1990) and control decay caused by pathogens (Mayberry and Hartz, 1992). The temperatures at which each one of the physiological processes are inhibited depend on the plant tissue (Maxie et al., 1974; Eaks, 1978; Lee and Young, 1984; Yakir et al., 1984; Klein and Lurie, 1990; Lurie and Klein, 1990).

High temperatures cause the rapid synthesis of a group of proteins known as heat-shock proteins, concomitant with a reduction in the rate of normal protein synthesis

(Czarnecka et al., 1984; Lurie and Klein, 1991). The production of heat-shock proteins confers thermotolerance on the tissue in which they were formed, so that subsequent exposure to higher or lower temperatures which normally will cause damage, will be harmless (Key et al., 1981; Krishman et al., 1989; Lurie and Klein, 1991).

2.2. Oxygen

Oxygen plays an important role in ethylene biosynthesis since it is a co-substrate of ACC oxidase (Pech et al., 1994). When the oxygen levels are reduced ethylene production goes down. However, a significant reduction in ethylene production requires generally O_2 levels lower than 1-1.5% (Marcellin, 1986; Nanos et al., 1992; Metzidakis and Sfakiotakis, 1993). In low-oxygen storage the reduction of ethylene production is mainly due to reduced ACC oxidase activity (Bufler and Bangerth, 1983; Blankenship and Richardson, 1986; Metzidakis and Sfakiotakis, 1993) although in some cases ACC levels and ACC synthase activity are reduced too (Bufler and Bangerth, 1983; Lau et al., 1984).

The Km of the partially purified ACC oxidase towards O_2 is around 6.4 μM (Kuai and Dilley, 1992). Low oxygen also reduces the expression of ethylene-regulated genes involved in fruit ripening (Kanellis et al., 1990).

Reduction of oxygen concentration (<10%) inhibited the effectiveness of propylene on autocatalytic ethylene production and ripening in kiwifruit (Stavroulakis and Sfakiotakis, 1997).

2.3. Carbon dioxide

High CO_2 levels are considered as competitive inhibitors of ethylene action and therefore limit the autocatalytic induction of ACC synthase (Buffler, 1984; 1986). Most of the effects of CO_2 on delaying fruit ripening are thought to be mediated through ethylene (Sisler and Wood, 1988).

Carbon dioxide concentrations up to 10% at 20°C notably reduced ethylene production rates of kiwifruit but did not affect ACC levels (Rothan and Nicolas, 1994). However, in the presence of saturating amounts of ACC, ethylene was stimulated by high CO_2

levels. The same authors found that the concentrations of ACC at which ACC oxidase was inhibited by high CO_2, are comparable to the concentrations found in the fruit. So, they suggested that the reduction of ethylene production in fruits caused by high CO_2 might be exerted at least partially at the level of the conversion of ACC to ethylene. However, it was found that CO_2 was required for ACC oxidase activity *in vitro* with an optimum around 2% (Dong et al., 1992; Smith et al., 1992; Yang et al., 1993). In kiwifruit pericarp tissue, the maximum ethylene production occurred at 0.2 to 2% CO_2 (Rothan and Nicolas, 1994). The level of CO_2 in fruits is generally high enough to allow full expression of ACC oxidase activity (Pech et al., 1994).

3. Fruit ripening

Fruit ripening involves a serial of biochemical and structural changes that make the fruit acceptable for eating. Tissue softening generally accompanies fruit ripening and is due to the dissolution of cell walls (mainly the pectins of the middle lamella) which results in ripening associated changes in cell wall polysaccharides (Rattanapanone et al., 1977). There is also an increase in soluble solids content (SSC) during fruit ripening. This increase can be due to the conversion of starch to soluble sugars and/or a release of cell wall bound neutral sugars (Ahmed and Labavitch, 1980; Chen et al., 1983). Chlorophyll usually degrades with fruit maturity and ripening and thus the carotenoids contribute more to the colour (Goodwin, 1976). Organic acids are important sources of respiration energy in the plant cell and flavour and aroma compounds, particularly in fruits (Zagory and Kader, 1989). Kiwifruit is considered to be eating-ripe when firmness is <1kgf and SSC ≥14% Brix (Lallu et al., 1989).

3.1. Climacteric and non-climacteric fruit

Fruit have been classified as either climacteric or non-climacteric depending on their respiration behaviour during ripening after the fruit matures (Biale and Young, 1981).

Non-climacteric fruit exhibit a fairly steady respiration rate during ripening and often change slowly as they ripen (Abeles, 1973). Treatment of non-climacteric fruit with ethylene causes an unnatural climacteric-like respiration increase (which subsides on removal of ethylene), but not an increase in endogenous ethylene.

Climacteric fruits are characterised by a surge of ethylene production at the onset of ripening, and it is recognised that ethylene plays an essential role in the ripening process (Pratt and Goeschl, 1969). Climacteric fruit shows a decrease in respiration rate as the fruit matures (the pre-climacteric minimum) followed by a large increase during ripening (Abeles, 1973). This is accompanied by marked changes in composition and texture, and finally a decrease in respiration rate and ethylene as the fruit enters a senescent decline. Treatment with sufficient concentration of ethylene in the pre-climacteric stage induces the climacteric response and ethylene production and this ripening process is irreversible after the endogenous ethylene increases beyond a threshold level of about 1µl/l.

McMurchie et al. (1972) found that propylene, an ethylene homologue, induced ethylene production in bananas (climacteric type) but not in citrus fruit (non-climacteric type). The same authors suggested two systems involved in ethylene biogenesis: System I, which is involved in the regulation of ageing processes and is associated with the low rate of ethylene production during growth and is present in climacteric and non-climacteric fruit, and system II which is responsible for the autocatalytic increase in ethylene production during ripening, inherent to climacteric fruit.

3.2. Respiration

The fruit respires at the expenses of substrates accumulated during fruit development. The presence of respiration enzymes and their substrates indicates that respiration will continue after harvest and storage products will be broken down (Burton, 1982). Within a given fruit species, a high respiration rate is commonly associated with a short storage life (Blanke, 1991). The rate of respiration is mostly temperature dependent and this relationship is expressed as the temperature coefficient (Q_{10}) (Blanke, 1991).

Chilling of fruits retards respiration by inhibiting the cytochrome oxidase pathway, leading to a larger contribution of the cyanide-insensitive pathway (Graham and Patterson, 1982). The increase in temperature increases respiration until a limit (dependent on fruit specie and time of exposure) above which heat injury occurs (Eaks, 1978; Lee and Young, 1984; Yakir et al., 1984; Inaba and Chachin, 1988; 1989).

4. Post-harvest storage physiology

4.1. Effect of temperature

The rates of progression of all vital processes, including respiration, ripening, moisture loss and decay, increase with a rise in temperature (McDonald, 1990).

The two post-harvest environmental factors that have the greatest effect on kiwifruit softening are temperature, which influences the rate of flesh softening, and the presence of ethylene during storage, which can hasten the softening process at a given temperature by approximately 50% (Arpaia et al., 1994b). The flesh firmness of kiwifruit (and therefore storage life) in CS or CA decreases with time, at a rate dependent on storage temperature (Arpaia et al., 1986; McDonald, 1990). Kiwifruit is usually stored at 0°C. A storage temperature below -0.5°C should be avoided for kiwifruit, in order to maintain an adequate margin of safety above the freezing point, which is -1.7 to -2.1°C (McDonald, 1990; Arpaia et al, 1994a).

4.2. Effects of controlled atmosphere (CA) and ultra low oxygen storage (ULO)

When cooling reaches the low temperature sensitivity limit of a particular fruit, storage and shelf-life can be prolonged by changing the ambient gas composition (Smock, 1979). Oxygen participates in respiration by the mediation of enzymes which can transfer electrons to it, the terminal oxidases (Blanke, 1991). Partial lowering of the oxygen level may first slow down respiration (Tucker and Laties, 1985). Oxygen concentrations below 2-5% may cause a switch to anaerobic processes such as fermentation and alcohol formation which reverse the reduction in respiration by producing CO_2 temporarily and raising its concentration to levels above those in air (Burton, 1982; Tucker and Laties, 1985). Fermentation may result in free radical formation and collapse, damaging the fruit tissue (Blanke, 1991).

The CO_2 effect results from reduced internal ethylene production and ethylene action in the fruit, thereby delaying fruit ripening and softening (Blanke, 1991). Elevating the CO_2 concentration can also have a suppressive effect on respiration metabolism, depending upon temperature, commodity and cultivar (Zagory and Kader, 1989).

The CA storage is used commercially for kiwifruit to allow greater flexibility for export marketing (McDonald, 1990; Arpaia et al., 1994b). The CA storage using a combination of 2%O_2+5%CO_2 has been shown to slow kiwifruit softening and development of decay (Arpaia et al., 1994b). Successful CA storage depends on rapid establishment (less than 1 week following harvest), careful temperature maintenance (0°C), exclusion of ethylene at all times during storage, and close attention to O_2 and CO_2 concentrations.

Although lowering the oxygen level will increase storage life, hypoxic conditions may cause injuries in plant tissues (Alpi et al., 1985). Hypoxic conditions or levels of CO_2 above the limits of tolerance of a particular commodity can result in accumulation of acetaldehyde and ethanol within the tissues indicating a shift to anaerobic respiration (Ke et al., 1990). Nanos et al. (1992) found high levels of ethanol and acetaldehyde, inhibition of loss of greenness and decreases in ethylene and CO_2 production in pears stored in 0.25% O_2 atmosphere. Thomai and Sfakiotakis (1997) reported that kiwifruit stored in ULO (O_2<1%) did not ripe normally during shelf-life.

4.3. Fruit softening

The kiwifruit is nonchilling sensitive and possesses a potential six months storage life at 0°C although softening will occur during this period (Arpaia et al., 1980; McDonald and Harman, 1982; Arpaia et al., 1986). Softening is accelerated by the presence of ethylene and reduced under CA conditions. Kiwifruit should be harvested when they are mature but hard and unripe, at a flesh firmness of 7-10 kgf (McDonald, 1990).

Atmospheres containing 4 to 10% CO_2 had an increased effect in retarding kiwifruit softening at 0°C, but additional CO_2 above 10% had no further effect on fruit firmness (McDonald and Harman, 1982). During shelf-life at 20°C, only fruit stored previously in a low O_2 atmosphere were firmer than air-stored fruit, being the advantages of the high CO_2 in air atmospheres been lost during the ambient storage period (McDonald, 1990).

4.4. Flesh colour

Elevated CO_2 and/or low O_2 levels reduce chlorophyll loss in many fruits and vegetables (Weichman, 1986). High cellular pH caused by elevated CO_2 may reduce the breakdown of chlorophyll to pheophytin (Zagory and Kader, 1989). Lowered sensitivity of plant tissues to ethylene in the presence of elevated CO_2 and/or low O_2 is presumably partly responsible for the reduced chlorophyll breakdown (Zagory and Kader, 1989). The CA reduced the loss of green colour in kiwifruit compared to CS (Harman and McDonald, 1983; 1989).

4.5. Soluble solids content (SSC)

Kiwifruit are normally fairly high in starch when harvested (5-8%) and this starch disappears in storage with a concomitant increase in SSC (Arpaia et al., 1980). A level of 6.2% Brix SSC at harvest is a suitable minimum maturity level for kiwifruit (Harman, 1981) and a minimum of 14% Brix is required for best consumer acceptance (Mitchell et al., 1991). The CA storage of $2\%O_2+5\%CO_2$ does not influence the increase in SSC as compared to CS (Arpaia et al., 1984; 1986; 1994b; Harman and McDonald, 1989).

5. Lipids in connection to ripening and ethylene

5.1. Lipid composition

Some treatments, such as acclimatisation, that increase the resistance of plant tissues to chilling injury also increase the percentage of unsaturated fatty acids in membrane lipids (Forney, 1990). An increase in fatty acids unsaturation during cold storage has been observed in potato and tomato (Spychalla and Desborough, 1990; Whitaker, 1994). However, Parkin and Kuo (1989) found little changes in fatty acids composition of cucumbers between control and chilled fruit.

Fatty acid unsaturation decreased slightly during ripening in apple, cucumber and tomato (Lurie and Ben-Arie, 1983; Parkin and Kuo, 1989; Whitaker, 1994) while it increased in 'Honey dew' muskmelons due to the increase of palmitoleic and oleic acids and coincidentally with the decrease in chilling sensitivity (Forney, 1990).

Brackmann et al. (1993) found the amount of fatty acids in the peel of apples to increase with storage time.

Only a small increase in linoleic acid was observed in chill-sensitive plants after hardening (Wilson and Crawford, 1974a). The degree of unsaturation of fatty acids associated with the phospholipid fraction increased during hardening of *Gossypium hirsatum* and *Phaseolis vulgaris*, but the treatment produced no effect on the fatty acid composition of the glycolipid fraction, agreeing with previous results that showed no increase in unsaturation of total leaf fatty acid on hardening (Wilson and Crawford, 1974b).

A decrease in the proportion of the unsaturated fatty acids was observed in chilling injured tomato plants (Senaratna et al., 1988). Linolenic and linoleic acid decreased and there was an increase in saturated fatty acid content. There appears to be contradicting evidence as to the role of fatty acid peroxidation in chilling injury (Senaratna et al., 1988). Peroxidation of fatty acids occurs at the double bonds of unsaturated fatty acids and the resulting peroxides and hydroperoxides degrade to smaller molecules. Consequently the relative proportion of unsaturated fatty acids decreases (Frankel, 1980).

Analyses of each lipid class in peach showed that the predominant fatty acids were palmitic and linoleic (Izzo et al., 1995). The double bond index showed a general increase during maturation being the main changes observed between the climacteric and postclimacteric stages.

5.2. Membrane permeability

Electrolyte leakage is a parameter that has often been used to indicate physical damage to the plasmalemma resulting from low-temperature stress (Senaratna et al., 1988; Parkin and Kuo, 1989). The same authors found substantial increases in electrolyte leakage only after fruit displayed visible signs of chilling injury. Membrane permeability of potato tubers was higher in storage at 3°C than at 9°C (Spychalla and Desborough, 1990). Peppers increased their membrane permeability, as measured by electrolyte leakage, as fruit approached the climacteric in consequence of senescence (Lurie and Ben-Yehoshua, 1986).

5.3. Lipids possibly related to chilling-induced ethylene production

Ethylene is often used as an indicator of stress. The decrease in fatty acid unsaturation precedes increases in rates of ethylene evolution, indicating that lipid peroxidation may be responsible for creating stress conditions in chilled cucumber fruit (Parkin and Kuo, 1989).

Since it was observed that lipid peroxidation increases when plant tissues are subjected to cellular damage (Galliard, 1978) or undergoing senescence (Dhindsa et al., 1981), it was suggested that the lipoxigenase activity is involved in the biosynthesis of ethylene under situations like senescence (Bousquet and Thirman, 1984), wounding or low-temperature stress (Kacperska and Kubacka-Zebalska, 1985). Although lipoxygenase may increase during the above-mentioned situations, considering the lack of affinity of the lipoxygenase system for the ACC and the low ACC concentration present in the plant tissue (<0.1 mM), it is very unlikely that the lipoxygenase system may produce ethylene to any substantial extent *in vivo* (Wang and Yang, 1987).

Bousquet and Thimann (1984) and Wang and Yang (1987) described an *in vitro* model system in which ACC was rapidly converted to ethylene in the presence of linoleic acid, pyridoxal-phosphate, manganese ion and lipoxygenase. The rate of ethylene production by the lipoxygenase system was highest during the first hour, gradually decreased thereafter, and essentially stopped after 24 hours (Wang and Yang, 1987). However, during the 24 hours of lipoxigenase-mediated reaction, 5% of ACC was converted to ethylene, whereas about 45% of the ACC was converted into other products, indicating that in this system the ACC reaction was not specific for ethylene production. Linoleic and oleic acid incubation enhanced ethylene production in oat leaves by enhancing ACC uptake, explaining their promotive effect on ethylene formation by the lipoxygenase system (Wang and Yang, 1987). Pirrung (1986) reports that the model system for ethylene biosynthesis developed by Bousket and Thimann (1984) has mechanistic features outwardly similar to those in plants. However, many of its characteristics concerning inhibitors and the function of individual components do not agree. So, caution should be exercised concerning any cell-free ethylene forming system requiring pyridoxal phosphate or manganese.

Gardner (1995) reports that lipoxygenase is activated by stress conditions and linoleic and linolenic acids are its preferable substrate. It has been suggested that stress ethylene is a direct product of lipid peroxidation (Mattoo et al., 1986). Linoleic and oleic acids incubation enhanced ethylene production in oat leaves by enhancing ACC uptake, explaining their promotive effect on ethylene formation by the lipoxygenase system (Wang and Yang, 1987). Wise and Naylor (1988) demonstrated that even under severe photooxidative stress, accompanied by lipid peroxidation, ethylene is synthesised exclusively by the ACC-dependent pathway. However, in the special case of copper toxicity, in which Cu+n plays several catalytic roles (Sandmann and Boger, 1980), ethylene can be shown to arise directly from lipid peroxidation (Mattoo et al., 1986).

Some biological effects of methyl jasmonate/jasmonic acid studied recently include ethylene biosynthesis (Chou and Kao, 1992; Sanz et al., 1993). Gene expression induced by methyl jasmonate has been compared with similar promoters, such as abcissic acid, desiccation, wounding and sucrose (Lorbeth et al., 1992; Mason et al., 1992; Reinbothe et al., 1992).

CHAPTER 3. THERMOREGULATION OF AUTOCATALYTIC ETHYLENE PRODUCTION AND RESPIRATION CLIMACTERIC INDUCED BY PROPYLENE IN RIPENING 'HAYWARD' KIWIFRUIT

ABSTRACT

It is known from a previous study that there is an inhibition of propylene induced ethylene production in kiwifruit bellow a critical temperature range of 11-14.8°C (Stavroulakis and Sfakiotakis, 1993). This work was undertaken to find whether kiwifruit behaves as a climacteric or non-climacteric fruit at temperatures above and below the critical temperature range for ethylene production.

In one experiment, 'Hayward' kiwifruit were treated continuously with 130μl/l propylene, with interruptions in propylene application and air free of propylene, at 10 and 20°C. In the other experiment, fruit were treated with concentrations of 0, 100, 400 and 1000μl/l propylene at the same temperatures. Ethylene production, respiration, and changes of firmness, SSC and flesh colour were measured.

Kiwifruit kept at 20°C in air started autocatalysis of ethylene production after they reached a threshold level of 0.2μl/kg/h in 19 days with a concomitant increase in respiration. Treating kiwifruit continuously with 130μl/l propylene stimulated autocatalysis of ethylene after 79 hours. Their respiration rate increased immediately after exposure to propylene, reached a maximum in 24 hours, slightly decreased, increased again with the climacteric rise in ethylene and decreased slightly thereafter. The application of 130μl/l propylene for 24 hours was enough to induce autocatalysis of ethylene production after a lag period of 68 hours. In this case, respiration decreased after removal of propylene, resuming with ethylene autocatalysis.

At 20°C, ethylene production and the respiration rise appeared earlier with increased propylene concentrations. The ethylene climacteric peaks were achieved after 180 hours in all propylene treatments and were lower at 100μl/l treatment than at 400 or 1000μl/l, which presented similar values. The respiration peak was increased with increasing propylene concentrations.

Kiwifruit at 10°C did not show autocatalysis of ethylene production. At 10°C, fruit exposed continuously to 130µl/l propylene increased their respiration rate reaching a maximum after 56 hours and remained almost constant thereafter. When propylene was removed, respiration was decreased resuming upon re-exposure. Respiration rate was increased together with propylene concentration.

Fruit in air did not ripe in 240-260 hours at any temperature. At 10°C, fruit to which propylene was removed for two periods were not completely ripe after 240-260 hours, while fruit treated continuously with propylene were ripe.

It is concluded that kiwifruit stored at 20°C behaves as a typical climacteric fruit, while at 10°C behaves like a non-climacteric fruit.

INTRODUCTION

Fruit are classified as climacteric and non-climacteric. Climacteric fruit are characterised by an increased rate of respiration that occurs at an early stage in the ripening process which is associated with a similar pattern of increased ethylene production. Non-climacteric fruit do not show any increase in respiration and ethylene during ripening (Biale and Yang, 1981). The application of exogenous ethylene to non-climacteric fruit results in an increased respiration rate proportional to the concentration of ethylene applied and declines to basal levels upon removal of the ethylene. In climacteric fruit the respiration peak is independent of the applied ethylene concentration. The main effect of applied ethylene, in this case, is the advancement in time of the fruit's respiration climacteric, this effect being proportional to the concentration of applied ethylene (Tucker and Grierson, 1987). The difference is caused by the lack of autocatalytic ethylene synthesis in non-climacteric fruit. The application of ethylene to a climacteric fruit, providing it is mature enough, will advance the onset of the climacteric. Once autocatalytic synthesis is triggered, ethylene levels will increase so that the final respiration rate is independent of the original exogenous ethylene concentration. The absence of any endogenous ethylene from non-climacteric fruit means that its respiration rate is proportional to the concentration of applied ethylene. Non-climacteric fruit have the ability to produce low levels of

ethylene, about 0.04μl/kg/h throughout ripening and can be induced to produce more ethylene under stress conditions. Although non-climacteric fruit do not produce autocatalytic ethylene, they respond to exogenous ethylene advancing ripening like climacteric fruit (Tucker and Grierson, 1987).

McMurchie et al. (1972) postulated two systems for ethylene production in fruit. System I makes background and wound ethylene, while system II is responsible for autocatalysis. Climacteric fruit have both systems I and II, while non-climacteric fruit have only system I.

Kiwifruit has been classified as a climacteric fruit whose ripening is mediated by ethylene (Pratt and Reid, 1974; McDonald and Harman, 1982; Arpaia et al., 1986; Arpaia et al., 1994a). Reid et al. (1982) have suggested that kiwifruit should be classified as a climacteric fruit because of the simultaneous increase in respiration rate and ethylene production. However, Pratt and Reid (1974) and Arpaia et al. (1994a) reported that the increased rates of respiration and ethylene production in kiwifruit do not occur until the fruit are full ripe, making it different from most climacteric fruit where ethylene production and ripening are coincident.

Ikoma et al. (1995) referred that ripening of kiwifruit seemed different from that of a typical climacteric fruit. The ripening-related ethylene production in kiwifruit rarely occurred for a long time after harvest without the help of exogenous ethylene. In addition, kiwifruit was less sensitive to ethylene for autocatalytic ethylene production than the other climacteric fruit. Yano and Hasegawa (1993a) found that when fruit were packed individually, most sound fruit did not start evolving ethylene till 40 to 60 days. The same authors postulated that the ethylene production in sound kiwifruit requires an exogenous induction factor such as ethylene gas or a diseased fruit nearby.

Arpaia et al. (1994a) reported that kiwifruit placed at 20°C after harvest take about 17±7 days to ripe. Ethylene production rate by mature kiwifruit at harvest is very low (0.1 to 1.0 μl/kg/h) and increases markedly with ripening from 50 to 100μl/kg/h. Hyodo and Fukasawa (1985) observed the autocatalytic production of ethylene in kiwifruit at 21°C after exceeding a threshold level of 0.1μl/kg/h. Sfakiotakis et al. (1989) reported the same autocatalytic ethylene production and ripening at 20°C after

external application of 130μl/l of propylene, which is equivalent to 1μl/l ethylene. The same authors found that propylene caused ripening of the fruit at 10 and 0°C at a slower rate, although it was not able to induce autocatalytic ethylene production. Stavroulakis and Sfakiotakis (1993) reported a critical temperature of 11 to 14.8°C below which ethylene production induced by propylene does not occur in kiwifruit. However, respiration was not assessed as well as the response to several propylene concentrations.

The objective of this study was to find whether kiwifruit behaves as climacteric or non-climacteric fruit, based on the studies of Stavroulakis and Sfakiotakis (1993) and the main parameters which classify a fruit as climacteric or non-climacteric: ripening, respiration and ethylene production. It was investigated the effect of temperatures above and below the critical range (11-14.8°C) for ethylene production induced by propylene on autocatalysis of ethylene production, respiration and ripening of kiwifruit.

MATERIAL AND METHODS

1. Plant material and treatments

1.1. Study on kinetics of temperature-associated inactivation and reactivation of ethylene and carbon dioxide production during 'Hayward' kiwifruit ripening

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 7.3 kgf flesh firmness and 5.9 (% Brix) SSC. After selection for uniformity of size and freedom from defects, 30 fruit were used the same day for analysis of quality parameters and the remaining were put in 5-litre jars through which a continuous, humidified, air stream was passed at a rate of 100ml/min. Each set of six jars was kept in a separate water bath at a constant temperature of 10 and 20°C. Experiments were set within 24 hours. The experimental design was a two-factor experiment distributed in a complete randomised design with the temperatures as first factor, propylene treatment as second and the jars as replications. Each treatment consisted of 4

replications with 15 fruit per replication. Fruit were kept in the air stream at the mentioned temperatures for 24 hours before propylene treatments started. The first treatment consisted of air free of propylene (control) and the second air+130μl/l propylene applied continuously. At 10°C, the third treatment consisted of air+130μl/l propylene applied for 24 hours, then switched to air for 48 hours, again to air+130μl/l propylene for 24 more hours and finally transferred to air, while at 20°C it consisted of air+130μl/l propylene applied for 24 hours, then fruit were transferred to air.

1.2. Study on propylene concentration-dependent ethylene and carbon dioxide production of 'Hayward' kiwifruit

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 9.9 kgf flesh firmness and 5.0 (% Brix) SSC. After selection for uniformity of size and freedom from defects, fruit were analysed and experiments were set as described above. The first treatment consisted of air free of propylene, the second air+100μl/l, the third air+400μl/l and the fourth air+1000μl/l propylene applied continuously. Each treatment consisted of 4 replications with 15 fruit per replication. Treatments were distributed in a complete randomised design as mentioned above.

2. Measurements

2.1. Ripening parameters

From the ripening parameters were measured firmness of flesh and core separately, SSC and green colour of flesh. Ripening parameters were performed within 24 hours after harvest and at the end of the experiment. A Minolta Chrome meter CR-200 was used to measure flesh colour using the a* value, whereby a change to more positive values indicates fading of the green colour. The SSC (% Brix) were measured by a digital Atago refractometer in juice from the equatorial zone of the fruit. Firmness was recorded by puncture with a Chatillon penetrometer fitted with a flat 8mm tip. The tip was inserted after skin removal, at the fruit equator to a depth of 7mm for flesh and 20mm for core firmness measurements.

2.2. Gas analyses

Ethylene and CO_2 production measurements started 1 hour after the experiment was installed and proceeded at regular intervals of 12 to 24 hours for ethylene and 2 to 8 hours for CO_2. Measurements were more often when fruit were transferred from air to propylene and vice-versa. Ethylene measurements were performed by withdrawing a 1ml-headspace gas-sample with a syringe and injecting it into a Varian 3300 gas chromatograph, equipped with a stainless steel column filled with Porapak, length 100cm, diameter 0.32cm, at 50°C and a flame-ionisation detector at 120°C. The carrier gas was N_2 at a flow rate of 20ml/min. Respiration was measured as CO_2 production automatically by an infrared gas analyser connected to a computer, in the gas phase of the jars.

3. Calculations and Statistical analysis

The Km values that correspond to the propylene concentration which causes half-maximal effect on CO_2 and ethylene production were calculated on the basis of the Lineweaver-Burk plot (Christensen and Palmer, 1967).

Statistical analysis were made with a SAS computer program. Two-way analyses of variance - ANOVA, Least Significant Difference and Duncan's Multiple-Range Test (a=0.05) for comparisons between treatments over time were conducted.

RESULTS

1. Study on kinetics of temperature-associated inactivation and reactivation of ethylene and carbon dioxide production during 'Hayward' kiwifruit ripening

1.1. Ethylene production at 20°C

Kiwifruit harvested at an early stage of maturity showed a clear climacteric pattern of ethylene production at 20°C (Fig. 3.1). Autocatalysis of ethylene production started

after the fruit reached a threshold level of 0.2µl/kg/h in 19 days (Fig. 3.1Aa). The climacteric of ethylene production followed a typical pattern reaching a peak in 24 days with almost a 20,000 fold increase of the rate of ethylene production (Fig. 3.1A).

Treating kiwifruit during 24 hours or continuously with propylene (130µl/l) stimulated autocatalytic ethylene production after the fruit reached a threshold level of 0.2-0.5µl/kg/h in 68 and 79 hours respectively (Fig. 3.2Aa). Values of both treatments were not significantly different till 170 hours (Fig. 3.2A). After 170 hours, fruit exposed continuously to propylene had significantly higher ethylene production than fruit exposed for only 24 hours. Fruit not treated with propylene did not produce ethylene during the experiment.

1.2. Carbon dioxide production at 20°C

Respiration of kiwifruit not treated with propylene did not change significantly before autocatalysis of ethylene production, then had a 2.5 fold increase of the rate of CO_2 and this was closely associated with the increase of ethylene production (Fig. 3.1).

Kiwifruit increased significantly their respiration rate immediately after they were exposed to 130µl/l propylene at 20°C, reaching a maximum in 24 hours (Fig. 3.2B). Respiration slightly decreased thereafter to a minimum in 55 hours, had again an increase coincident with the climacteric rise in ethylene production and then decreased slightly through time. When propylene was removed respiration decreased significantly, increased again with the commencement of autocatalysis of ethylene production and decreased slightly thereafter. Kiwifruit not treated with propylene did not change their respiration pattern through time.

1.3. Ethylene production at 10°C

Fruit at 10°C did not show autocatalysis of ethylene production in none of the treatments (Fig. 3.3A). Although differences in ethylene production were statistically significant higher in air+130µl/l propylene than in the other treatments after 170 hours, they are negligible when compared with autocatalysis of ethylene production at 20°C (Fig. 3.2A).

1.4. Carbon dioxide production at 10°C

At 10°C, respiration of kiwifruit started to increase significantly after 10 hours exposure to propylene, reached a maximum after 56 hours and remained almost constant thereafter (Fig. 3.3B). Fruit kept continuously in air free of propylene did not significantly change their respiration rate through time.

When propylene was removed from the stream after 24 hours application, CO_2 production decreased significantly and then remained constant (Fig. 3.3B). However, values were significantly higher than in fruit continuously in air. Re-exposing the fruit to propylene resulted in a significant increase in respiration to values close to those of fruit kept continuously with propylene. By removing again propylene, respiration rate decreased significantly.

1.5. Associated changes of firmness, soluble solids content (SSC) and flesh colour

Kiwifruit firmness of flesh and core decreased significantly with time in all treatments except in air at 10°C (Table 3.1). Fruit treated with propylene showed much lower values than fruit in air at both temperatures. Fruit treated continuously with propylene or fruit where propylene was removed for some periods showed similar values at 20°C. However, at 10°C, values were significantly higher in fruit where propylene was removed for some periods than in fruit exposed continuously to propylene. Firmness was significantly higher at 10 than at 20°C within each treatment, except for flesh firmness of fruit treated continuously with propylene were values were similar at both temperatures.

At 10°C, SSC and fading of flesh green colour did not change significantly with time in air, but were significantly higher in fruit continuously in propylene followed by fruit where propylene was removed for some periods (Table 3.1). At 20°C, SSC and fading of flesh green colour increased significantly with time in all treatments. After 240 hours storage, values were similar in both propylene treatments and significantly higher than in air treatment (Table 3.1).

2. Study on propylene concentration-dependent ethylene and carbon dioxide production of 'Hayward' kiwifruit

2.1. Ethylene production at 20°C

Kiwifruit treated with 1000µl/l propylene at 20°C started autocatalytic ethylene production after reaching a threshold level of 0.5µl/kg/h in 24 hours of exposure, while 400µl/l and 100µl/l treatments needed 72 hours to start it and a threshold level of 0.4µl/kg/h (Fig. 3.4Aa). Ethylene production was significantly higher in 1000µl/l propylene than in the other treatments for the first 120 hours of exposure to propylene. After this, its values became similar with the 400µl/l propylene treatment (Fig.3.4A). Values of the 100µl/l propylene treatment were significantly lower than the other propylene treatments except at the end of the experiment. The peak in ethylene production occurred after 180 hours exposure to propylene for all treatments. Fruit not treated with propylene did not produce ethylene during the experimental time.

2.2. Carbon dioxide production at 20°C

The respiration rate of kiwifruit at 20°C increased significantly when fruit were treated with propylene reaching the respiration peak after 55 hours exposure in all treatments (Fig. 3.4B). Carbon dioxide production increased with increased propylene concentration till 120 hours exposure. After that, values were similar in all propylene treatments, coinciding with the increase in ethylene production (Figs. 3.4A and B). Respiration increased earlier in fruit treated with 1000µl/l propylene followed by 400µl/l and 100µl/l (Fig. 3.4B). Carbon dioxide production of kiwifruit from 1000µl/l treatment was significantly higher than that of fruit from 400µl/l treatment between 5 and 30 hours of exposure to propylene and was significantly higher than 100µl/l treatment between 5 and 120 hours. Fruit kept continuously in air free of propylene did not significantly change their respiration rate through time.

2.3. Ethylene production at 10°C

Propylene concentration did not induce autocatalysis of ethylene production at 10°C (Fig. 3.5A). Although there was statistically significant increase in ethylene production

after 100 hours exposure to propylene, its values were negligible when compared with autocatalysis of ethylene production at 20°C (Fig. 3.4A and 3.5A).

2.4. Carbon dioxide production at 10°C

At 10°C, the respiration rate of kiwifruit increased significantly with the increase in propylene concentration, showed a wide peak and remained almost constant after 200 hours exposure (Fig. 3.5B). Fruit not treated with propylene almost did not change their respiration rate during the experiment. Fruit treated with 1000µl/l propylene showed significantly higher CO_2 production than fruit treated with 100µl/l after 30 hours of exposure. Respiration of fruit in 400µl/l propylene was always lower than fruit of 1000µl/l treatment with significant differences between 120 and 200 hours of exposure. Fruit in 100µl/l propylene had significantly lower CO_2 production than the other propylene treatments.

2.5. Calculated Km values for propylene induced ethylene and CO_2 production

At 20°C, the ethylene production peak had a great increase from 0 to 100µl/l propylene with little further effect at higher concentrations (Fig. 3.6A). The calculated Km which gives half-maximal ethylene production induced by propylene at 20°C was 33.3µl/l (Fig. 3.6Aa).

Maximum CO_2 production increased with propylene concentration mostly from 0 to 400µl/l and had higher values at 20 than at 10°C (Fig. 3.6B). The calculated Km values were 83.9 and 37.6µl/l propylene at 10 and 20°C, respectively (Fig. 3.6Bb).

2.6. Associated changes of firmness, soluble solids content (SSC) and flesh colour

Kiwifruit not treated with propylene did not significantly change firmness, SSC and flesh green colour in air free of propylene at 10°C during the experiment (Table 3.2). After 260 hours at 10°C fruit significantly decreased their firmness when treated with propylene, showing similar values of flesh firmness in all propylene treatments. The firmness of the core was significantly decreased with propylene concentration. The SSC and loss of flesh green colour of kiwifruit at 10°C were significantly higher after 260 hours propylene treatment than at harvest, but were not significantly different among propylene concentrations (Table 3.2).

Fruit significantly decreased their firmness, increased their SSC and lost flesh green colour with time in all treatments at 20°C (Table 3.2). Values were similar in all propylene concentrations and had great differences from air.

Firmness was significantly higher at 10°C than at 20°C in all treatments (Table 3.2). The SSC and fading of flesh green colour did not have significant differences between temperatures in air but were significantly higher at 20 than at 10°C in the propylene treatments.

DISCUSSION

Kiwifruit showed a typical climacteric pattern at 20°C by autocatalitically increasing ethylene production after reached a threshold level of ethylene production of 0.1-0.2µl/kg/h in approximately 19 days, in agreement with previous work (Pratt and Reid, 1974; Hyodo and Fukasawa, 1985; Arpaia et al., 1994a). By increasing the concentration of propylene, kiwifruit showed earlier the respiration rise and autocatalysis of ethylene but similar values in the peaks as shown for typical climacteric fruit (Tucker and Grierson, 1987).

Brady (1987) and Tucker and Grierson (1987) reported that the increase in respiration during the climacteric appears to be a consequence of the increase in endogenous ethylene because, in most fruit, respiration climacteric occurs after autocatalysis of ethylene production (Tucker, 1993). However, in some fruit the burst of ethylene either coincides with or, more rarely, follows the respiration climacteric (Tucker, 1993). Kiwifruit not treated with propylene at 20°C showed an increase in respiration coincident with the autocatalysis of ethylene production. Kiwifruit treated continuously with propylene showed an immediate increase in respiration, a small decrease and a new increase coincident with the start of autocatalysis of ethylene production. When propylene was removed after 24 hours exposure, respiration decreased and resumed again only when autocatalysis of ethylene production started after 70 hours. This last observation is typical of a nonclimacteric fruit or a climacteric fruit that lacks the maturity to ripe (Brady, 1987) and is in the system I of ethylene production (McMurchie et al., 1972; Sfakiotakis and Dilley, 1973; Sfakiotakis et al., 1989). The

above observations are in accordance with the concept that the respiration increase during ripening result simply as a general response to the ethylene produced by the fruit or the application of exogenous ethylene (Tucker, 1993).

It was previously observed that kiwifruit strongly inhibits its ethylene production (induced by propylene) at temperatures below 11-14.8°C (Stavroulakis and Sfakiotakis, 1993). We observed that by treating kiwifruit with propylene at 10°C the respiration rate increased proportionally to propylene concentration, and ethylene production was very low not showing autocatalysis. This is characteristic of non-climacteric fruit (Tucker and Grierson, 1987; Tucker, 1993). When propylene was removed from kiwifruit after 24 hours exposure at 10°C, CO_2 production decreased, resumed upon re-exposure to propylene after 72 more hours and declined again when propylene was removed after 48 hours. This behaviour is similar with that of non-climacteric fruit (Brady, 1987; Tucker and Grierson, 1987).

The results of this study on autocatalytic ethylene production are compatible with the concept that two systems of ethylene production are involved in the ripening process of climacteric fruit. System I represents the low level of ethylene present in climacteric fruit before the onset of ripening and in non-climacteric fruit and system II represents the autocatalytic burst of ethylene production which accompanies the ripening process unique to climacteric fruit (McMurchie et al., 1972; Sfakiotakis and Dilley, 1973). Sfakiotakis et al. (1989) suggested that the application of propylene or ethylene in pre-climacteric fruit changes the status from system I to II. In kiwifruit temperature plays an important role since low temperatures as 10°C strongly inhibit the conversion from system I to II. However, by altering temperature in avocado fruit, only a moderated reduction on autocatalytic ethylene production was found (Metzidakis and Sfakiotakis, 1993), suggesting that the controlling mechanism of ethylene production in kiwifruit and avocado is different.

Yang et al. (1986) suggested the existence in pre-climacteric fruit of a 'ripening inhibitor' which prevents the affinity of ethylene to bind to the receptors and/or the increase in the receptors. According to this concept the low level of system I ethylene produced by the climacteric fruit plays an essential role by accelerating the destruction of the 'ripening inhibitor'. When the ethylene-receptor complex reaches a critical

concentration, it induces the synthesis of both ACC oxidase and ACC synthase resulting in autocatalytic system II ethylene production, and the synthesis of other enzymes associated with ripening. It has been recently reported that the plant senses ethylene by a protein kinase cascade and that ethylene sensors are encoded by multigene families with members that are differentially expressed during plant growth and development (Theologis, 1995). Low temperatures as 10°C seem to interfere with the destruction of the 'ripening inhibitor' or the expression of genes responsible for the formation of ethylene sensors in kiwifruit preventing the conversion of system I to system II.

Respiration of kiwifruit showed to be sensitive to propylene as reported by other authors (Given, 1993; Yano and Hasegawa, 1993b). Besides, respiration rate was higher at 20 than at 10°C. This agrees with Arpaia et al. (1994a) who reported that kiwifruit respiration is low starting at about 3 to 7mg/kg/h at 0°C and has a temperature coefficient (Q_{10}) near 3.0.

The propylene concentration that allows for a half-maximal CO_2 production (Km) showed values of 83.9µl/l at 10°C and 37.6µl/l at 20°C. According to the concept of Christensen and Palmer (1967), in kiwifruit the process saturates for propylene concentrations of 839µl/l at 10°C and 376µl/l at 20°C. These results indicate that as temperature increases the propylene concentration that gives maximal CO_2 production decreases. The Km for ethylene production was 33.3µl/l propylene at 20°C showing that the process saturates at propylene concentrations of 333µl/l.

Treating kiwifruit with propylene at 10°C advanced ripening similar to non-climacteric fruit (Tucker and Grierson, 1987). Kiwifruit at 10°C, in which propylene was removed for some periods, did not completely ripe since ripening was caused only by external propylene. At 20°C, fruit were equally ripe when continuously in propylene or when propylene was applied for only 24 hours because both induced internal autocatalysis of ethylene production. Since at 10°C fruit ripened in response to external propylene but there was no induction of autocatalysis of ethylene production, the present work suggests that ripening and ethylene biosynthesis in kiwifruit are regulated by two independent mechanisms, in agreement with Stavroulakis and Sfakiotakis (1993).

The present study suggests that kiwifruit stored at room temperature behaves as a typical climacteric fruit in reference to respiration and ethylene production, while at temperature low as 10°C it behaves like a non-climacteric fruit.

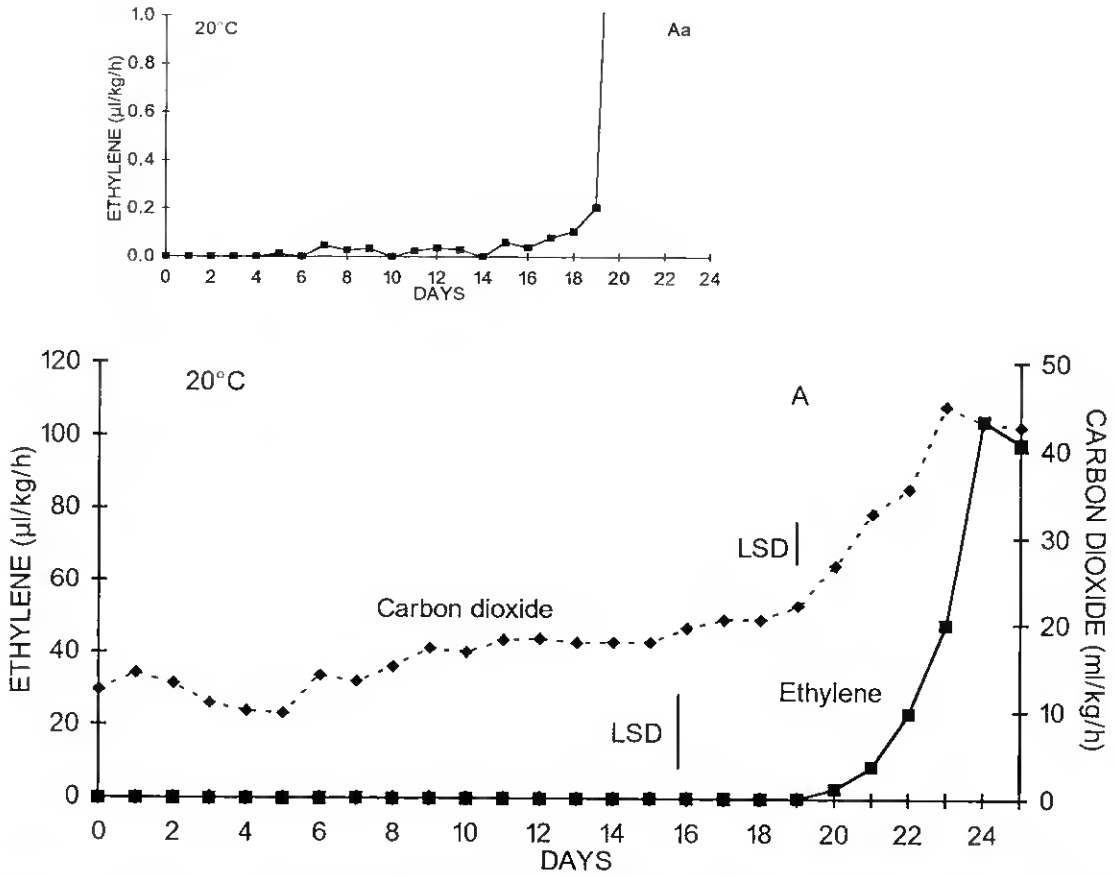
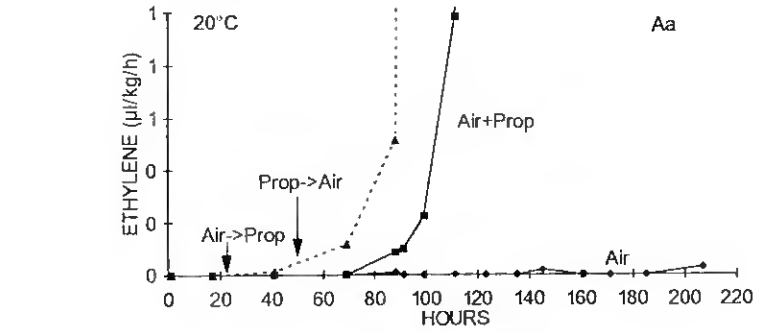
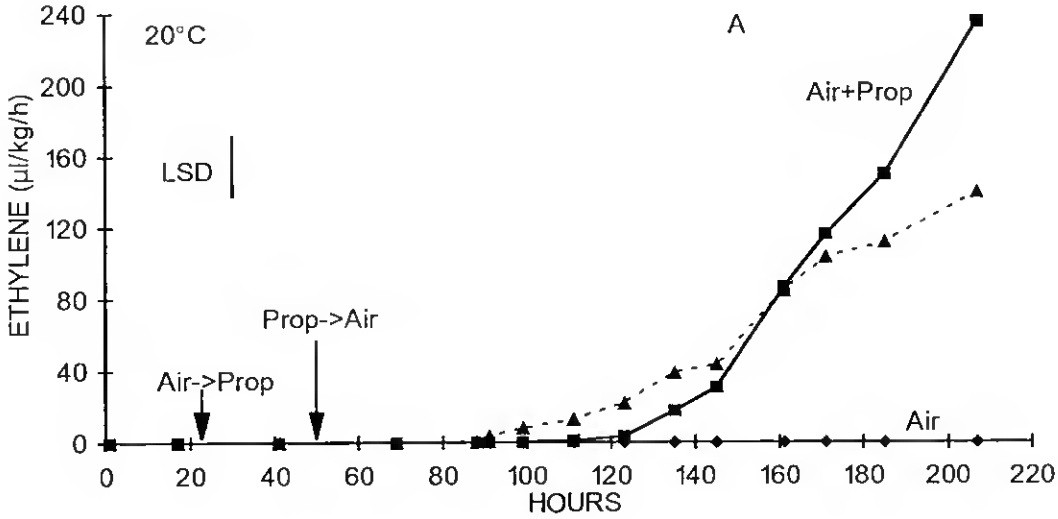


Figure 3.1. Ethylene and CO_2 production of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.





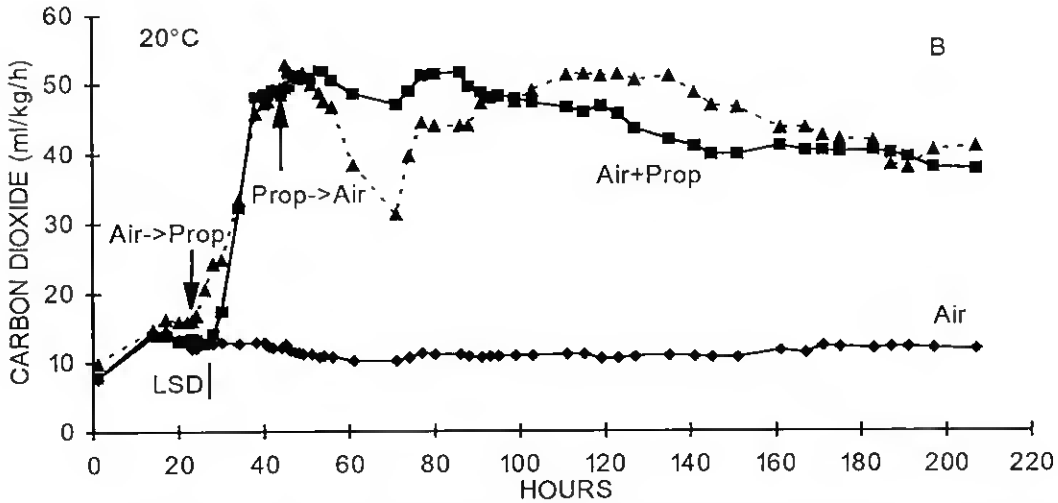


Figure 3.2. Time course study of ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept at 20°C in a continuous, humidified, air stream, air+130µl/l propylene continuously and air+130µl/l propylene for only 24 hours. The arrows represent the switch from air to propylene (Air->Prop) and from propylene to air (Prop->Air). The first arrow shows when propylene treatments started after the setting of the experiment. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis. LSD at α=0.05.

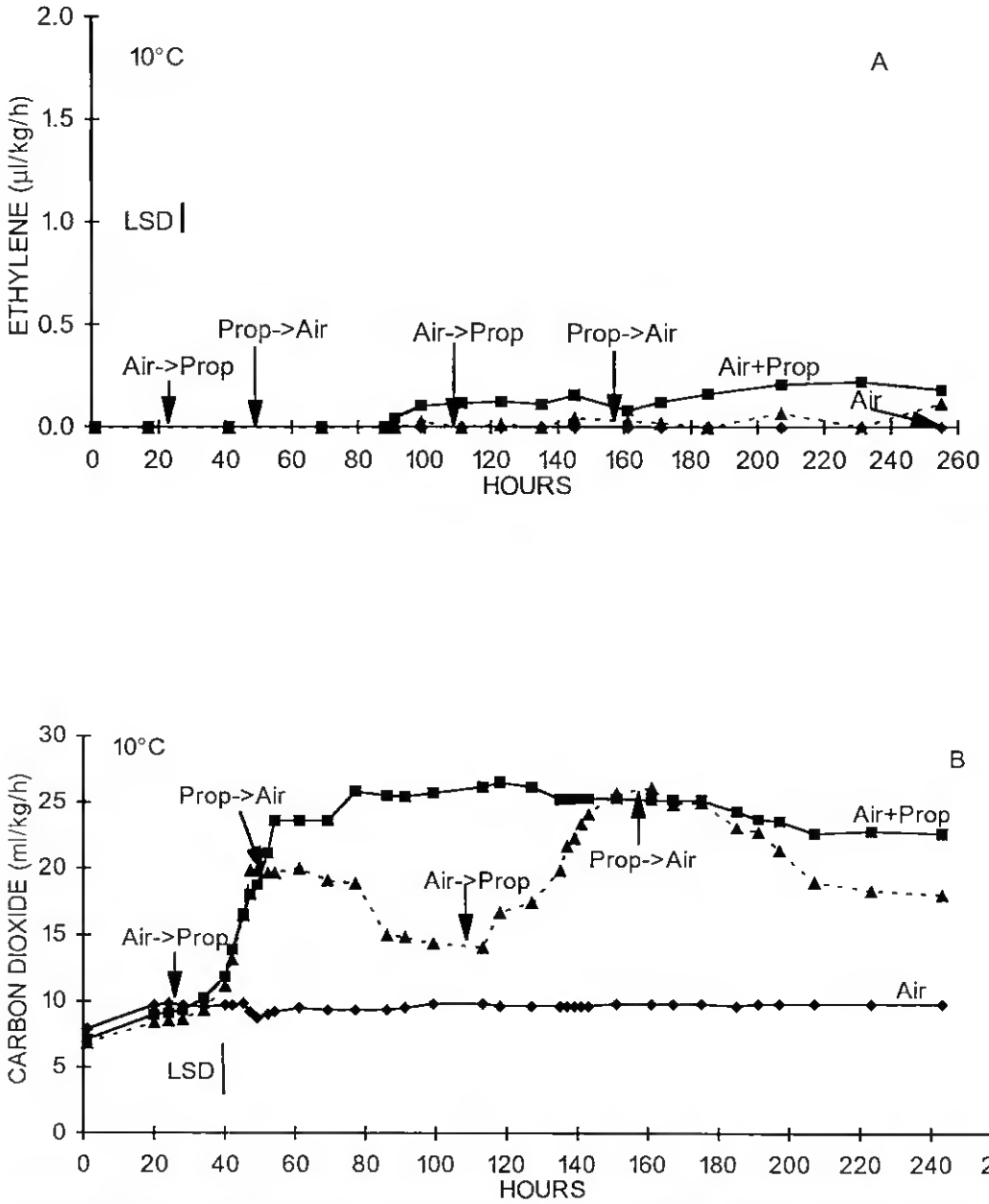
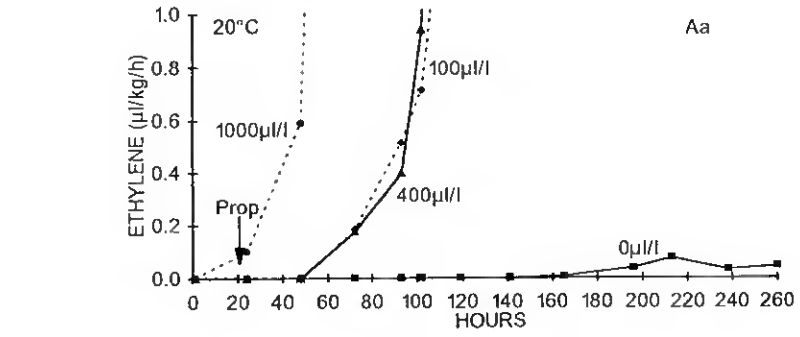
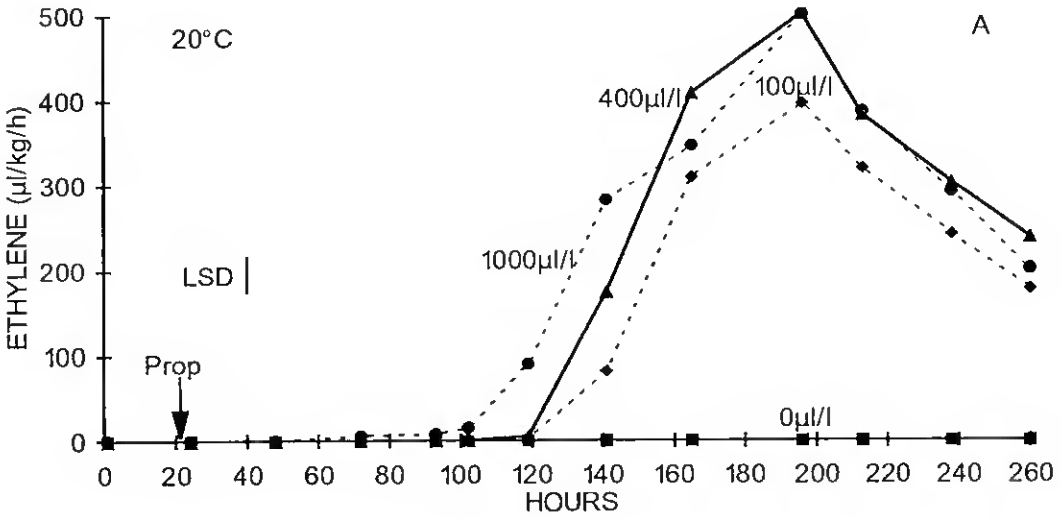


Figure 3.3. Time course study of ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept at 10°C in a continuous, humidified, air stream, air+130µl/l propylene continuously and air+130µl/l propylene only for 24 hours, then transferred to air for 48 hours, in air+130µl/l propylene for 24 more hours and finally transferred to air. The arrows represent the switch from air to propylene (Air->Prop) and from propylene to air (Prop->Air). The first arrow shows when propylene treatments started after the setting of the experiment.

LSD at α=0.05.





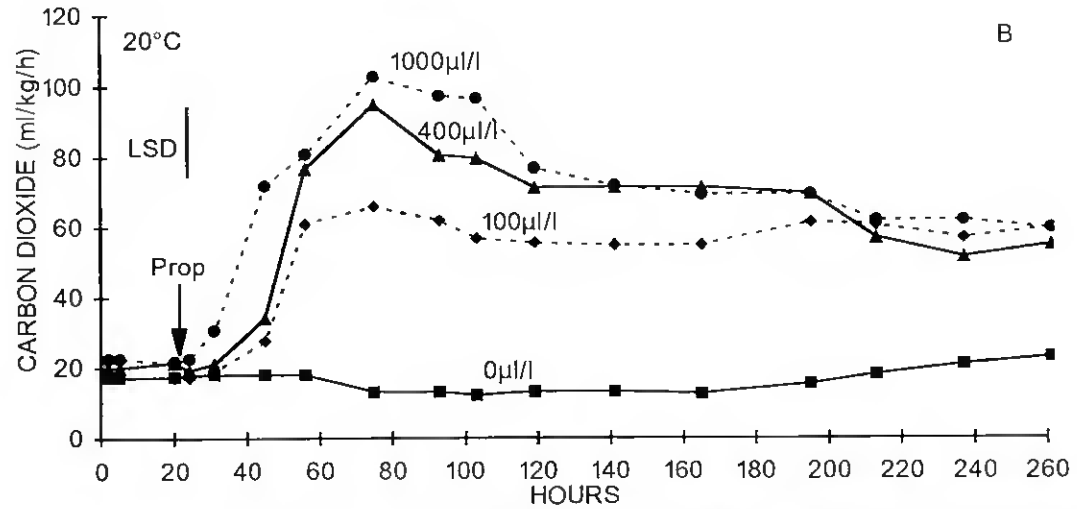


Figure 3.4. Ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept at 20°C in a continuous, humidified, air stream with different propylene concentrations: 0, 100, 400 and 1000µl/l. The arrow shows when propylene treatments (Prop) started after the setting of the experiment. The graph Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.

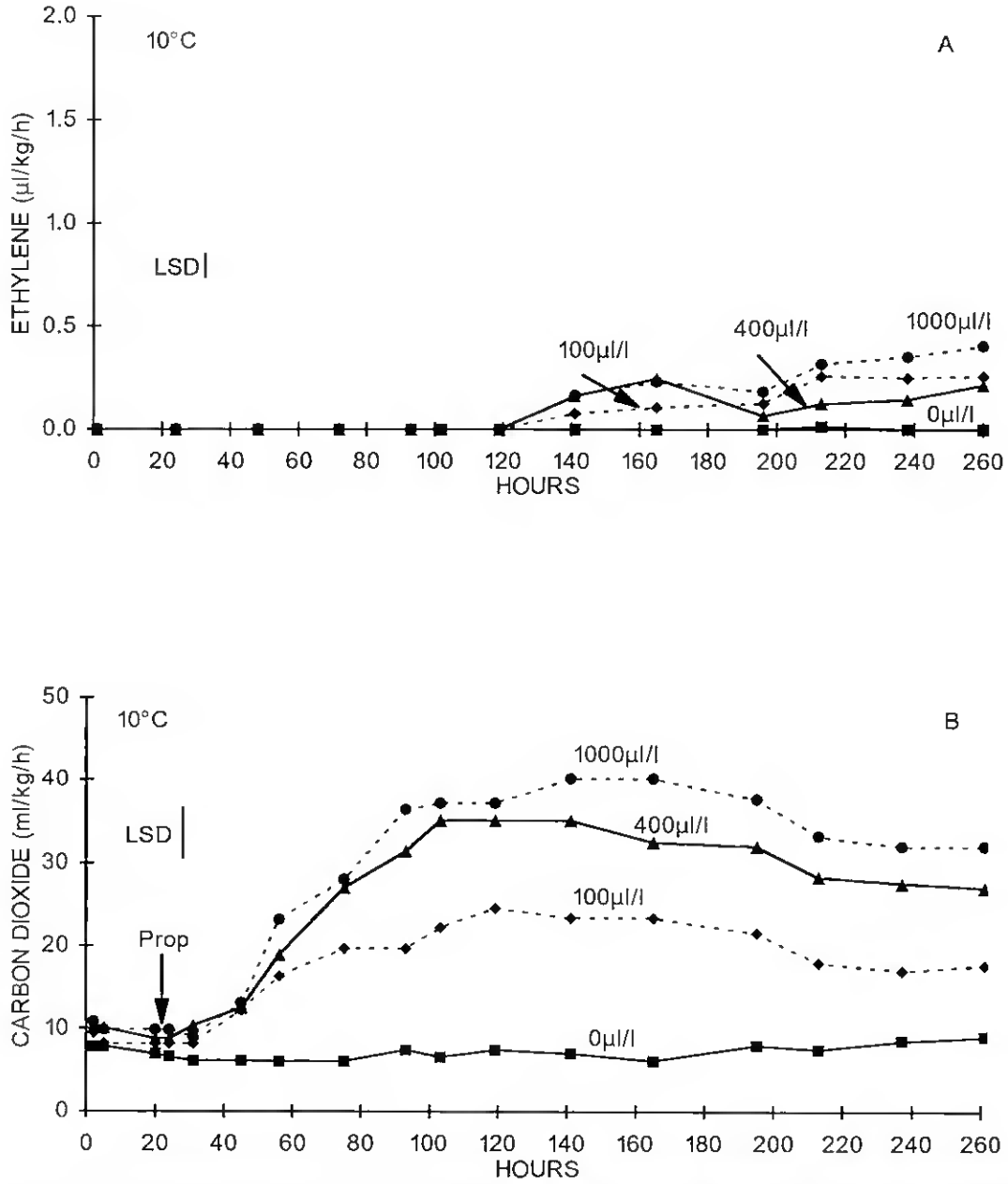


Figure 3.5. Ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept at 10°C in a continuous, humidified, air stream with different propylene concentrations: 0, 100, 400 and 1000µl/l. The arrow shows when propylene treatments (Prop) started after the setting of the experiment.
LSD at α=0.05.

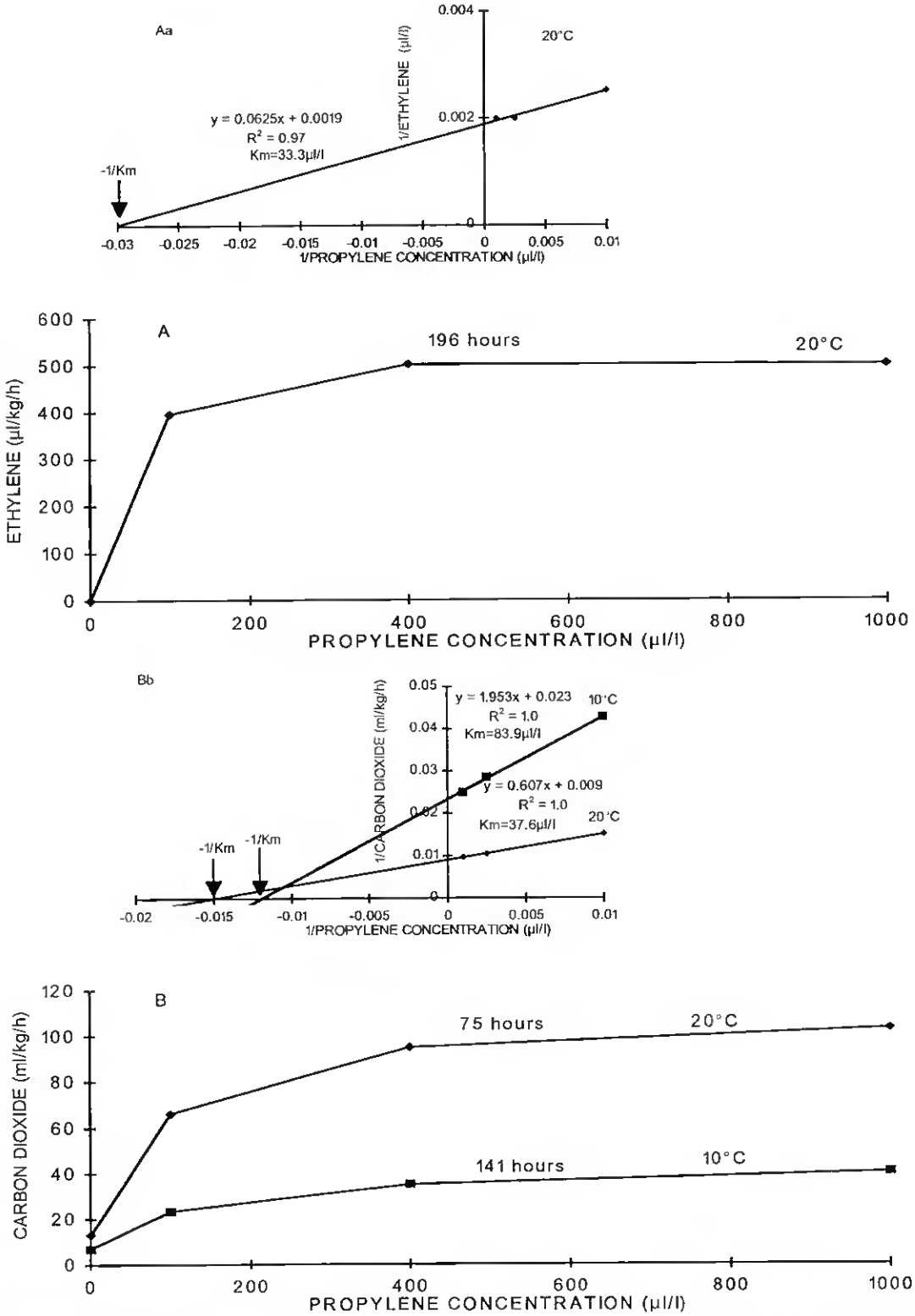


Figure 3.6. Effect of propylene concentration on ethylene production at 20°C (A) and CO_2 production at 10 and 20°C (B) of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream. Values used correspond to the maximum production during the experiment. The graphs Aa and Bb represent the Lineweaver-Burk plot of graphs A and B respectively in order to calculate the propylene concentration that corresponds to the half-maximal (Km) ethylene and CO_2 production.

Table 3.1. Ripening parameters of 'Hayward' kiwifruit at harvest and after 240 hours at 10 and 20°C under a continuous, humidified, air stream (Air), air+130µl/l propylene continuously (Propylene) and air+130µl/l propylene for 24 hours followed by air for 48 hours, air+130µl/l propylene for 24 hours and finally transferred to air (Prop->Air).

Hours	Temperature (°C)	Treatments	Flesh firmness (kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0 (Harvest)			7.25 a**	16.60 a	5.92 e	-17.94 d
240	10	Air	6.62 a	15.93 a	6.57 de	-17.83 d
		Prop->Air	1.89 c	4.84 c	10.53 c	-16.35 c
		Propylene	0.92 d	3.82 d	12.46 b	-14.68 b
	20	Air	5.93 b	15.19 b	7.39 d	-16.21 c
		Prop->Air	0.36 d	0.44 e	13.53 a	-11.04 a
		Propylene	0.38 d	0.46 e	13.42 ab	-10.77 a

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

Table 3.2. Ripening parameters of 'Hayward' kiwifruit at harvest and after 260 hours at 10 and 20°C under a continuous, humidified, air stream, air+100µl/l, air+400µl/l, and air+1000µl/l propylene.

Hours	Temperature (°C)	Propylene (µl/l)	Flesh firmness (Kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0 (Harvest)			9.97 a**	17.03 a	5.50 c	-19.07 e
260	10	0	9.07 a	16.63 a	5.13 c	-18.64 e
		100	1.83 c	6.40 c	8.87 b	-17.79 c
		400	1.50 c	4.77 d	9.07 b	-18.09 cd
		1000	1.27 cd	3.20 e	9.60 b	-17.82 c
	20	0	7.53 b	15.90 b	5.80 c	-18.48 de
		100	0.40 d	0.63 f	10.80a	-12.69 ab
		400	0.40 d	0.57 f	10.90a	-12.32 a
		1000	0.37 d	0.57 f	11.23a	-13.00 b

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

CHAPTER 4. EFFECT OF LOW TEMPERATURE ON THE PROPYLENE INDUCED AUTOCATALYTIC ETHYLENE BIOSYNTHESIS, RESPIRATION AND RIPENING OF 'HAYWARD' KIWIFRUIT

ABSTRACT

Stavroulakis and Sfakiotakis (1993) reported that kiwifruit lacks the ability for autocatalysis of ethylene production below 11-14.8°C due to low ACC availability, rather than ACC oxidase activity. We showed in chapter 3 that kiwifruit has a climacteric pattern above the mentioned temperature range and a non-climacteric pattern below it. The aim of this research was to identify the biochemical basis of inhibition of the propylene-induced ethylene production in kiwifruit, below the above mentioned critical temperature range.

'Hayward' kiwifruit were treated with 130μl/l propylene and air free of propylene at 10 and 20°C. Ethylene and CO_2 production, ACC content and ACC synthase and ACC oxidase activities, as well as changes in SSC and flesh firmness were measured during a period of 312 hours. Northern blot hybridisation using specific probes for ACC synthase and ACC oxidase were performed with total RNA, 192 hours after the commencement of the experiment.

Kiwifruit treated at 20°C with propylene, resulted in induced ripening and ethylene production. Ripening proceeded immediately after propylene treatment, while ethylene autocatalysis needed a lag period of 72 hours. The latter event was attributed to the delay found in the induction of ACC synthase activity and consequently to the delayed increase of ACC content. In contrast, propylene treatment induced ACC oxidase activity with no lag period. Moreover, transcription of ACC synthase and ACC oxidase genes was only active in ethylene-producing kiwifruit at 20°C. Respiration rate in propylene-treated fruit at 20°C increased with almost no lag period and showed a climacteric peak between 72 and 91 hours, while ethylene production showed a climacteric peak 220 hours after the initiation of the propylene treatment.

Contradictory, kiwifruit treated at 10°C with propylene, resulted in a strong inhibition of ethylene production which was attributed to the low activities of both ACC synthase and ACC oxidase as well as the low initial ACC level. Interestingly, fruit treated with propylene at 10°C appeared to be able to transcribe the ACC oxidase but not the ACC synthase gene. However, propylene induced ripening of that fruit with almost the same rate found for the propylene-treated fruit at 20°C.

It should be noted that during the whole experimental period (312 hours) the control fruit (treated with air free of propylene) showed no ripening or ethylene production at either 10 or 20°C.

We propose that the main reasons for the inhibition of the propylene induced (autocatalytic) ethylene production in kiwifruit at low temperature (≤10°C), are primarily the suppression of the propylene-induced ACC synthase gene expression and the possible post-transcriptional modification of ACC oxidase.

INTRODUCTION

The onset of ripening in climacteric fruit is marked by a burst of ethylene production. Autocatalytic ethylene production in climacteric fruit can be caused by application of exogenous ethylene above a threshold level (Abeles, 1973). Hyodo and Fukasawa (1985) and Sfakiotakis et al. (1989) observed the autocatalytic production of ethylene in kiwifruit at 20-21°C after exceeding a threshold level of 0.1µl/kg/h. It is not clear if the threshold level causes ripening or stimulates ethylene production to physiologically active levels, which in turn cause fruit ripening (Sfakiotakis et al., 1989).

Kiwifruit is very sensitive to the exogenous application of ethylene or propylene (McDonald and Harman, 1982; Arpaia et al., 1986; Sfakiotakis et al., 1989). However, contrarily to most fruit, ethylene autocatalysis induced by propylene proceeded in kiwifruit at 20°C with a lag period relatively long (80 hours), while ripening started within a short period (<20 hours), suggesting that for autocatalysis of ethylene production there is a requirement for *de novo* synthesis rather than the activation of pre-existing enzymes (Stavroulakis and Sfakiotakis, 1995).

Stavroulakis and Sfakiotakis (1993) reported that kiwifruit is an unique climacteric fruit which, at temperatures below a critical range of 11-14.8^{o}C, lacks the ability for autocatalysis of ethylene production even when induced by propylene, the limiting factor being the ACC availability rather than the ACC oxidase activity. Once induced the autocatalysis at temperatures above 11-14.8^{o}C, low temperature reduces ethylene production by reducing the rate of the enzymatic reaction by itself.

Ethylene biosynthesis is strictly regulated, and is induced by a number of different signals, including mechanical trauma, pathogen infection, auxins and developmental factors in senescing flowers and ripening fruit (Yip et al., 1992). Tucker (1993) reported that the conversion of methionine to SAM, which is used in other biochemical pathways, is considered to be constant throughout the development and ripening of the fruit. Thus, the two key control enzymes for the biosynthesis of ethylene are ACC synthase and ACC oxidase. The conversion of SAM to ACC, catalysed by ACC synthase, is generally regarded as the rate limiting step (Yip et al., 1992). Moreover, Kende and Boller (1981) and Yoshii and Imaseki (1982) reported that ACC synthase is a labile enzyme with an apparent half-life of about 0.5 hours. Thus, the activity of ACC synthase in the tissue is regulated by both the synthesis and the decay of the enzyme (Yang and Hoffman, 1984). Though ACC oxidase is expressed constitutively in most tissues, its synthesis increases during ripening in tomato (Picton et al., 1993).

ACC synthase is encoded by multigene families in all species examined, and differential regulation of the individual genes has been reported (Huang et al., 1991; Olson et al., 1991). Multiple ACC oxidase genes have been isolated from both tomato and petunia, and these also are differentially regulated (Tang et al., 1994; Barry et al., 1996).

The mechanisms of initiation, regulation and the co-ordination of the diverse biochemical changes during ripening are not yet well understood. However, they must rely on an interplay between the regulation of gene expression and enzyme activity (Tucker, 1993).

The objective of the present work was to identify the biochemical basis of inhibition of the propylene-induced ethylene production in kiwifruit, below the above mentioned

critical temperature range. It was investigated the effect of temperatures below and above the critical range for ethylene production (11-14.8°C) reported by Stavroulakis and Sfakiotakis (1993) on ethylene autocatalysis induced by propylene, ACC synthase and ACC oxidase activities, ACC content and CO_2 production, as well as changes in SSC and flesh firmness. Also, isolation of RNA and northern blotting using the complementary probes for ACC synthase and ACC oxidase transcripts was done to identify if low temperature exerts its inhibitory effect on these enzyme genes at the transcription level.

MATERIAL AND METHODS

1. Plant material and treatments

Kiwifruit (cv. Hayward) were harvested from a commercial orchard in Pieria-North Greece with 10.0 kgf flesh firmness and 6.7 (% Brix) SSC. After selection for uniformity of size and freedom from defects, fruit were placed in 5-litre jars through which a continuous, humidified, air stream with 130μl/l propylene or air free of propylene was passed at a rate of 100ml/min. Each set of six jars was kept in a separate water bath at a constant temperature of 10 and 20°C. Experiments were set within 24 hours. The experimental design was a two-factor experiment distributed in a complete randomised design with the temperatures as first factor, propylene treatment as second and the jars as replications. Each treatment consisted of 4 replications with 30 fruit per replication.

At intervals of 0, 48, 120, 192, 240 and 312 hours, 6 fruit per replication were removed from the jars and ACC content, ACC synthase and ACC oxidase (*in vivo*) activities, flesh firmness and SSC were measured. Ethylene and CO_2 production were measured at intervals of 24 hours. Total RNA extraction and Northern blotting were performed 192 hours after the experiment started.

2. Measurements

2.1. Gas analysis and ripening parameters

Gas analysis (ethylene and CO_2 production) and ripening parameters (flesh firmness and SSC) were measured as described in chapter 3.

2.2. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) activity

The ACC synthase was extracted from flesh tissue without seeds and its activity was assayed by the method described by Bufler (1984) with the following modifications: 150mM Tricine-KOH (pH 9.0) were used and 0.5mM PMSF and 150μM Iodoacetate were added in the extraction buffer, in order to bring the pH of the extract to 8.5 and to inhibit the protease actinidin, respectively. The extract was filtered through 4 layers of cheesecloth and centrifuged for 20 min at 10700g. Aliquots of enzyme preparation, after sephadex G-50 column separation, were assayed in the presence and absence of 50μM SAM. One unit of ACC synthase activity was defined as the formation of 1 nmol of ACC/2hrs at 30°C.

2.3. 1-aminocyclopropane-1-carboxilic acid (ACC) content

For determination of ACC levels in the tissue, after the centrifugation step, aliquots (0.4μl) of the homogenate prepared for the ACC synthase assay were analysed directly by the method of Lizada and Yang (1979) in accordance with Kende and Boller (1981).

2.4. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activity

The ACC oxidase activity was determined *in vivo* by measuring the conversion of added ACC to ethylene (Cameron et al., 1979). Three cylinders of 0.5g (7mm diameter) flesh tissue without seeds were cut and incubated for 1.5 min, under vacuum, in vials containing 350 mM manitol in the presence or absence of 1mM ACC. The excess solution was removed, the vials were sealed, and gas samples were taken after 30 min at 20°C for ethylene analysis by gas chromatography.

2.5. RNA extraction

Total RNA was isolated from flesh tissue without seeds based on the method of Slater et al. (1985).

Five grams of frozen kiwifruit flesh (free of seeds) were homogenised using a mortar and pestle in a 5ml solution of 6% (w/v) p-amino salicylic acid, 1% (w/v) Tri-isopropyl naphthalene sulphonic acid - sodium salt (TNS), 5% (v/v) phenol reagent and 50mM Tris-HCl (pH 8.5). Five ml of phenol/chloroform solution were also added.

The homogenate was spun at 10K in a SS34/MSE rotor for 15 min. The supernatant was transferred to new tubes and re-extraction of the organic phase occurred once more with homogenising buffer. Nucleic acids were precipitated with 0.1 volume of 3M NaOAc and 2 volumes ethanol at -20°C for 1h, then spun for 10 min at 5K. Supernatant was discarded and the pellet washed with 80% ethanol. The pellet was re-suspended in 1ml of sterile water and extracted with the same volume of 2x cetyltrimethylammonium bromide (CTAB) extraction buffer (2% (w/v) CTAB, 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0 and 1.4M NaCl). Two volumes of precipitation buffer (1% (w/v) CTAB, 50mM Tris-HCl pH 8.0 and 10mM EDTA pH 8.0) were added. The precipitate was then spun down at 7.5K for 45 min at 9°C.

The pellet was then re-suspended in 1ml of 1x CTAB extraction buffer and the same volume of precipitation buffer was added. The solution was spun at 7.5K for 20 min, pellet was re-suspended in 2ml sterile water and precipitated with an equal volume of 6M LiCl at -20°C for 1.5 hours. Following, it was spun at 7.5K for 20 min and the pellet re-suspended in 300µl of sterile water. A 0.1volume of 3M NaOAc and 2 volumes of ethanol were added and precipitation occurred at -20°C overnight.

Spun occurred in a microfuge at full speed for 15 min and pellet was washed with 80% ethanol. The RNA pellet was re-suspended in 100µl sterile water and quantified by measuring its absorbance at 260nm. Concentration was adjusted to 2µg/µl and then stored at -80°C.

2.6. Preparation of radioactively labelled nucleic acid probes

The ACC synthase probe was a 311bp cDNA (KWACC1) from ethylene-treated kiwifruit (Ikoma et al., 1995). It was used the whole insert gently ceded by Dr. Ioshino from the Fruit Tree Research Station, Okitsu-Japan. The ACC oxidase probe used was a 1230bp cDNA clone (pMEL1) from a climacteric melon fruit (Balague et al., 1993). The insert used was from the 378 to 1079bp. Both KWACC1 and pMEL1 cDNA inserts were labelled using a nick-translation kit (Amersham) in the presence of (α-^{32}P) dATP and (α-^{32}P) dCTP, as described by the manufacturer and used as probes.

2.7. Northern blot hybridisation

Total RNA (10µg) was fractionated on a formaldehyde-agarose gel before blotting onto a Hybond-N membrane (Amersham) (Sambrook et al., 1989). The membrane was then baked at 80°C for 2 hours to fix the RNA. Prehybridisation and hybridisation were performed with the Church buffer (1M $NaHPO_4/NaH_2PO_4$ pH 7.0) (Church and Gilbert, 1984) at 52°C for ACC synthase and 55°C for ACC oxidase. Prehybridisation occurred for 30 min and hybridisation overnight. The blots were washed three times with a solution of 0.15M NaCl+15mM sodium citrate NaOH pH 7.0, 0.1% SDS, for 20 min at the hybridisation temperature. After drying, the blots were exposed to X-ray films with an intensifying screen at -80°C.

3. Statistical analysis

Statistical analysis were performed as described in the methods of chapter 3.

RESULTS

1. Firmness

Flesh firmness of kiwifruit treated with propylene decreased significantly during the first 120 hours at both 10 and 20°C (Fig. 4.1A). Fruit softened to a ripe value (<1kgf) in 220 hours at 10 and in 120 hours at 20°C. Although firmness of fruit treated with

propylene was always higher at 10 than at 20°C, differences were not statistically significant.

At both 10 and 20°C, fruit not treated with propylene did not ripe during the experiment showing a flesh firmness significantly higher than the propylene treatments (Fig. 4.1A). During the experiment, although fruit at 20°C did not completely ripe they decreased significantly their flesh firmness, while at 10°C firmness remained almost constant.

2. Soluble solids content (SSC)

The SSC of fruit treated with propylene increased significantly reaching a ripe value (14% Brix) after 120 hours at 20°C and 240 hours at 10°C (Fig. 4.1B). Values were significantly lower at 10 than at 20°C.

In air free of propylene, kiwifruit did not show significant changes of SSC at any temperature (Fig. 4.1B).

3. Ethylene production

For fruit kept at 20°C, autocatalysis of ethylene production was induced by propylene after the fruit reached a threshold level of ethylene production of 0.2µl/kg/h and with a lag period of 72 hours (Fig. 4.2Aa). For fruit kept in air at 10 and 20°C and for fruit treated with propylene at 10°C ethylene production did not proceed over 0.2µl/kg/h.

At 20°C, ethylene production reached a peak of 300µl/kg/h after 216 hours exposure to propylene and decreased significantly thereafter (Fig. 4.2A).

4. Carbon dioxide production

At 10°C, the respiration rate of fruit treated with propylene increased significantly within 48 hours and remained constant thereafter (Fig. 4.2B). Fruit at 20°C had a significant increase in respiration within 24 hours of exposure to propylene, reached a peak after 72 hours and decreased to a minimum after 168 hours. As ethylene was

reaching the climacteric peak, respiration rate increased again significantly and then remained almost constant (Fig. 4.2A and B).

For fruit treated with propylene, CO_2 production was significantly higher in fruit kept at 20 than at 10°C, with a difference of 30-40ml/kg/h (Fig. 4.2A). Respiration of fruit treated with propylene at 10°C was significantly higher, with a difference of 10-15ml/kg/h, than of fruit kept in air at 20°C. The respiration rate of fruit not treated with propylene almost did not change during the experiment, although it was slightly higher at 20 than at 10°C.

5. 1-aminocyclopropane-1-carboxilic acid (ACC) content

The ACC content of kiwifruit was almost null at harvest, but increased significantly after 120 hours in fruit treated with propylene at 20°C (Fig. 4.3A). The ACC reached a maximum level after 240 hours and decreased significantly thereafter.

Fruit treated with propylene at 10°C and fruit in air free of propylene at both 10 and 20°C were able to produce only small amounts of ACC without significant increases during the experiment (Fig. 4.3A).

6. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) activity

ACC synthase activity was very low at harvest, but increased significantly when fruit were treated with propylene at 20°C after a lag period of about 120 hours (Fig. 4.3B). Peak values were achieved after 240 hours and decreased significantly thereafter.

Fruit treated with propylene at 10°C and fruit in air free of propylene at 10 and 20°C showed very low ACC synthase activity without significant increase during the experiment (Fig. 4.3B).

7. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activity

In fruit treated with propylene at 20°C, ACC oxidase activity was almost null at harvest, started to increase significantly after 48 hours, reached a peak after 240 hours and decreased sharply thereafter (Fig. 4.3C). Fruit treated with propylene at 10°C and fruit in air free of propylene at 10 and 20°C did not show a significant increase in ACC oxidase activity during the experiment.

8. ACC synthase and ACC oxidase gene transcription

After 192 hours exposure to propylene, it was found ACC synthase and ACC oxidase gene transcription in ethylene producing kiwifruit at 20°C (Fig. 4.4). Kiwifruit treated with propylene at 10°C did not show transcription of ACC synthase gene up to 192 hours, but it showed for ACC oxidase. The expression of ACC oxidase was stronger at 20 than at 10°C.

Fruit not treated with propylene did not show transcription of ACC synthase or ACC oxidase genes at any temperature, as well as, just harvested fruit (Fig. 4.4).

DISCUSSION

The application of propylene enhanced ripening of mature fruit by initiating a rapid softening and increasing the total sugar level, while the burst of endogenous ethylene biosynthesis at 20°C occurred very late in ripening, just before fruit senescence as reported by Stavroulakis and Sfakiotakis (1993) and Whittaker et al. (1997). Temperature of 10°C slowed down a little the ripening but inhibited drastically autocatalysis of ethylene production as shown previously (Stavroulakis and Sfakiotakis, 1993). The temporal separation of ethylene sensitivity and climacteric ethylene production in kiwifruit suggests that both ripening and ethylene autocatalysis are regulated by two independent mechanisms (Stavroulakis and Sfakiotakis, 1993). The burst in CO_2 production that occurred immediately after propylene application

seems to be a response to the stress induced by exogenous propylene and the second increase a response to the endogenous ethylene production (Tucker, 1993).

Although kiwifruit is classified as a climacteric fruit when ripened at 20°C (chap 3), its behaviour is somewhat atypical in the way it is ripening in response to exogenous propylene prior to autocatalysis of ethylene occurs. In this respect, kiwifruit differs from avocado and tomato, for instance, which are characterised by a surge in ethylene production, respiration and ripening upon exposure of mature fruit to external ethylene (Metzidakis and Sfakiotakis, 1989; Abeles et al., 1992).

It is believed that the rate limiting step in ethylene biosynthesis pathway is catalysed by ACC synthase (Theologis, 1992; Yip et al., 1992). However, the activity of ACC oxidase also increases in some plants in response to internal or external factors that induce ethylene formation (Woodson et al., 1992; Kende, 1993). In the present work, transcripts of ACC synthase and ACC oxidase genes were found after 192 hours propylene treatment at 20°C, corresponding to the climacteric rise of ethylene production. Similar results were obtained by Ikoma et al. (1995) for ACC synthase and Whittaker et al. (1997) for ACC synthase and ACC oxidase in kiwifruit. In addition, the latter found ACC synthase gene transcription to increase with climacteric ethylene production in ripe fruit, while ACC oxidase transcripts were induced earlier, immediately after treatment with exogenous ethylene, reaching a maximum before the ethylene burst. This may explain the fact that ACC oxidase activity starts immediately after propylene treatment, while ACC synthase needs a lag period before to start its activity. Ikoma et al. (1995) found transcription of KWACC1 (a gene induced by ethylene in kiwifruit) after 48 hours exposure to ethylene with increasing levels of expression till 144 hours.

Whittaker et al. (1997) support the concept that ACC synthase has a controlling role in ethylene biosynthesis early in the climacteric. Later in the post-climacteric, ACC synthase transcript levels remain high, suggesting that ACC oxidase activity is impaired late in ripening as for other fruit (Abeles et al., 1992). In the present work, it was found that late in the climacteric, the decline in ethylene production was due to decreased ACC synthase and ACC oxidase activities. However, it is still possible that

ACC oxidase is impaired before ACC synthase since there was some accumulation of ACC in the post-climacteric.

ACC synthase activity of kiwifruit treated with propylene at 20°C increased significantly only after a lag period of about 120 hours coincident with the climacteric rise of ethylene production, while ACC oxidase activity started almost after propylene treatment. Based on these findings, we confirm that ACC synthase is the responsible for the atypical behaviour of kiwifruit at room temperature (≅20°C) which needs a lag period of about 68-79 hours prior to autocatalysis of ethylene production as postulated by Stavrolakis and Sfakiotakis (1995) and Whittaker et al. (1997).

Kiwifruit has been classified as an unique climacteric fruit which at low temperature lacks the ability for autocatalysis of ethylene production (induced by propylene) being the limiting factor the ACC availability rather than the ACC oxidase activity (Stavroulakis and Sfakiotakis, 1995). We found a non-climacteric behaviour of kiwifruit at 10°C (chapter 3). The present research showed that inhibition of ethylene production at 10°C was associated with low activities of ACC synthase and ACC oxidase.

An interesting observation was the fact that we found no transcription of ACC synthase gene in kiwifruit treated with propylene at 10°C, while there was transcription of ACC oxidase. It seems that temperature plays a crucial role in controlling the gene for ethylene induced ACC synthase and, as a consequence, the biosynthesis of endogenous ethylene in kiwifruit, making it different from the other climacteric fruit. However, it was found that kiwifruit infected with *Botrytis cinerea* produced ethylene and showed increased levels of ethylene at 0 and 10°C (Niklis et al., 1993), suggesting that the gene for wound induced ACC synthase is not affected by low temperature. More research is needed to clarify this point.

There is an emerging picture that ACC synthase is encoded by a highly divergent multigene family (Theologis, 1992). In tomato and zucchini, the enzyme is encoded by a divergent multigene family differentially expressed during ripening, wounding and auxin treatment (Nakajima et al., 1990; Van Der Staeten et al., 1990; Huang et al., 1991; Olson et al., 1991; Rottman et al., 1991; Sato et al., 1991; Yip et al., 1992;

Botella et al., 1995). Similarly, in *Arabidopsis*, ACC synthase genes are differentially expressed in response to developmental, hormonal and environmental stimuli (Liang et al., 1992). Like ACC synthase, ACC oxidase is encoded by a multigene family whose expression seems to be differentially regulated (Bouzayen et al., 1992). Ikoma et al. (1995) isolated two ACC synthase genes from kiwifruit: KWACC1 which was expressed after wounding and ethylene treatment and, KWACC2 which was expressed only after wounding.

Our results suggest that temperature as low as 10°C inhibits the expression of the ACC synthase gene that is induced by propylene. For ACC oxidase, low temperature exerts its effect mostly on reducing enzyme activity or maybe by inhibiting translation of the mRNA and the synthesis of the enzyme. This is supported by Wang (1989) who postulated that in tissues where there is accumulation of transcripts of the enzymes of ethylene biosynthesis pathway at low temperatures, but ethylene is not produced until after being transferred to warmer temperatures, the translation and the synthesis of a new protein were not completed at chilling temperatures.

Mature unripe kiwifruit not treated with propylene did not show transcription of ACC synthase or ACC oxidase for up to 192 hours at 10 or 20°C. Thus, in kiwifruit, as ACC synthase (Theologis, 1992; Woodson et al., 1992; Gaudiere and Vendrell, 1993), ACC oxidase is not a constitutive enzyme as presumed by Acaster and Kende (1983) and Yang and Hoffman (1984), but is induced by ethylene treatment or other stimuli as for a range of other tissues (Maunders et al., 1987; McGarvey et al., 1992; Ross et al., 1992; Woodson et al., 1992; Kende, 1993; Kim and Yang, 1994; Tang et al., 1994).

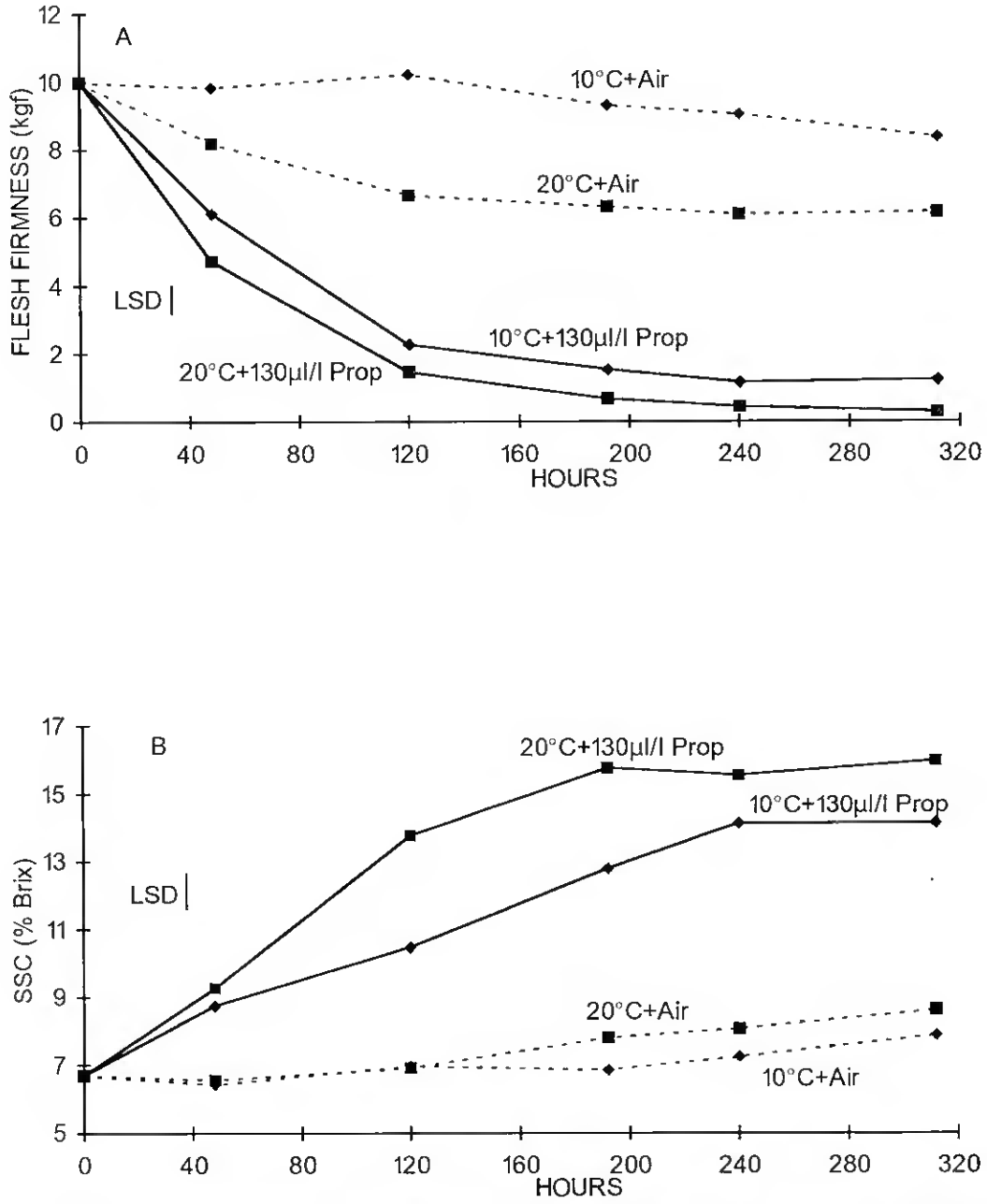
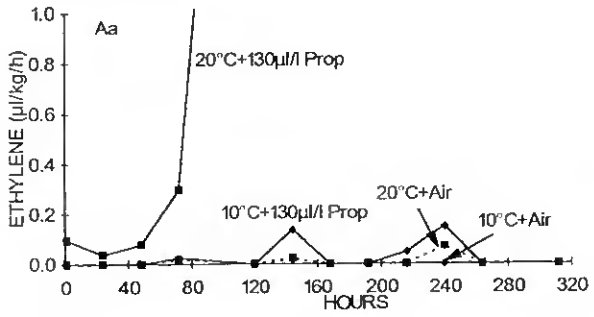
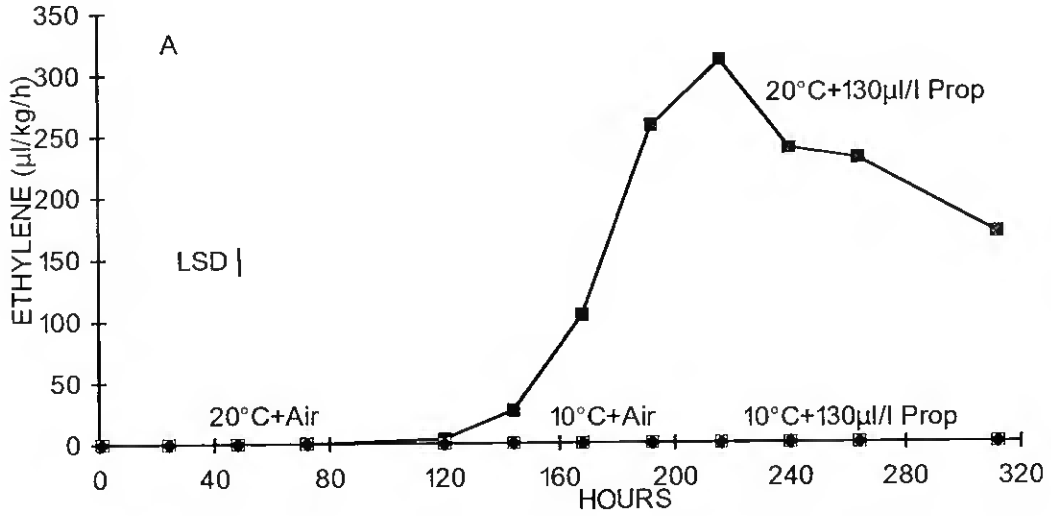


Figure 4.1. The effect of temperature (10 and 20°C) and propylene (130µl/l) on firmness (A) and SSC (B) of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream.
LSD at α=0.05.





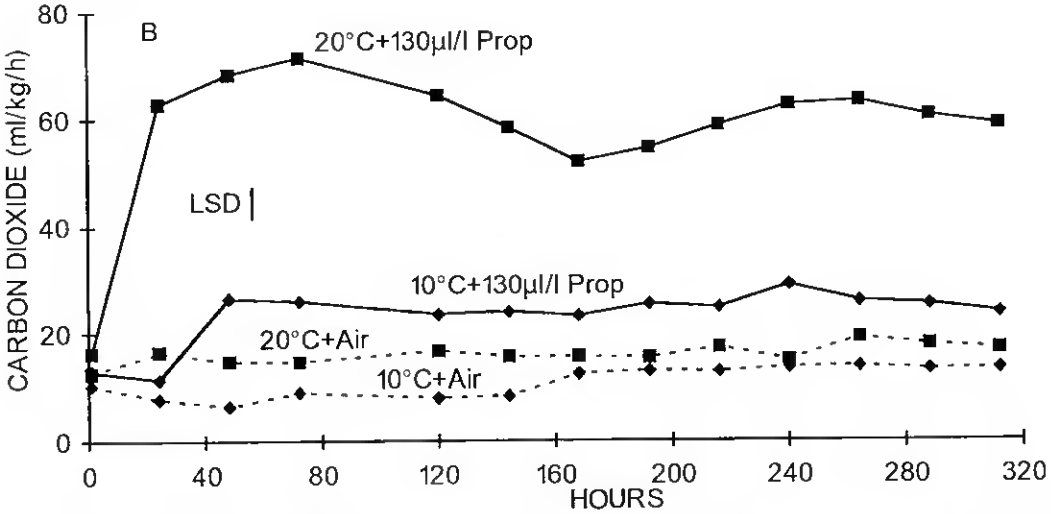


Figure 4.2. The effect of temperature (10 and 20°C) and propylene (130µl/l) on ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.

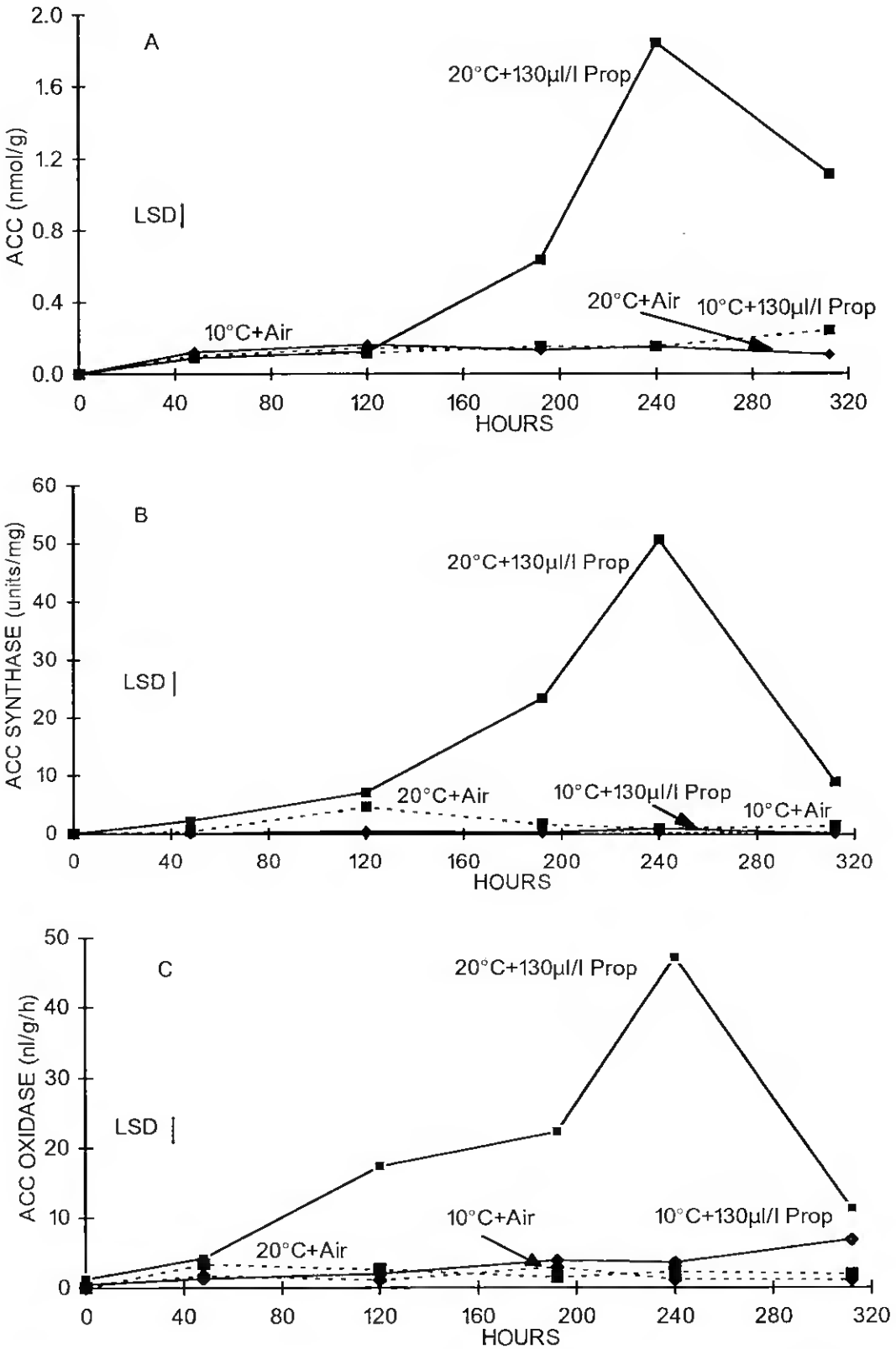
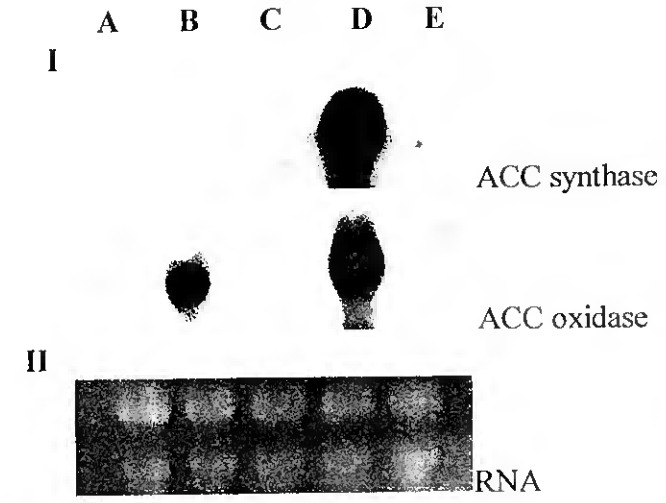


Figure 4.3. The effect of temperature (10 and 20°C) and propylene (130µl/l) on ACC content (A) and ACC synthase (B) and ACC oxidase (C) activities of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream.
1 unit/mg = 1pmol ACC/mg protein/2hours.
LSD at α=0.05.



III

0.5	0.3	1.7	23.5	0.0	ACC synthase activity (units/mg)
2.8	3.9	1.5	22.4	0.9	ACC oxidase activity (nl/g/h)
0.1	0.1	0.2	0.7	0.0	ACC (nmol/g)
0.0	0.0	0.0	258	0.0	Ethylene (µl/kg/h)

Figure 4.4. The effect of temperature (10 and 20°C) on ACC synthase and ACC oxidase transcription in 'Hayward' kiwifruit after 192 hours exposure to a continuous, humidified, air stream at 10°C+air free of propylene (A), 10°C+130µl/l propylene (B), 20°C+air free of propylene (C), 20°C+130µl/l propylene (D) and at harvest time (E). **I**- Northern blot hybridisation. Total RNA from each sample was isolated and analysed by Northern blot hybridisation using the cDNA probes for ACC synthase (KWACC1) and ACC oxidase (pMEL1). **II**- Agarose-gel electrophoresis of total RNA stained with ethydium bromide. **III**- Corresponding ACC synthase and ACC oxidase activities, ACC content and ethylene production.
1 unit/mg = 1pmol ACC/mg protein/2hours.

CHAPTER 5. EFFECT OF HIGH TEMPERATURE STRESS ON ETHYLENE BIOSYNTHESIS, RESPIRATION AND RIPENING OF 'HAYWARD' KIWIFRUIT

ABSTRACT

Temperatures up to 35°C have been shown to increase ethylene production and ripening of the propylene treated kiwifruit (Stavroulakis and Sfakiotakis, 1993). 'Hayward' kiwifruit were treated with 130µl/l propylene and air free of propylene at temperatures of 30, 34, 38, 40, 42 and 45°C in order to study the effect of high temperature stress on ethylene and CO_2 production and ripening.

At 30-40°C, ethylene and CO_2 production, ACC content and ACC synthase and ACC oxidase activities, as well as changes in flesh and core firmness and SSC were measured during a period of 120 hours. At 38-45°C, CO_2 production, flesh and core firmness, SSC and flesh colour were measured during a period of 72 hours.

Propylene induced ripening as evaluated by the increase in softening and SSC of kiwifruit at 30-34°C. Fruit failed to ripe normally at 38°C showing the core hard when the flesh was soft, and above 40°C ripening was inhibited.

Propylene also induced autocatalysis of ethylene production after a lag period of 24 hours at 30-34°C with concomitant induction of ACC production and ACC synthase and ACC oxidase activities. Induction of ethylene production occurred after 72 hours at 38°C. ACC production was similar at 30-38°C and was very low at 40°C. ACC oxidase and ACC synthase activities decreased with temperature increase above 30°C, but ACC oxidase decreased at a faster rate than ACC synthase. ACC oxidase activity declined after 96 hours at 30-34°C, while ACC synthase remained almost constant. Ethylene production was drastically reduced at 38°C and almost null at 40°C. This was attributed to the reduced activity of ACC oxidase rather than the reduced activity of ACC synthase or ACC production. Fruit not treated with propylene showed no ripening response nor ethylene and ACC production or ACC synthase and ACC oxidase activities.

In propylene-free atmosphere, kiwifruit respiration rate increased with temperature till 45°C reaching the respiration peak in 10 hours. At temperatures up to 38°C propylene treatment enhanced the respiration rate. Above this temperature, CO_2 production was similar in fruit treated or not with propylene. After 48 hours at 45°C fruit showed injury symptoms and started then a great decrease in CO_2 production and a faster fading of green colour.

The results suggest that high temperature stress inhibits ripening by inhibiting ethylene production and sensitivity while respiration proceeds until the breakdown of the tissues. Exposing kiwifruit to unusual high temperatures (>38°C) causes uneven ripening and has a detrimental effect on fruit quality parameters such as firmness, SSC and flesh colour.

INTRODUCTION

High temperatures (above 35°C) have been reported to inhibit ripening of many fruits (Mitchell, 1986). The effect of high temperature on inhibition of ethylene production applied as heat shock has been studied in the last few years (Klein and Lurie, 1990; Lurie and Klein, 1990; 1991). However, this technology may lead to physiological disorders (Pech et al., 1994).

Ethylene production usually exhibits a Q_{10} value of about 2 between 20 to 40°C, with further increases in temperature generally resulting in a decline in the rate of ethylene production (Field, 1985). However, inhibition of ethylene production at temperatures up to 40°C does not appear to be associated with permanent tissue damage, since return of the tissue to a permissive lower temperature results in the resumption of ethylene production (Field, 1985).

The failure of fruit to ripe normally at high temperatures has been attributed to the reduction of ethylene biosynthesis at these temperatures (Eaks, 1978). The stress resulting from high temperature appears to inhibit more ACC oxidase than ACC synthase (Yu et al., 1980; Field, 1985). Apelbaum et al. (1981) proposed that high temperature causes impairment of ethylene production by perturbing cellular

membranes, resulting in the inhibition of the membrane-associated ACC oxidase. This finding was supported by Yu et al. (1980) but in contrast to Horiuchi and Imaseki (1986) and Biggs et al. (1988) who found ACC synthase more sensitive to high stress temperature than ACC oxidase. However, Biggs et al. (1988) reported that the removal of high temperature stress resulted in more rapid recovery of ACC synthase than ACC oxidase activity.

Many climacteric fruit are inhibited from ripening or exhibit abnormal ripening at high temperatures. The respiration rate and ethylene production of harvested pears and avocados were suppressed at temperatures above 30-35°C and fruit suffered from heat injury (Maxie et al., 1974; Lee and Young, 1984). At temperatures above 30°C, colour development, softening, respiration rate and ethylene production of tomatoes were suppressed (Yakir et al., 1984; Inaba and Chachin, 1988). Inaba and Chachin (1989) reported a maximum ethylene production in tomatoes at 25°C, a decrease thereafter and only small amounts of ethylene at 40°C.

Eaks (1978) found typical climacteric patterns of respiration in avocado from 20 to 35°C, with the climacteric maximum increasing with temperature and a respiration rate decreasing with time at 40°C. Inaba and Chachin (1989) reported that the peak respiration rate of tomato fruit was higher at 30°C, even though the level of ethylene production was lower than at 25°C.

Stavroulakis and Sfakiotakis (1993) found increasing values of ethylene production (induced by propylene) in kiwifruit from 17 to 35°C. Ripening was induced and was similar between 20 to 35°C. Hence, a systematic study to understand bases of inhibition of ethylene production and ripening under heat stress has not been conducted.

The purpose of this study was to investigate the effect of high temperature stress on ACC synthase and ACC oxidase activities, ACC content, ethylene and CO_2 production and ripening of 'Hayward' kiwifruit induced or not by propylene. The temperature limits for the inhibition of the above were also defined.

MATERIAL AND METHODS

1. Plant material and treatments

1.1. High temperature limit for ethylene production

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 5.5 kgf flesh firmness and 12.1 (% Brix) SSC. After selection for uniformity of size and freedom from defects, fruit were placed in 5-litre jars through which a continuous, humidified, air stream with 130μl/l propylene or air free of propylene was passed at a rate of 100ml/min. Each set of six jars was kept in a separate water bath at a constant temperature of 30°, 34°, 38° and 40°C. Experiments were set within 24 hours. The experimental design was a two-factor experiment distributed in a complete randomised design with the temperatures as first factor, propylene treatment as second and the jars as replications. Each treatment consisted of 4 replications with 30 fruit per replication.

1. 2. High temperature limit for respiration

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 3.5 kgf flesh firmness and 11.7 (% Brix) SSC. After selection for uniformity of size and freedom from defects, fruit were analysed and experiments set as described above, but at temperatures of 38, 42 and 45°C. Each treatment consisted of 4 replications with 24 fruit per replication.

2. Measurements

For definition of high temperature limit for ethylene production, 6 fruit per replication were removed from the jars at intervals of 0, 48, 72, 96 and 120 hours. Measurements of ACC content, ACC synthase and ACC oxidase (*in vivo*) activities, firmness of flesh and core and SSC took place on these fruit. Ethylene production was measured daily. Respiration was measured three times in the first day and once per day thereafter.

For definition of high temperature limit for respiration, firmness of flesh and core, SSC and flesh colour (a* value) were measured at intervals of 0, 24, 48 and 72 hours.

Ethylene production was measured daily. Carbon dioxide production was measured four times in the first day and once per day thereafter.

2.1. Ripening parameters and gas analysis

Ripening parameters (flesh and core firmness, SSC and flesh colour) and gas analysis (ethylene and CO_2 production) were measured as described in chapter 3.

2.2. ACC content, ACC synthase and ACC oxidase activities

ACC content and ACC synthase and ACC oxidase activities were measured as described in chapter 4.

3. Statistical analysis

Statistical analysis were performed as described in the methods of chapter 3.

RESULTS

1. High temperature limit for ripening and ethylene production

1.1. Firmness

Flesh firmness of kiwifruit treated with propylene significantly decreased during 72 hours at 30-38°C, while at 40°C the decrease was significant only between 0 and 96 hours (Fig. 5.1A). Fruit at 30-34°C reached a ripe firmness value (<1kgf) in 72 hours, while at 38°C needed 120 hours. Fruit at 40°C did not ripe during the experiment showing a flesh firmness significantly higher than the other treatments after 72 hours.

Core firmness of kiwifruit treated with propylene followed the same pattern as flesh firmness at 30 and 34°C (Fig. 5.2A). However, at 38°C fruit failed to ripe normally; the core was still hard when flesh had softened to eating-ripeness, presenting values significantly higher than at 30 and 34°C. The 40°C treatment significantly reduced core softening in comparison with the other temperatures.

There were no significant changes in flesh and core firmness in kiwifruit not treated with propylene during the experimental time at any temperature (Figs. 5.1B and 5.2B).

1.2. Soluble solids content (SSC)

The SSC increased significantly during 72 hours when fruit were treated with propylene in all temperatures (Fig. 5.3A). Although values were always lower at 40°C than in the other treatments, differences were not significant. In air free of propylene, kiwifruit did not have significant changes of SSC during the experimental time at any temperature (Fig. 5.3B).

1.3. Ethylene production

Autocatalysis of ethylene production was induced by propylene after kiwifruit reached a threshold level of 0.1-0.3µl/kg/h ethylene production with a lag period of 24 hours at 30 and 34°C and 48 hours at 38°C (Fig. 5.4Aa).

Temperature of 38°C significantly reduced ethylene production induced by propylene (Fig. 5.4A). After 120 hours exposure to propylene, fruit reached a value of 40µl/kg/h ethylene production at 38°C, while at 30 and 34°C values were of 320 and 380µl/kg/h, respectively. Ethylene was drastically inhibited at 40°C, where its production did not proceed over 2µl/kg/h (Fig. 5.4Aa).

Kiwifruit not treated with propylene presented very low values of ethylene production and were not able to induce autocatalysis during the experiment (Fig. 5.4B).

1.4. 1-aminocyclopropane-1-carboxilic acid (ACC) content

The ACC levels of kiwifruit treated with propylene increased significantly after 48 hours at 30, 34 and 38°C without significant differences between them (Fig. 5.5A). At 40°C, fruit were able to produce only small amounts of ACC without significant increase during the experiment.

Kiwifruit in air free of propylene showed only trace amounts of ACC for all temperatures (Fig. 5.5B).

1.5. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) activity

The ACC synthase activity significantly increased when fruit were treated with propylene after 48 hours in all temperatures (Fig. 5.6A). Values were significantly higher at 30°C followed by 34, 38 and 40°C. At 40°C, values were very low in comparison with the other temperatures. ACC synthase activity increased significantly from 48 to 72 hours at 30, 34 and 38°C and remained almost constant thereafter. At 40°C, the increase in ACC synthase activity was constant till 96 hours and then had a decrease.

In air free of propylene, kiwifruit ACC synthase activity was very low in all temperatures (Fig. 5.6B).

1.6. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activity

ACC oxidase activity was almost null at harvest and started to increase with no lag period when fruit were treated with propylene in temperatures from 30 to 38°C (Fig. 5.7A). Values were significantly higher at 30°C followed by 34, 38 and 40°C. At 30 and 34°C, ACC oxidase activity had the highest increase between 48 and 96 hours and decreased significantly thereafter. At 38°C, fruit had a constant increase in ACC oxidase activity, but its values were significantly lower than at 34°C except after 120 hours. ACC oxidase activity of kiwifruit at 40°C did not increase significantly during the experiment.

Kiwifruit not treated with propylene presented very low values of ACC oxidase activity during the experiment (Fig. 5.7B).

1.7. Carbon dioxide production

The respiration peak increased significantly with temperature presenting the lowest values at 30°C and the highest at 40°C in fruit treated or not with propylene (Fig. 5.8).

The respiration peak of fruit treated with propylene was reached after 10 hours in all temperatures and presented no significant differences between treatments except between 30 and 40°C (Fig. 5.8A). Thereafter, CO_2 production decreased to values of

about 80 to 100 ml/kg/h until 55 hours had passed and then remained constant in all temperatures.

Respiration rate of fruit not treated with propylene increased significantly reaching a simultaneous peak after 4-10 hours at 30 to 38°C and after 10 hours at 40°C (Fig. 5.8B). Peak values were significantly higher at 40°C followed by 38, 34 and 30°C. The 30 and 34°C treatment did not show significant differences between respiration rates in the respiration peak. Carbon dioxide production significantly decreased thereafter to values of 40 to 70ml/kg/h until 30 hours had passed and remained constant thereafter. After 30 hours, there were no significant differences between treatments except at 30°C which had significantly lower values than the other treatments.

Values of CO_2 production in fruit not treated with propylene were lower than in fruit treated with propylene (Fig. 5.8). However, differences were decreased with temperature increase. Differences in CO_2 production, in the respiration peak, between fruit treated and not treated with propylene were of 48ml/kg/h at 30°C, 51ml/kg/h at 34°C, 41ml/kg/h at 38°C and 5ml/kg/h at 40°C.

2. High temperature limit for respiration

2.1. Carbon dioxide production

Respiration rate increased significantly in all treatments reaching a peak after 10 hours (Fig. 5.9). At 38 and 42°C, CO_2 production slightly decreased from 10 to 24 hours and then remained constant. Values of respiration were always significantly higher at 42 than at 38°C. At 45°C, fruit presented significantly higher values than the other treatments for the first 10 hours, but then respiration rate decreased sharply and after 48 hours respiration values were significantly lower than for fruit at the other temperatures. After 72 hours at 45°C, fruit showed a respiration rate lower than in the beginning of the experiment. There were no significant differences between fruit treated or not with propylene at any temperature.

2.2. Flesh colour

The fading of flesh green colour was small, without significant differences between fruit at 38 and 42°C (Fig.5.10). During the first 24 hours, fruit at 45°C had similar flesh colour to the other treatments. Thereafter, kiwifruit at 45°C had a significant increase in the loss of flesh green colour coincident with the great decrease in fruit respiration (Figs. 5.9 and 5.10). There were no significant differences in flesh colour between fruit treated or not with propylene at any temperature (Fig.5.10).

2.3. Firmness and soluble solids content (SSC)

Fruit treated with propylene at 38°C had a significant decrease in firmness and increase in SSC after 72 hours (Table 5.1). However, fruit did not ripe normally; the core was still hard when flesh had softened to eating-ripeness. Fruit not treated with propylene at 38°C did not show significant alterations of firmness and SSC.

Temperatures of 42 and 45°C inhibited the fruit to ripe normally independently of being treated or not with propylene (Table 5.1). Kiwifruit were destroyed after 72 hours at 45°C.

DISCUSSION

In the present investigation, it was shown that at temperatures of 30-34°C there is an induction of ethylene production and ripening, by propylene, as reported by Stavroulakis and Sfakiotakis (1993). However, as reported by these authors, the lag period for ethylene production was shorter at 30 and 34°C (24 hours) than at 20°C (68-79 hours) (chapter 3 and 4). The increase in ethylene production was accompanied by the increase in ACC content and ACC synthase and ACC oxidase activities. Ripening induced by propylene started at the above temperatures with no lag period confirming that kiwifruit senses ethylene for ripening prior to its autocatalysis (chapter 4; Stavroulakis and Sfakiotakis, 1993; Whittaker et al., 1997).

During the experiment, fruit not treated with propylene and at temperatures that normally induce ethylene production did not ripe, did not produce ethylene and did not

show ACC synthase or ACC oxidase activities. This is explained by the fact that autocatalysis of ethylene production and ripening of kiwifruit starts around 19 days after harvest as it was shown in chapter 3 and, by Hyodo and Fukasawa (1985) and Arpaia et al. (1994a).

High temperature stress decreased the rate of ripening and ethylene production in kiwifruit, while respiration was increased. Similar results were observed for avocado (Eaks, 1978), tomato (Yakir et al., 1984; Inaba and Chachin, 1988) and apple (Lurie and Klein, 1990). However, the upper temperature limits vary from species to species.

By exposing kiwifruit to high stress temperatures (>38°C) there was a detrimental effect on fruit quality parameters such as firmness, SSC and flesh colour. In kiwifruit high temperature stress affected more firmness than SSC in the same way as it was reported for apples (Lurie and Klein, 1990). Kiwifruit treated with propylene at temperatures up to 40°C attained a SSC of about 14% Brix considered to be the minimum for a mature fruit (Mitchel, 1994). Temperatures above 42°C inhibited fruit ripening in terms of SSC.

Kiwifruit are ripe for eating at a firmness value of 0.5-0.8 kgf (McDonald, 1990). Ripe kiwifruit should have equal flesh and core firmness. Temperature of 38°C inhibited normal ripening of kiwifruit induced by propylene since the core was still hard when flesh had softened to eating-ripeness. A physiological disorder with similar symptoms has been observed in kiwifruit stored at 0°C in CO_2 concentrations above 14% (Arpaia et al., 1994a). Over 40°C, softening was significantly reduced and at 45°C stress gradually accumulated, injuring the fruit (Inaba and Chachin, 1989). The characteristic flesh green colour of kiwifruit was affected, turning yellow-brown, only at temperatures of 45°C.

The failure of kiwifruit to ripe normally at temperatures above 38°C was due not only to the reduction of ethylene biosynthesis at these temperatures, as reported by Eaks (1978), but also due to the decreased sensitivity of the fruit to propylene application (Maxie et al., 1974). Another reason may be the inhibition of the synthesis or activity of cell-wall degrading enzymes (Klein and Lurie, 1990; Lurie and Klein, 1990).

There is a decrease in total protein synthesis during the course of the heat treatment, while at elevated temperatures heat shock proteins are synthesised (Key et al., 1981; Lurie and Klein, 1990). The strong inhibition of fruit softening and ethylene production at high stress temperatures may be a consequence of the inhibition of protein synthesis related to ripening and ethylene production (Brady, 1987; Lurie and Klein, 1990).

The present study showed that from 30 to 34°C CO_2 production was increased by propylene. This is similar to the behaviour at lower temperatures (chapter 3 and 4) and seems to be a response to the stress induced by exogenous propylene (Tucker, 1993). The small differences in respiration rate between fruit treated and fruit not treated with propylene at 38°C and the practical absence of differences over 40°C suggest that kiwifruit does not sense ethylene at high temperatures. Maxie et al. (1974) found that pears in ethylene-free atmosphere showed an initial respiration rate increased with temperature up to 40°C and a decrease at 50°C. However, when ethylene was applied respiration increased with temperature until 50°C. The same authors reported that the failure of pears to ripen at 40°C when exposed to ethylene is an example of stimulation of respiration by this gas without affecting other biochemical events associated with ripening.

Kiwifruit reached the respiration peak after 10 hours while the peak of ethylene production was not attained during the experiment. This observation confirms the result presented in chapter 3, that kiwifruit treated with propylene shows the respiration peak before the peak of ethylene production.

In fruit treated or not with propylene mostly at the respiration peak, the present research showed an increase in the respiration rate with temperature, while ethylene production induced by propylene was inhibited at the same high temperatures. These results, together with previous studies in tomatoes and apples (Inaba and Chachin, 1989; Klein and Lurie, 1990; Lurie and Klein, 1991) indicate that the respiration rate of some fruit is not directly controlled by the level of ethylene production (Inaba and Chachin, 1989).

It seems that respiration increases with temperature, from the beginning of storage, as a response to stress, until a level where physiological processes are stooped. At 45°C, kiwifruit showed the highest respiration rate from the beginning of storage, indicating severe stress at this temperature, that finally resulted in heat injury after 48 hours exposure. This implies that the stress gradually accumulates and alters the physiological processes of the fruit after a certain time of exposure. Similar results were shown for tomatoes at 40°C after 4 days exposure (Inaba and Chachin, 1989). In the present study, it was found that heat injury in kiwifruit was expressed by a strong reduction in respiration, increase in fading of flesh green colour and a general flesh breakdown. Lee and Yang (1984) and Yakir et al. (1984) reported suppression of ethylene and CO_2 production as well as heat injury of pears and avocados at 30-35°C.

For kiwifruit treated with propylene, the decrease in ethylene production at 38°C was caused by low activity of ACC oxidase, since ACC synthase activity and ACC content had similar values with fruit at 30-34°C. Inhibition of ethylene production at 40°C was associated with low activities of ACC synthase and ACC oxidase. Biggs et al. (1988) reported a reduction in ACC synthase and ACC oxidase activities in tomatoes at high temperatures. However, the decline in ACC synthase activity with temperature rise was faster than the decline in ACC oxidase activity. Since in our work, when fruit were kept at temperatures above 30°C, ACC oxidase activity decreased at a faster rate than ACC synthase, it seems that ACC oxidase in kiwifruit is more affected by high temperatures than ACC synthase as it was reported for apple tissue and auxin-treated mung-bean hypocotyls (Yu et al., 1980). This explains the accumulation of ACC at 38°C when ethylene production was very low. The decline in ACC oxidase activity observed in kiwifruit after long exposure to high temperature was also observed in tomato by Biggs et al. (1988).

Treating pericarp discs from heat-stressed pears with cycloheximide showed no recovery of ethylene production upon transfer to 25°C, while control fruit produced ethylene (Biggs et al., 1988). These results indicate the requirement of *de novo* synthesis of at least one of the enzymes involved in ethylene biosynthesis (Biggs et al., 1988; Lurie and Klein, 1990). Lurie and Klein (1990) hypothesised that heat treatment differentially affects processes which normally increase simultaneously during fruit

ripening, by inhibiting those processes which require *de novo* protein synthesis and enhancing those that do not. Lurie and Klein (1991) support this theory with the finding that protein synthesis normally associated with ripening was depressed in tomatoes kept at high temperatures, while fruit accumulated heat-shock proteins. It is know that ethylene production requires continuous protein synthesis (Grierson et al., 1986) making ethylene production to be apparently one of the most sensitive indicators of heat stress (Lurie and Klein, 1990). Tucker et al. (1980) and Tucker and Grierson (1982) showed that cell wall-degrading enzymes undergo synthesis at the onset of ripening. This may explain the suppression of ethylene production and ripening of kiwifruit at high stress temperatures while respiration was still increasing. According to Lurie and Klein (1990), we suppose that the turnover of the enzymes involved in respiration may be such that heat treatment up to 42°C did not appreciably affect their activity in kiwifruit up to 72 hours. High CO_2 production at 45°C may be a response to high stress that gradually accumulates resulting in heat injury after 48 hours exposure and a consequent decrease in respiration.

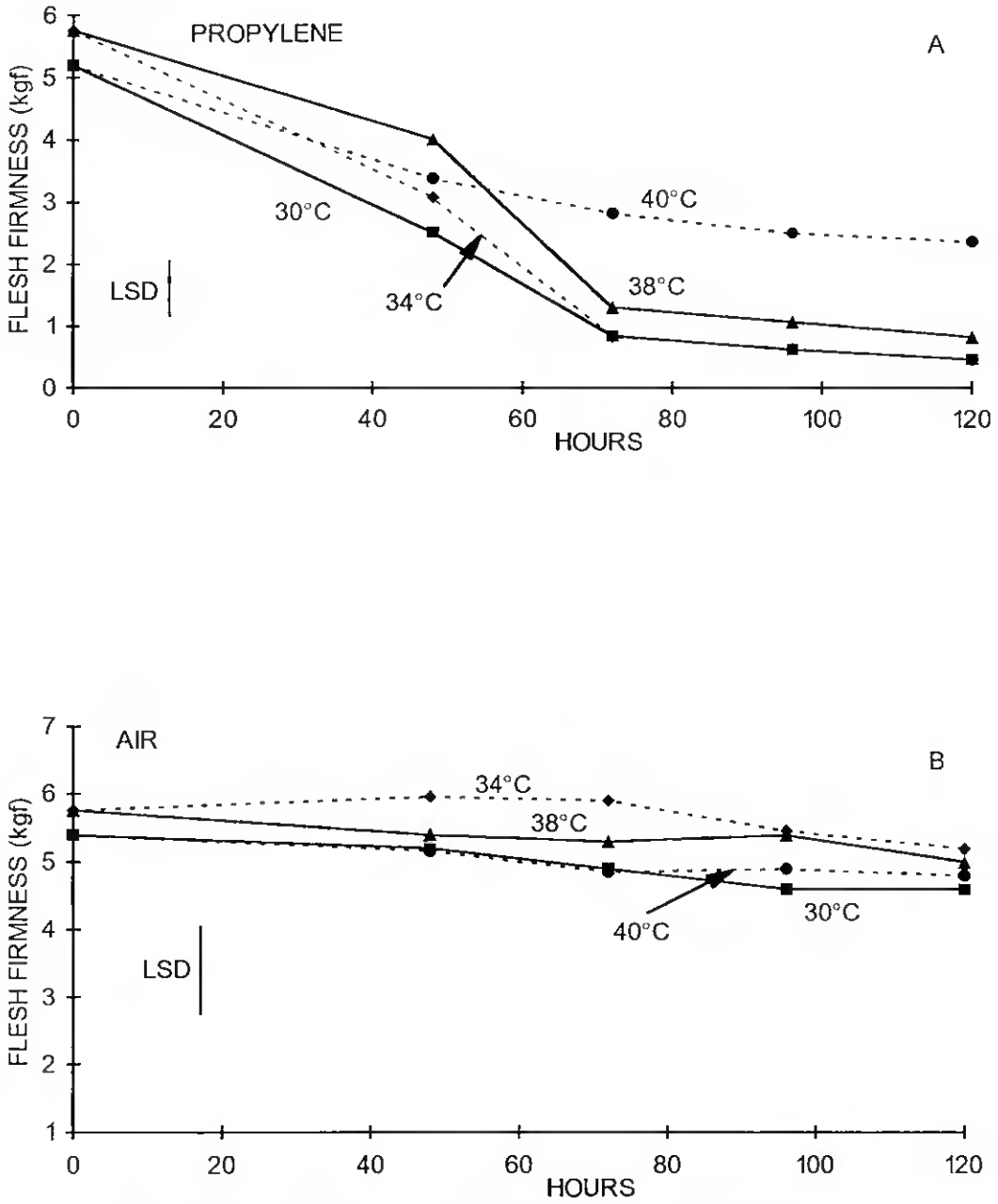


Figure 5.1. The effect of temperature (30, 34, 38 and 40°C) on flesh firmness of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

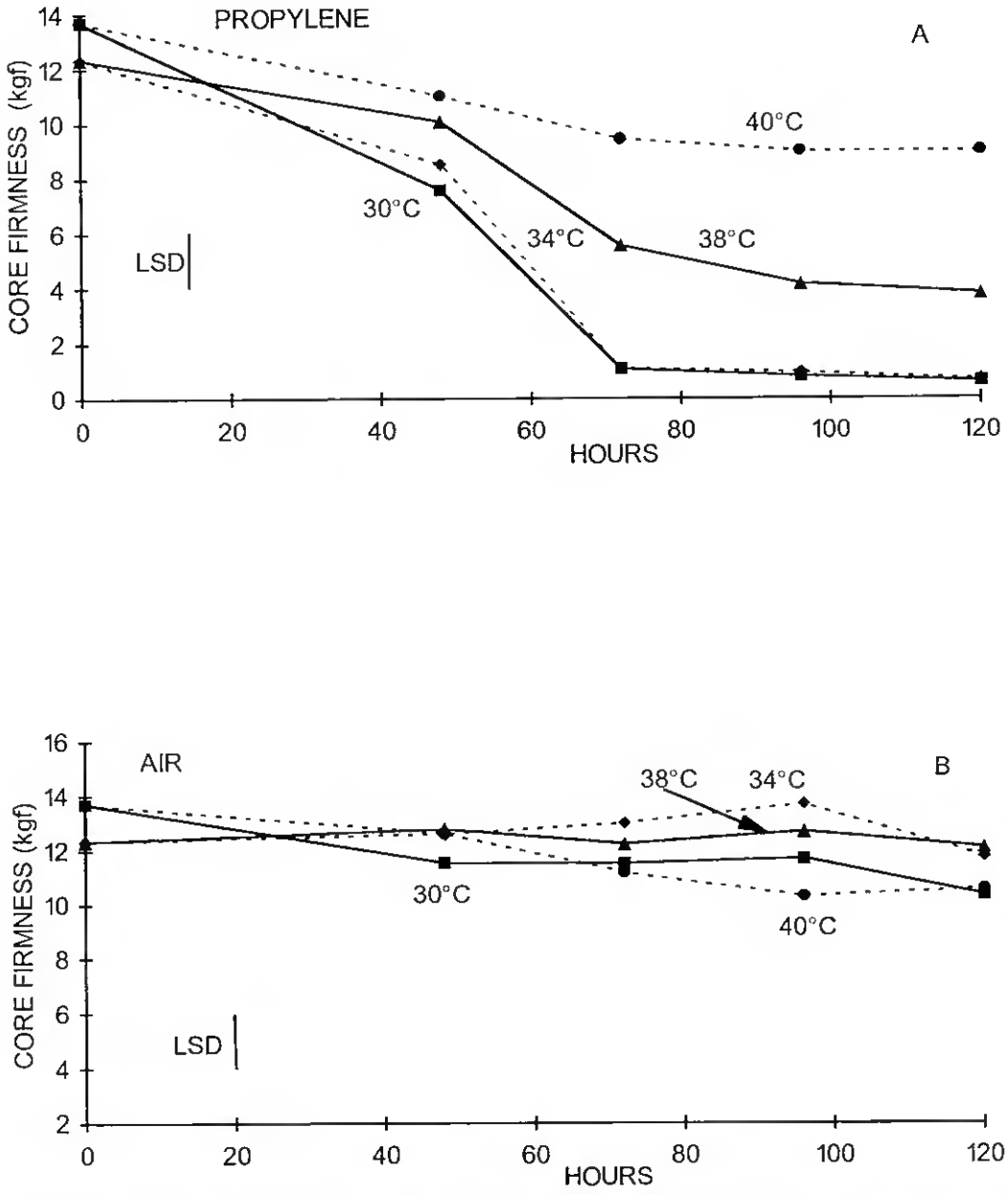


Figure 5.2. The effect of temperature (30, 34, 38 and 40°C) on core firmness of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

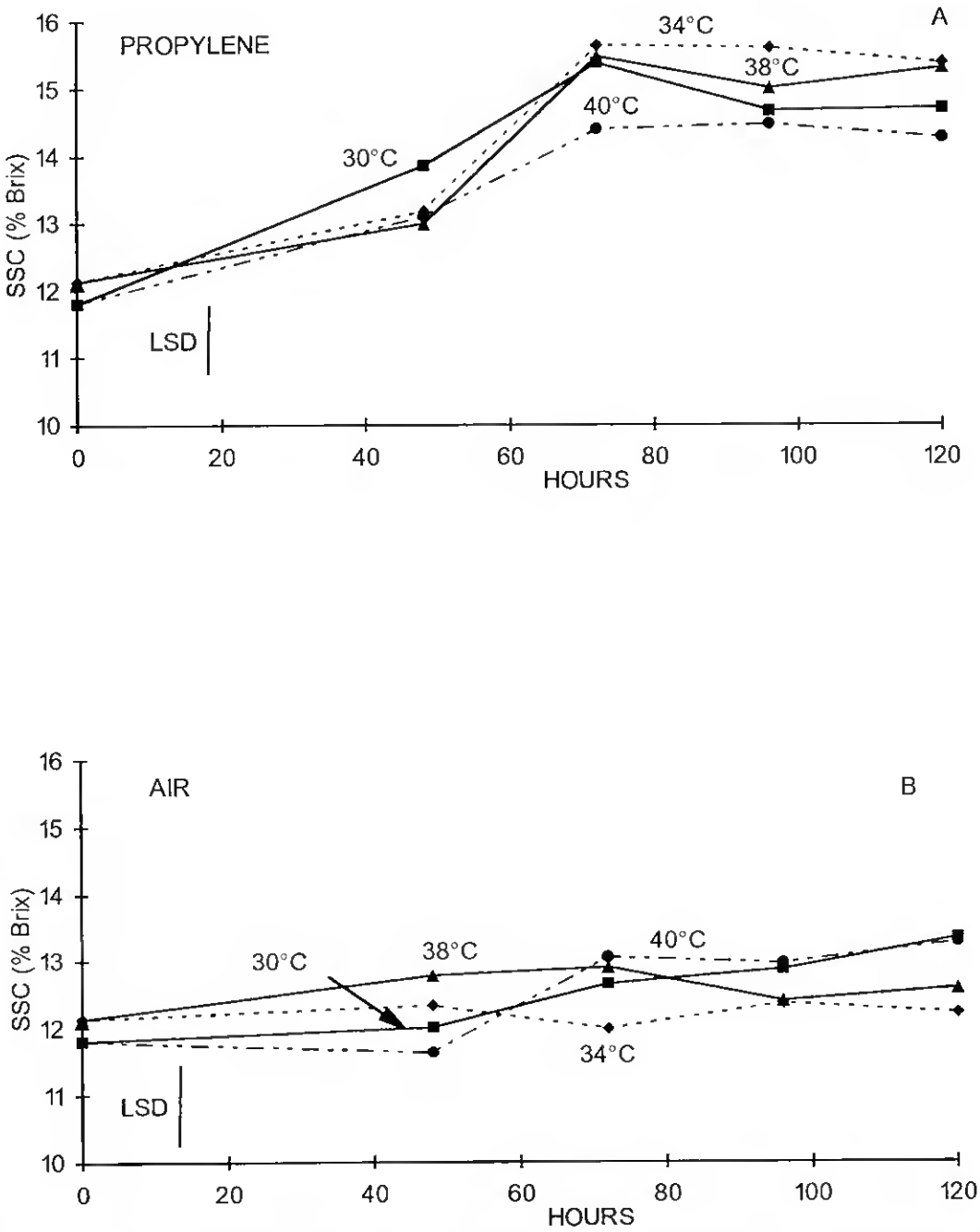
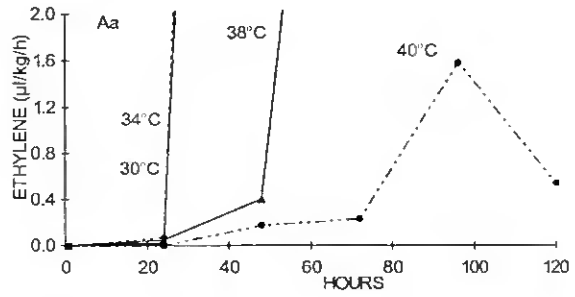
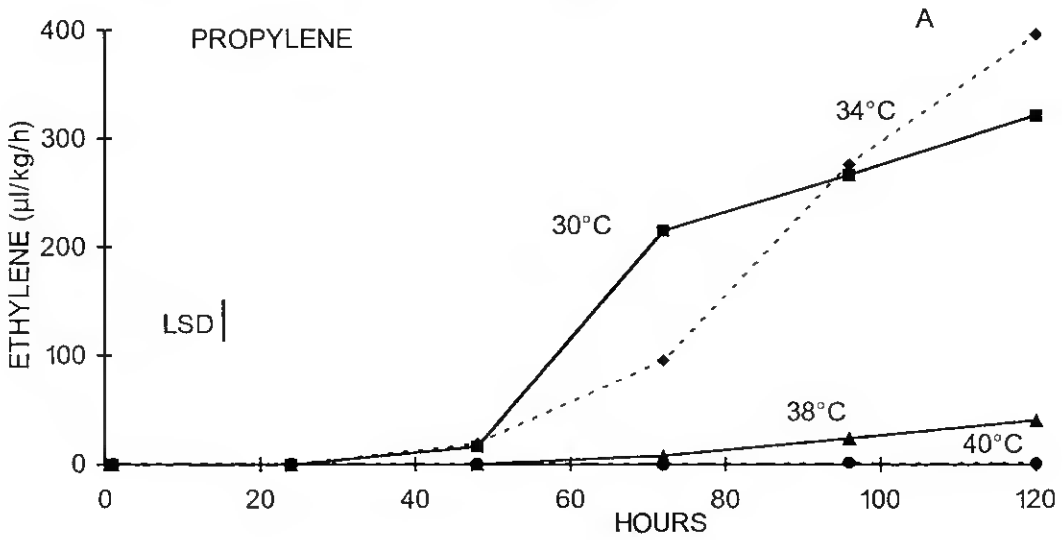


Figure 5.3. The effect of temperature (30, 34, 38 and 40°C) on SSC evolution of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.





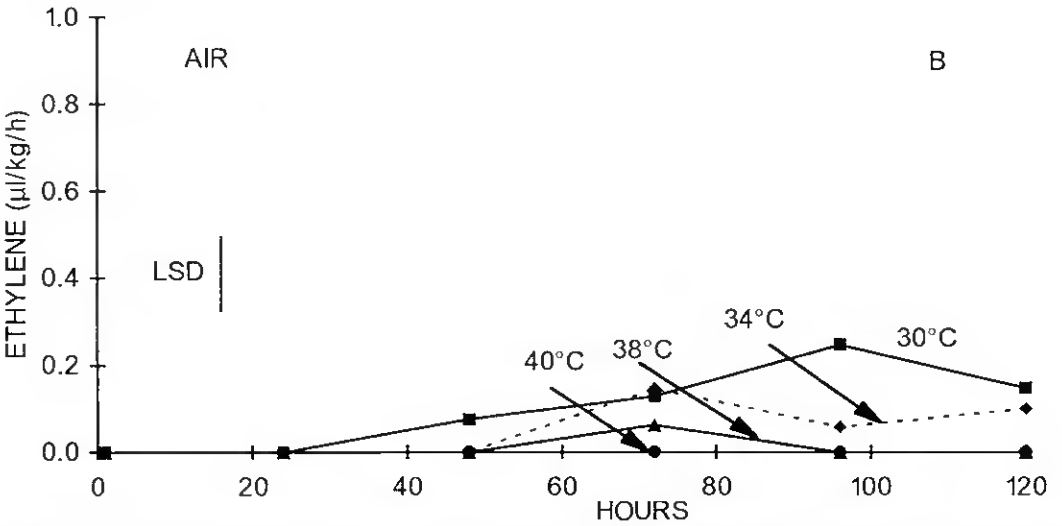


Figure 5.4. The effect of temperature (30, 34, 38 and 40°C) on ethylene production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

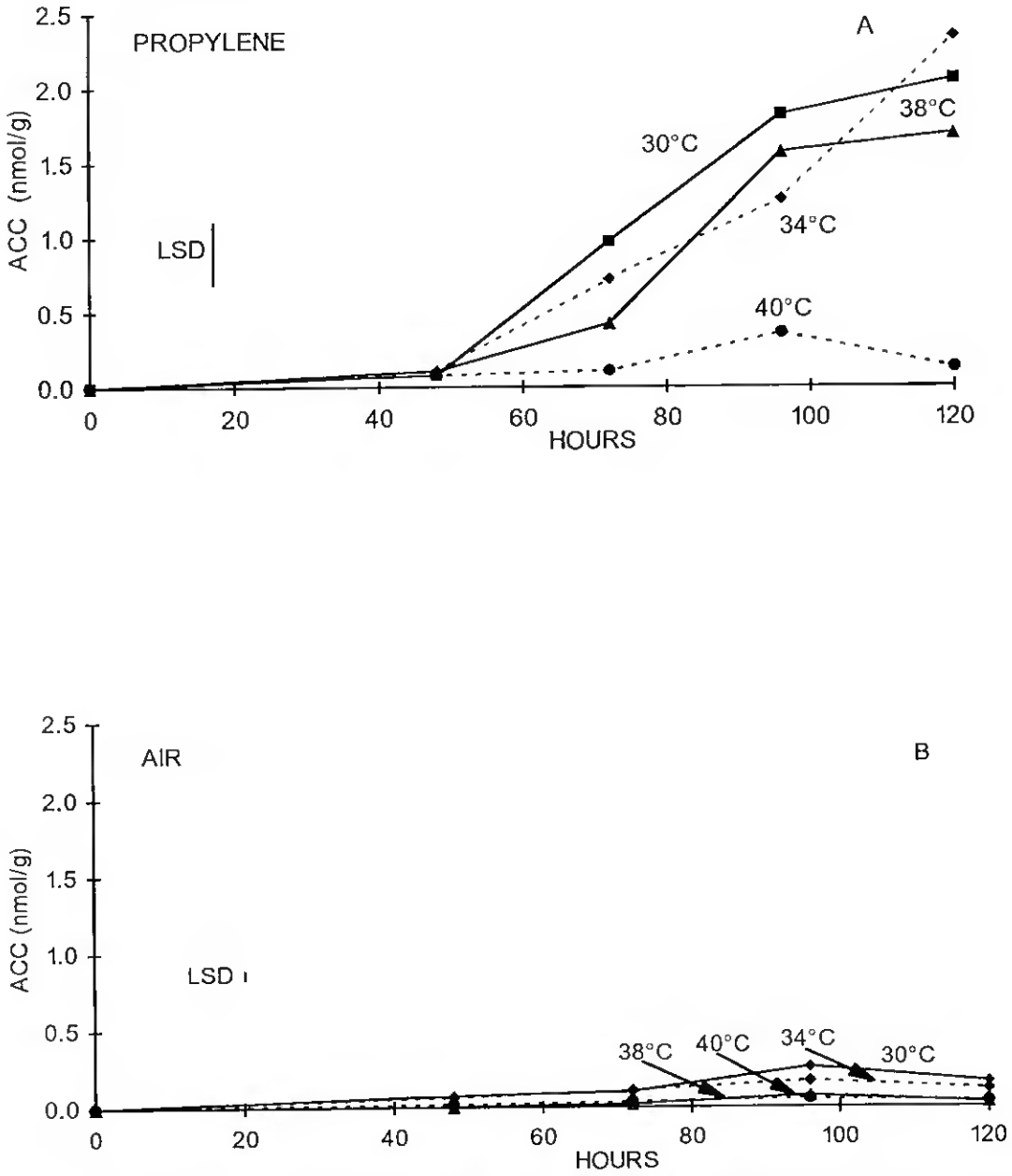


Figure 5.5. The effect of temperature (30, 34, 38 and 40°C) on ACC content of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

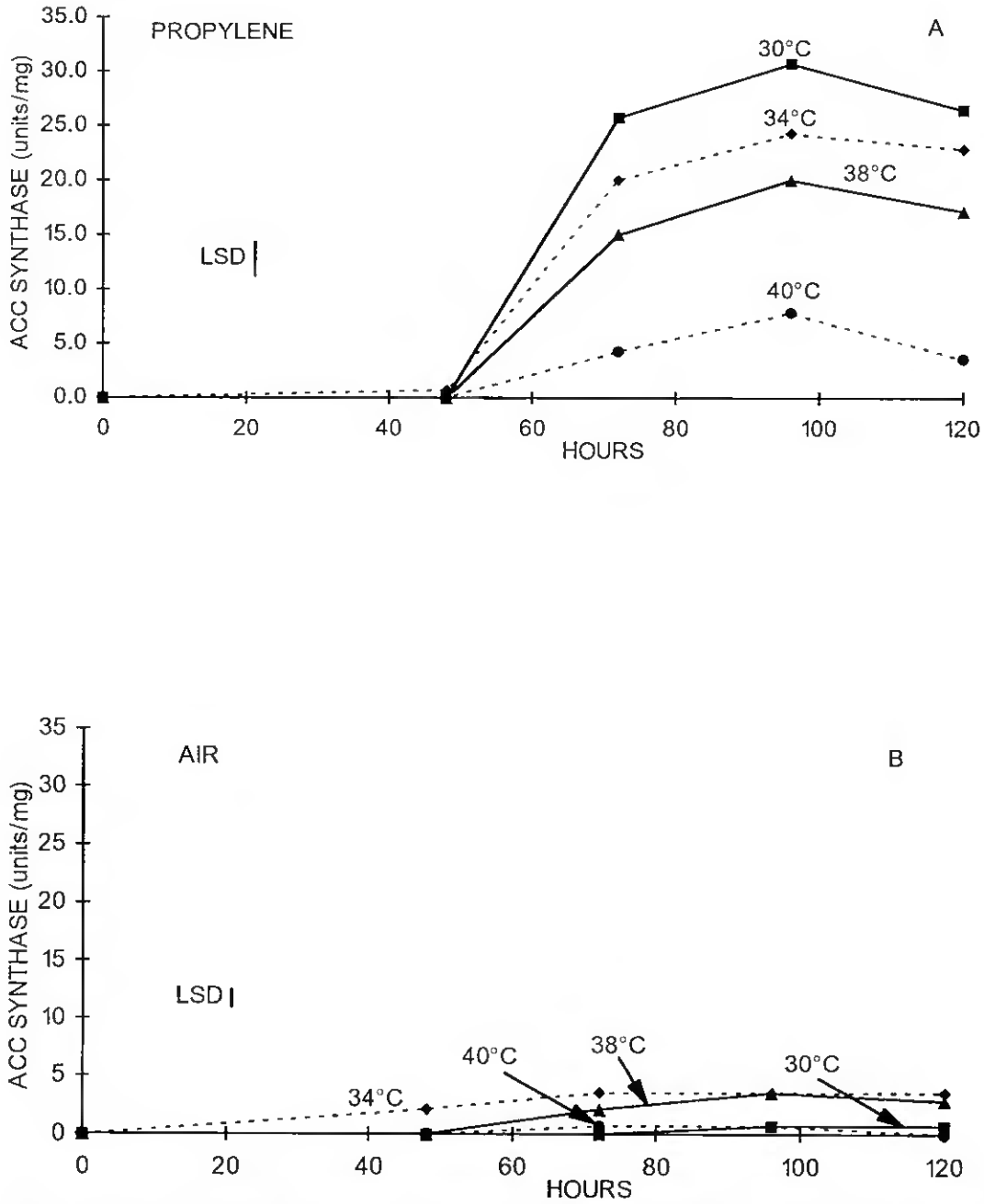


Figure 5.6. The effect of temperature (30, 34, 38 and 40°C) on ACC synthase activity of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
1 unit/mg = 1pmol ACC/mg protein/2hours.
LSD at α=0.05.

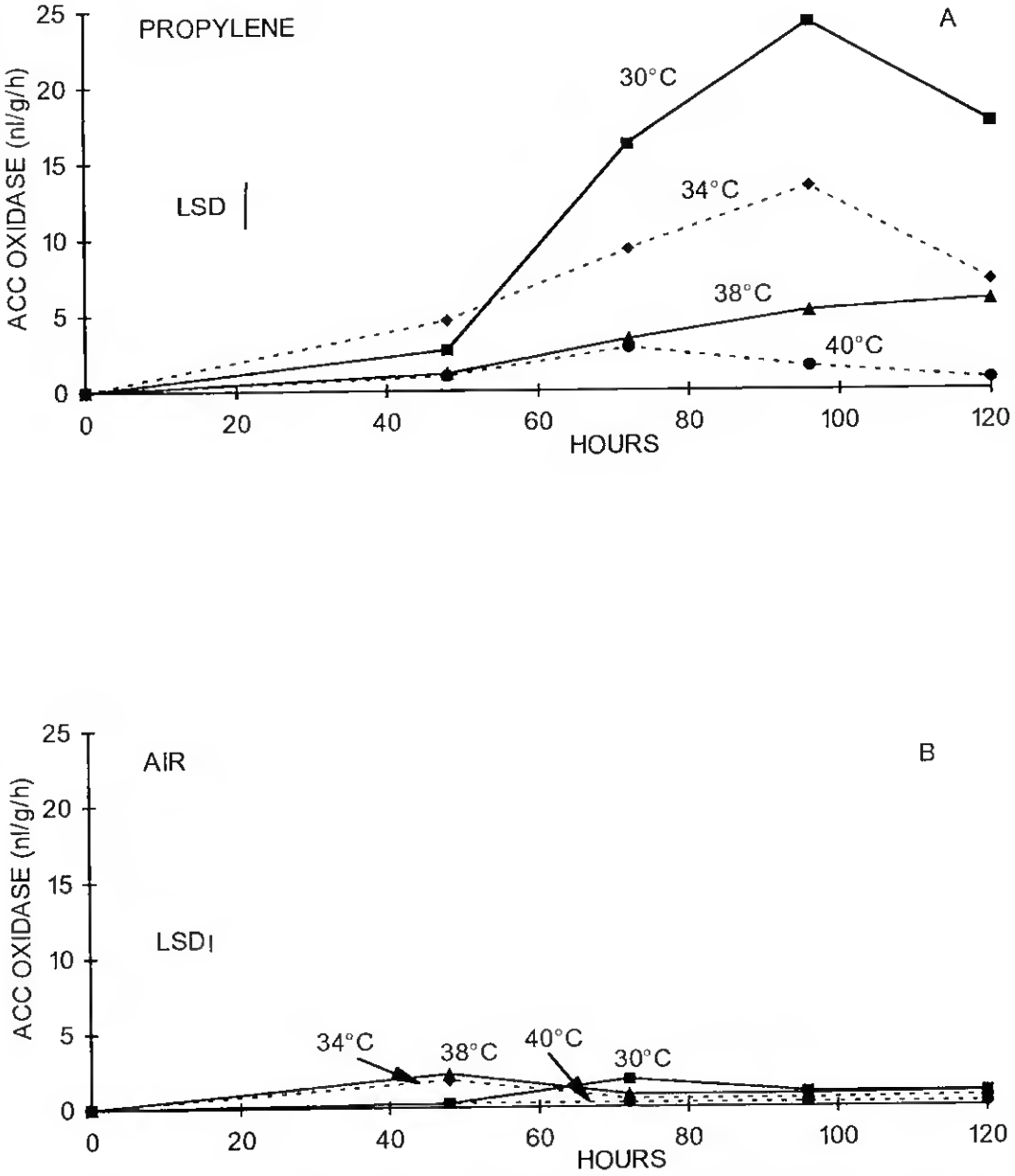


Figure 5.7. The effect of temperature (30, 34, 38 and 40°C) on ACC oxidase activity of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

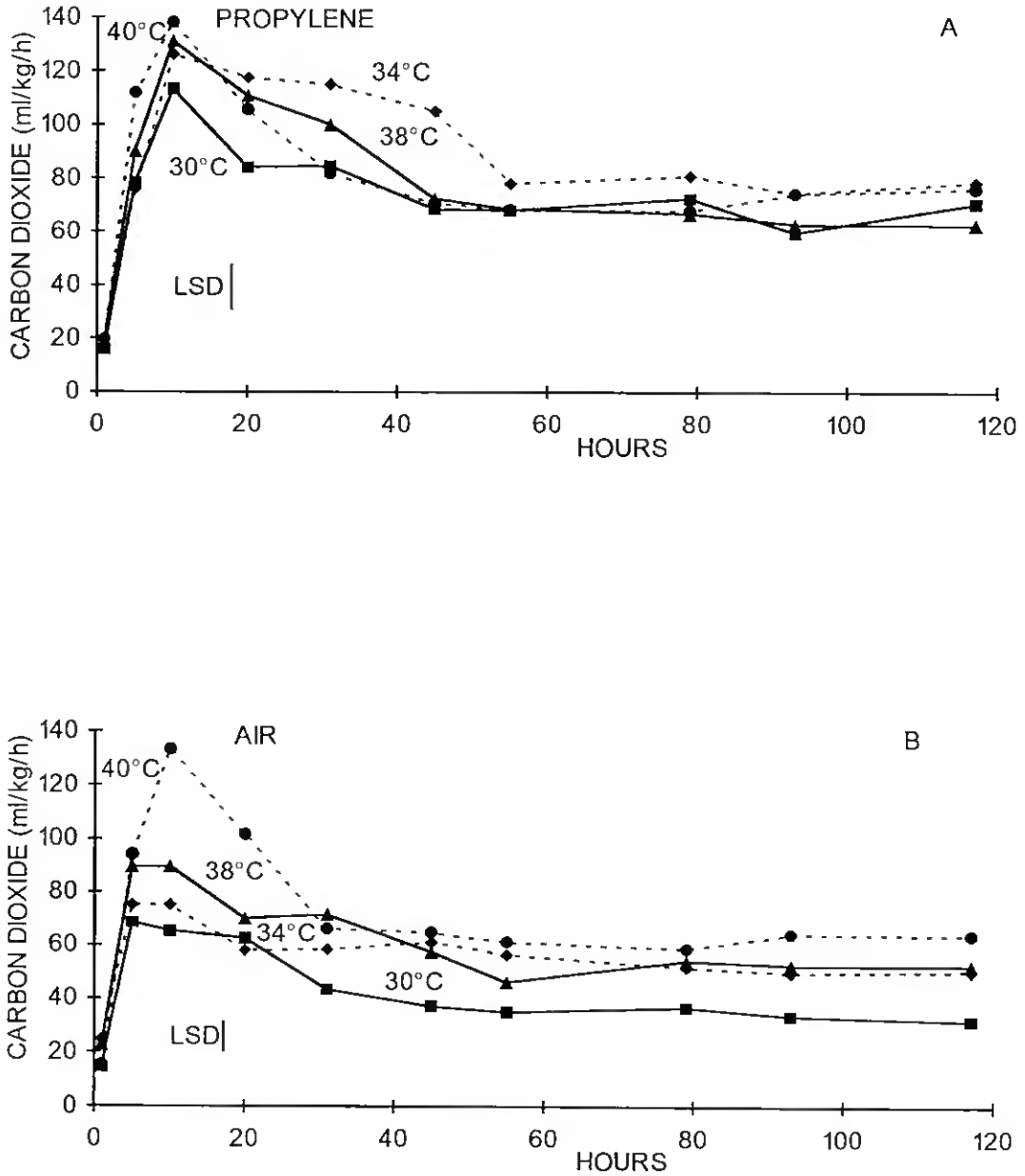


Figure 5.8. The effect of temperature (30, 34, 38 and 40°C) on CO_2 production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene (A) or air free of propylene (B).
LSD at α=0.05.

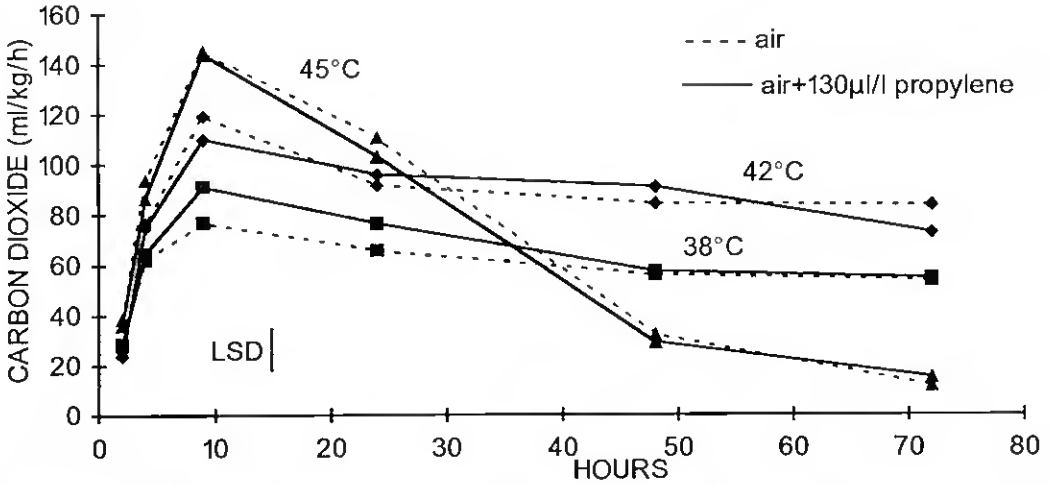


Figure 5.9. The effect of temperature (38, 42 and 45°C) on CO_2 production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene or air free of propylene.
LSD at α=0.05.

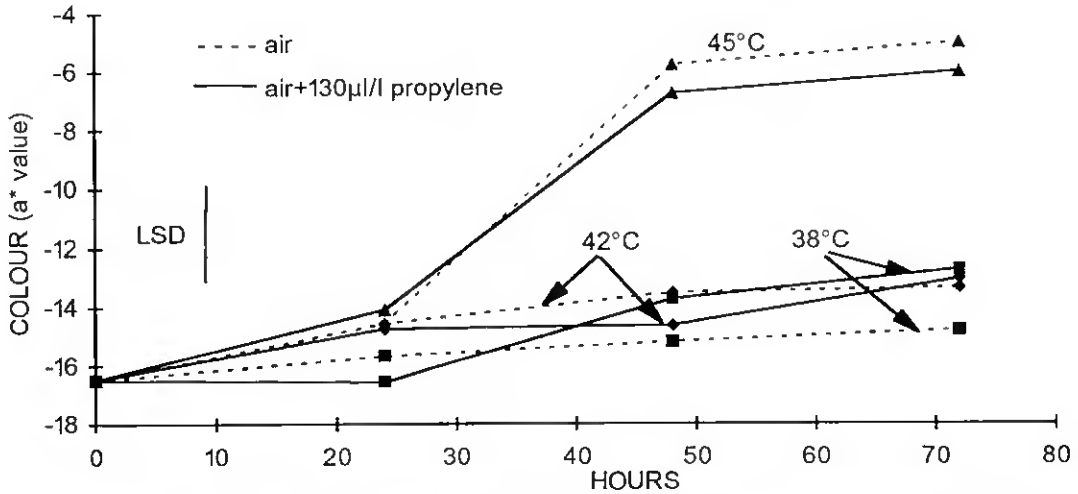


Figure 5.10. The effect of temperature (38, 42 and 45°C) on flesh colour of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream with 130µl/l propylene or air free of propylene.
LSD at α=0.05.

Table 5.1. Ripening parameters of 'Hayward' kiwifruit at harvest and, after 72 hours at 38 and 42°C and after 48 hours at 45°C in a continuous, humidified, air stream with 130µl/l propylene and air free of propylene.

Hours	Temperature (°C)	Propylene (µl/l)	Flesh firmness (kgf)	Core Firmness (kgf)	SSC (% Brix)
0			3.5 a**	11.7 a	11.7 c
72	38	0	2.5 ab	8.7 ab	12.2 bc
		130	0.97 b	5.9 b	13.5 ab
	42	0	2.43 ab	8.7 ab	12.2 bc
		130	2.43 ab	8.6 ab	12.0 bc
48	45	0	2.03 ab	8.5 ab	11.6 c
		130	2.77 a	8.8 ab	11.7 bc

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

CHAPTER 6. EFFECT OF CHILLING ON THE INDUCTION OF ETHYLENE BIOSYNTHESIS AND ASSOCIATED CHANGES OF RESPIRATION, RIPENING PARAMETERS AND FATTY ACIDS COMPOSITION OF 'HAYWARD' KIWIFRUIT

ABSTRACT

The chilling requirements for ethylene production and ripening as well as changes in fatty acids and membrane permeability were studied in 'Hayward' kiwifruit. Fruit were stored at 0, 5, 10, 15 and 20°C for 5, 12 and 17 days before rewarming to 20°C for 10 more days. Measurements of ethylene and CO_2 production, ACC content, ACC synthase and ACC oxidase activities, SSC, flesh and core firmness, flesh colour, fatty acid composition and electrolyte leakage were performed during the experiment.

Kiwifruit stored at 0, 5, 10 and 15°C did not ripe, produce ethylene or show ACC synthase or ACC oxidase activity during the 17 days. Kiwifruit rewarmed after 5 days storage at the above temperatures followed the same pattern. After 12 and 17 days storage, rewarmed fruit started autocatalysis of ethylene production within 24 hours, followed by the ripening of the fruit. Autocatalysis of ethylene production during shelf-life at 20°C was correlated with the increase in ACC content, and the activities of ACC synthase and ACC oxidase. Ethylene production after rearming, for fruit stored for 12 days, was higher in fruit stored at 0°C, while for fruit stored for 17 days it was higher in fruit kept at 15°C. Fruit placed continuously at 20°C started autocatalysis of ethylene production after 19 days with concomitant increase in ACC content, ACC synthase and ACC oxidase activities and ripening. Respiration increased after rewarming, coincidentally with the increase in ethylene production.

Membrane permeability and unsaturated/saturated fatty acid ratio increased during storage in all treatments. The highest increase was during the first 5 days and at the lowest temperatures. The increase in unsaturated/saturated fatty acid ratio was caused mainly by a decrease in palmitic and an increase in oleic acids. Stearic, linoleic and linolenic acids had insignificant changes during storage. There was no correlation

between fatty acid unsaturation or membrane permeability and ethylene production or ripening in kiwifruit.

INTRODUCTION

At 21°C, kiwifruits (cv. Hayward) begin to produce ethylene at an increasing rate (exponentially) after exceeding a threshold level of 0.1 µl/kg/h (Hyodo and Fukazawa, 1985; Hyodo et al. 1987; Arpaia et al., 1994a). Hyodo and Fukasawa (1985) suggested that chilling stress in kiwifruit advances the onset of ethylene production in the fruit when transferred to 21°C possibly by stimulating the formation of ACC as for other fruit.

Various chilling sensitive plants respond differently to chilling stress regarding the stimulation of ethylene production (Wang, 1989). Some tissues such as pears and 'Honey dew' melons accelerate their ethylene production during the chilling stress (Sfakiotakis and Dilley, 1974; Wang et al., 1985; Lipton and Wang, 1987), while others do not show any stimulation until after being transferred to warmer temperatures, as for example, cucumbers (Wang and Adams, 1982; Andersen and Kent, 1983), and zucchini squash (Mencarelli et al., 1983).

It was observed that chilling induces ethylene biosynthesis in cucumber (Wang and Adams, 1982) and bean leaf (Field, 1984) at the ACC synthesis level, the later being increased with transference to warm temperatures. Wang and Adams (1982) and Knee (1987) referred a reduced activity of ACC synthase activity at low temperatures in cucumbers and pears, its activity increasing only upon rewarming of the fruit. In Granny Smith apples at low temperatures, ACC accumulated while ACC oxidase activity was low (Gaudierre and Vendrell, 1993). The sharp increase in ethylene production observed upon rewarming was a consequence of the activation of ACC oxidase activity rather than the increase in ACC. This enhancement is probably the result of an activation of ACC synthase related to a stress response, or an inhibition of ACC oxidase probably due to the cold-induced alterations of the membrane properties. Similar results were observed by Jobling et al. (1991) in apples stored for more than 8 days at 0°C. Lelievre et al. (1995) reported that ACC oxidase, in addition to ACC

synthase, is induced during chilling of preclimacteric Granny Smith apples and accumulates before any transfer of the fruit to warmer temperatures.

Electrolyte leakage is a parameter that has often been used to indicate physical damage to the plasmalemma resulting from low-temperature stress (Parkin and Kuo, 1989). Chilling injury was evaluated in tomato leaves by the rate of electrolyte leakage that increased following chilling treatment, suggesting some degree of injury to the plasmalemma (Senaratna et al., 1988).

The ratio of unsaturated to saturated fatty acids increased with ripening in 'Honey dew' muskmelons coincidentally with the decrease in chilling sensitivity that has been associated with this factors (Forney, 1990). In apple flesh, however, the degree of unsaturation of fatty acids decreased as fruit ripened (Lurie and Ben-Arie, 1983). Heureux et al. (1993) found increasing electrolyte leakage and fatty acid unsaturation of tomato during storage at 1°C. Schirra and Sass (1994) found significant changes in fatty acid composition during storage of grapefruit at temperatures from 4 to 12°C with the proportion of linolenic and palmitoleic acids increasing and palmitic, stearic and oleic acids decreasing.

Since it was observed that lipid peroxidation increases when plant tissues are subjected to cellular damage (Galliard, 1978) or undergoing senescence (Dhindsa et al., 1981), it was suggested that the lipoxigenase activity is involved in the biosynthesis of ethylene under situations like senescence (Bousquet and Thirman, 1984), wounding or low-temperature stress (Kacperska and Kubacka-Zebalska, 1985).

Gerasopoulos (1988) reported that the timing of the chilling requirement of pears satisfactory correlates with the increase in unsaturated/saturated fatty acid ratio. Also, some biological effects of methyl jasmonate/jasmonic acid studied recently include ethylene biosynthesis (Chou and Kao, 1992; Sanz et al., 1993).

The purpose of this study was to investigate the chilling requirements for ethylene production and ripening of mature unripe 'Hayward' kiwifruit. Thus, it was studied which chilling temperature and its duration was more effective in developing competency to produce ethylene upon rewarming of the fruit. The changes in fatty acid

composition and membrane permeability and their possible relation to chilling-induced ethylene production were also investigated.

MATERIAL AND METHODS

1. Plant material and treatments

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 8.5 kgf flesh firmness and 5.1 (% Brix) SSC. After selection for uniformity of size and freedom from defects, fruit were placed in 5-litre jars through which a continuous, humidified, air stream free of ethylene was passed at a rate of 100ml/min. Each set of six jars was kept in a separate water bath at a constant temperature of 0, 5, 10, 15 and 20°C. Experiments were set within 24 hours.

At intervals of 0, 5, 12 and 17 days, 20 fruit per replication were removed from the jars. One half was used immediately for measurements of ACC content, ACC synthase and ACC oxidase (*in vivo*) activities, flesh and core firmness, SSC, flesh colour, electrolyte leakage and fatty acid analysis. The other half was put at 20°C in the same conditions for 10 more days. After this period of time the same measurements were performed, except for electrolyte leakage and fatty acid analysis. In fruit continuously at 20°C, the above mentioned measurements were done at intervals of 0, 8, 15 and 26 days. Ethylene and CO_2 production were measured daily. Ethylene was measured during all the experiment, while respiration was measured only when fruit were rewarmed.

The experimental design was a two-factor experiment distributed in a complete randomised design with the temperatures as the first factor, the number of days in storage as second and the jars as replications. Each treatment consisted of 4 replications with 60 fruit per replication.

2. Measurements

2.1. Ripening parameters and gas analysis

Ripening parameters (flesh and core firmness, SSC and flesh colour) and gas analysis (ethylene and CO_2 production) were measured as described in chapter 3.

2.2. ACC content, ACC synthase and ACC oxidase activities

ACC content and ACC synthase and ACC oxidase activities were measured as described in chapter 4.

2.3. Electrolyte leakage

Electrolyte leakage measurements were based on the methods described by Lester et al. (1988) and Parking and Kuo (1989) with modifications. Freshly excised discs (6 discs with a total of 4g) of mesocarp tissue were placed in 20 ml destilled water, shacked and incubated at ambient temperature. Conductivity of the suspending solution was measured after 5 hours. Then, the flasks were put in an oven at 100°C for 1 hour, cooled to room temperature, conductivity was measured again and taken as total electrolyte leakage. Values are presented as percentage of total.

2.4. Fatty acids analysis

Lipid extraction procedure and fatty acids analysis were based on the methods of Folch et al. (1957) and AOAC official methods of analysis (1984). Peeled kiwifruit tissue (20g without seeds) was homogenised in 100ml of 2:1 chloroform/methanol solvent with a polytron homogeniser. The homogenate was filtered through Whatman #1 filter paper in a separator funnel and the filtered residue was washed with 50ml of the same solvent. Twenty five ml of 0.58% NaCl were added to the filtrate in the separator funnel which was shaken well and left for 4 hours to separate the two phases. The total lipids were obtained in the chloroform phase which was concentrated to oil in a rotary evaporator under reduced pressure at 40°C. The oil was placed in an oven at 105°C for 20 min to eliminate any trace of water, dissolved in 2 ml chloroform and transferred to 8.5ml Teflon lined screw cap test tubes. Tubes were filled to the top with chloroform and stored at -20°C for further analysis of fatty acids.

For the fatty acids analysis, the oil/chloroform extract was transferred to evaporation tubes and the chloroform phase was evaporated with a rotary evaporator at 40°C. Concentrated oil was placed in an oven at 60°C for 30min for further drying. Four ml of 0.5N methanolic NaOH solution was added to the flasks which were then attached to a condenser and the mixture was boiled by placing the tubes in a boiling water-bath until fat globules disappeared (5-10min); 5ml of BF3/methanol were added with a pipette through the condenser and boiling was continued for another 2 minutes. Two ml heptane were added through the condenser and boiling was further continued for 1 min. Heat was removed from the condenser and 5ml of saturated NaCl solution were added to float heptane phase to the neck of the flask. The upper heptane layer was transferred to glass test tubes. Two µl of the heptane solution were injected into a Varian 3700 gas chromatograph for methyl esters fatty acids identification. The gas chromatograph was equipped with a 4mm x 2m column packed with 10% DEGS on 80/100 mesh Chromosorb WAW at 190°C, an injector at 220°C and a flame-ionisation detector at 250°C. The carrier gas was N_2 at a flow rate of 20ml/min.

Fatty acids are described in the nomenclature by the number of carbon atoms of the chain (n) and the number of the double bonds following the colon (n:#) (Lehninger, 1982). The unsaturated/saturated fatty acid ratio was calculated by the formula:

(18:1+18.2+18.3)/(16:0+18:0)

16:0 = Palmitic acid
18:0 = Stearic acid
18:1 = Oleic acid
18:2 = Linoleic acid
18:3 = Linolenic acid

3. Statistical analysis

Statistical analysis were performed as described in the methods of chapter 3.

RESULTS

1. Ripening behaviour of kiwifruit after 5 days storage

1.1. Ethylene production

Ethylene production at harvest was almost null and did not increase significantly during storage at 0, 5, 10 and 15°C for 5 days at any temperature or upon transference to 20°C for 10 more days (fig. 6.1A). Values did not pass 0.15µl/kg/h.

1.2. Carbon dioxide production

Respiration increased significantly upon rewarming of the fruit to 20°C in the first 2 days and then remained constant for all treatments (Fig. 6.1B). The increase in CO_2 production did not exceeded 10ml/kg/h. After 5 days at 20°C, respiration rate was always higher in fruit from 15°C storage than in the other treatments, but differences were not significant.

1.3. ACC content, ACC synthase and ACC oxidase activities

ACC content, ACC synthase and ACC oxidase activities were very low at harvest (Table 6.1). Their values did not increase significantly after 5 days storage at 0, 5, 10 and 15°C neither after the 10 following days at 20°C. Although values of ACC and ACC oxidase activity showed statistical significant increases during storage, their maximum values of 0.065 nmol/g and 2.94 nl/g/h, respectively, are negligible when compared with fruit autocatalytically producing ethylene.

1.4. Changes of firmness, soluble solids content (SSC) and flesh colour

Firmness of the flesh and core did not decrease significantly during 5 days storage at any temperature or upon rewarming of the fruit for 10 more days (Table 6.2). It was observed a small increase in SSC with time. However, this increase did not exceed 2% Brix in any treatment, staying the fruit unripe at the end of the experiment. Similar pattern was observed for the green colour of the flesh.

2. Ripening behaviour of kiwifruit after 12 days storage

2.1. Ethylene production

Ethylene production was almost null during storage for 12 days at 0, 5, 10 and 15°C (fig. 6.2A). When fruit were transferred to 20°C, they started autocatalysis of ethylene production in 24 hours for all treatments (Fig. 6.2Aa). Ethylene production did not show statistical significant differences between treatments except for fruit stored at 0°C which had significantly higher values than the other treatments after 6 days shelf-life at 20°C (Fig. 6.2A).

2.2. Carbon dioxide production

Respiration increased upon rewarming of the fruit to 20°C in all treatments (Fig. 6.2B). The increase was significant in all treatments except for fruit transferred from 15 to 20°C. Values were higher in fruit previously treated with 0°C followed by 5, 10 and 15°C. However, differences were significant only between fruit pre-treated with 0 and 10 or 15°C, and between 5 and 15°C after 1 day shelf-life. Carbon dioxide production was almost constant in all treatments from 4 to 10 days shelf-life at 20°C. Fruit producing more ethylene had generally the highest values of respiration (Fig. 6.2A and B).

2.3. ACC content, ACC synthase and ACC oxidase activities

ACC content, ACC synthase and ACC oxidase activities did not increase significantly during 12 days storage at 0, 5, 10 and 15°C (Table 6.3). However, their values were significantly higher after 10 days at 20°C than at removal from storage, for all treatments. The ACC content was the highest in fruit from 0°C storage followed by fruit from 10, 5 and 15°C. The ACC synthase and ACC oxidase activities showed significantly lower values in fruit from 15°C than from the other treatments, which were not significantly different among them.

2.4. Changes of firmness, soluble solids content (SSC) and flesh colour

Firmness of the flesh and core did not decrease significantly during 12 days except at 10 and 15°C (Table 6.4). However, the decrease in firmness was not significant in

terms of ripening. Kiwifruit decreased significantly flesh and core firmness upon rewarming of the fruit for 10 days at 20°C. Fruit were ripe after 10 days shelf-life except for fruit from 15°C storage which had significantly higher values of core firmness than the other treatments.

The SSC increased significantly after 12 days storage in all treatments except at 15°C (Table 6.4). However, the increase was not significant in terms of ripening. After 10 days shelf-life, SSC increased significantly in all treatments. Values were significantly higher in fruit pre-stored at 15°C than in fruit pre-stored at 0, 5 and 10°C, which showed no significant differences among them.

Kiwifruit did not significantly lost the green colour of the flesh after 12 days storage at any temperature (Table 6.4). After 10 days shelf-life at 20°C, fruit had a significant increase in the loss of green colour in all treatments without significant differences among treatments, except for fruit pre-stored at 10°C, which showed significantly lower values than fruit pre-stored at 15°C.

3. Ripening behaviour of kiwifruit after 17 days storage

3.1. Ethylene production

Ethylene production was almost null during storage for 17 days at 0, 5, 10 and 15°C (fig. 6.3A). When fruit were transferred to 20°C, they followed the same pattern as for 12 days storage, starting autocatalysis of ethylene production without delay in all treatments (Fig. 6.3Aa). Ethylene production did not show significant differences between treatments except for fruit from 0°C storage, which had significantly lower values than the other treatments after 6 days shelf-life at 20°C (Fig. 6.3A).

3.2. Carbon dioxide production

Respiration was significantly higher after 1 day shelf-life at 20°C than at removal from 0, 5, 10 and 15°C storage and then remained almost constant for all treatments (Fig. 6.3B). Values were not significantly different among fruit pre-stored at 5, 10 and 15°C. Between 2 and 6 days shelf-life, CO_2 production for fruit from 0°C storage was

significantly lower than for fruit from 10 or 15°C. Fruit producing more ethylene had generally the highest values of CO_2 production (Fig. 6.3A and B).

3.3. ACC content, ACC synthase and ACC oxidase activities

ACC content, ACC synthase and ACC oxidase activities did not increase significantly after 17 days storage at 0, 5, 10 and 15°C (Table 6.5). The ACC content, ACC synthase and ACC oxidase activities were significantly higher after 10 days shelf-life at 20°C than at removal from storage for all treatments. The ACC, ACC synthase and ACC oxidase activities showed significantly lower values in fruit pre-stored at 0°C than in the other treatments.

3.4. Changes of firmness, soluble solids content (SSC) and flesh colour

Firmness of the flesh and core did not decrease significantly during 17 days storage at any temperature except at 10°C (Table 6.6). However, as it was observed after 12 days storage, the decrease in firmness was not significant in terms of ripening. After rewarming, kiwifruit decreased significantly flesh and core firmness, becoming ripe during shelf-life at 20°C.

The SSC increased significantly after 17 days storage at 0, 5, 10 and 15°C (Table 6.6). Values were significantly lower at 15°C than in the other treatments, which were not significantly different among them. After 10 days shelf-life, SSC increased significantly in all treatments. Values were significantly higher in fruit pre-stored at 10 and 15°C, without significant differences between them, followed by fruit pre-stored at 5 and 0°C.

After 17 days storage, kiwifruit did not significantly lost the green colour of the flesh at any temperature (Table 6.6). After 10 days shelf-life at 20°C, fruit had a significant fading of flesh green colour in all treatments, with fruit pre-stored at 0°C loosing less colour than the other treatments.

4. Ripening behaviour of fruit kept continuously at 20°C

4.1. Ethylene and carbon dioxide production

Kiwifruit kept continuously at 20°C started autocatalysis of ethylene production after ethylene reached a threshold level of 0.2μl/kg/h after 19 days post-harvest (Fig. 6.4Aa). The ethylene production peak was achieved after 24 days at 20°C and then ethylene production decreased (Fig. 6.4A).

Carbon dioxide production did not change significantly till just before start of autocatalysis of ethylene production (Fig. 6.4A). Respiration increased significantly after the fruit started to produce ethylene, reached a maximum after 23 days at 20°C and then decreased.

4.2. ACC content, ACC synthase and ACC oxidase activities

For kiwifruit stored continuously at 20°C, ACC content, ACC synthase and ACC oxidase activities were very low and did not increase significantly in the first 15 days, but had a significant increase from 15 to 26 days at 20°C (Table 6.7).

4.3. Changes of firmness, soluble solids content (SSC) and flesh colour

Firmness of the flesh and core did not decrease significantly for the first 8 days in kiwifruit stored continuously at 20°C (Table 6.8). However, it showed a decrease from 8 to 15 days and a high significant decrease from 15 to 26 days.

The SSC and the fading of flesh green colour did not increase significantly till 15 days storage at 20°C (Table 6.8). Their values had a highly significant increase from 15 to 26 days storage.

5. Effect of chilling on fatty acid composition

Figures 6.5 and 6.6 show that, generally, the major fatty acid component (35-40%) consisted of linolenic acid, followed by oleic acid (26-36%), palmitic acid (15-22%), linoleic acid (9-11%) and stearic acid (2-5%).

Palmitic acid levels had an insignificant decrease during 17 days storage at 20°C, while in the other treatments the decrease was significant and occurred mostly in the first 5 days (Fig. 6.5A). Values were always higher at 20°C followed by 15, 10, 5 and 0°C. Differences were not significant between 20 and 15°C or among 10, 5 and 0°C.

Stearic acid did not change significantly with storage time at any temperature (Fig. 6.5B). Values were significantly higher at 15°C than at 0°C after 5 days. After 12 and 17 days storage there were not significant differences between any treatment.

Oleic acid had a significant increase in the fist 5 days storage for all treatments (Fig. 6.6A). Values were significantly higher at 0, 5 and 10°C than at 15 and 20°C. The percentage of oleic acid continued to increase thereafter at 0 and 5°C while in the other treatments did not significantly change. After 12 days storage, the percentage of oleic acid was significantly higher at 0 and 5°C than at 15 and 20°C. In the end of the experiment, oleic acid was significantly higher at 0 and 5°C than in the other treatments which did not show significant differences among them.

Linoleic acid did not significantly change during storage in any treatment (Fig. 6.6B). After 5 and 12 days storage values were significantly higher at 20°C than at 0°C but after 17 days differences among treatments were not significant.

Linolenic acid did not change or show significant differences between treatments during the experiment (Fig. 6.6C).

6. Effect of chilling on fatty acid unsaturation/saturation ratio

Fruit stored at 0, 5 and 10°C had a significant increase in the unsaturated/saturated fatty acid ratio after 5 days storage and remained almost constant thereafter (Fig. 6.7A). Unsaturated/saturated fatty acid ratio increased significantly at 15°C only from 0 to 12 days storage, and at 20°C the increase was insignificant during all experiment.

After 5 days storage, unsaturated/saturated fatty acid ratio was significantly higher at 0°C followed by 5, 10, 15 and 20 being the last 3 not significantly different among them (Fig. 6.7A). After 12 days storage, values were only significantly higher at 0 than at 15 or 20°C. After 17 days storage, the unsaturated/saturated fatty acid ratio was

significantly higher at 0 and 5°C followed by 10, 15 and 20°C. Values were significantly different between 0 or 5°C and 15 or 20°C.

7. Effect of chilling on membrane permeability

Membrane permeability of kiwifruit, expressed by the change in electrolyte leakage, had a significant increase during the first 5 days storage in all treatments (Fig. 6.7B). Values were higher at 0°C followed by 5 and 10°C without significant differences between them. Fruit at 15 and 20°C showed values of electrolyte leakage significantly lower than the other treatments.

From 5 to 17 days storage, the increase in electrolyte leakage of kiwifruit was higher at 15 and 20°C than at 0, 5 and 10°C. However, after 12 days storage, electrolyte leakage was significantly lower at 20 than at 0°C, while after 17days storage it was significantly lower at 20°C than in the other treatments, which did not show significant differences among them.

DISCUSSION

Kiwifruit harvested at an early stage of maturity produced ethylene in about 19 days at room temperature like a climacteric fruit confirming the results of chapter 3. The increase in ethylene was accompanied by the rise in respiration and SCC, the loss of green colour and the decrease of firmness. Similar results were observed by Hyodo and Fukasawa (1985). The ethylene production was a result of the increase in ACC synthase and ACC oxidase activities.

Five days storage at low temperature were not enough to induce autocatalytic ethylene production upon transference of kiwifruit to 20°C. A period of 12 days at temperatures from 0 to 15°C satisfied the requirement of kiwifruit to autocatalyticaly produce ethylene 24 hours after rewarming. A longer period of 17 days induced ethylene production with no delay after rewarming of the fruit. This is in agreement with other authors who reported that the lag period of kiwifruit to produce ethylene at room

temperature become shorter as the storage period at 0°C is extended (Hyodo and Fukazawa, 1985; Hyodo et al. 1987; Arpaia et al., 1994a).

Kiwifruit, as cucumber (Wang and Adams, 1982) and contrasting with apples, (Larriguadiere and Vendrell, 1993) did not produce ethylene and did not show any activity of ACC synthase or ACC oxidase during a chilling period up to 17 days. Some plant tissues have a reduced ACC synthase activity at low temperatures increasing only upon rewarming (Wang and Adams, 1982; Knee, 1987), while in others tissues, ACC accumulates at low temperature, the increase in ethylene production observed upon rewarming being mainly a consequence of the activation of ACC oxidase activity (Jobling et al., 1991; Gaudierre and Vendrell, 1993). It is suggested that a message involving RNA and protein synthesis is produced or unmasked during the chilling period and probably involves mRNA coding for ACC synthase (Wang and Adams, 1982; Wang, 1989). In tissues where ethylene is not produced until after being transferred to warmer temperatures, the translation and the synthesis of a new protein were not completed at chilling temperatures. Although the signal is turned on by chilling stress, ethylene is not produced until after the completion of the translation and the formation of ACC synthase at warmer temperatures (Wang and Adams, 1982). However, as for ACC synthase, kiwifruit did not show ACC oxidase activity during the chilling period, suggesting a similar behaviour for both enzymes.

It was shown in chapter 4 (fig. 4.4) that there was no gene transcription of ACC synthase or ACC oxidase up to 8 days exposure to low temperatures, when fruit were not treated with propylene. This may explain why the 5 days chilling were not enough to induce ethylene production. Twelve days chilling were probably enough to activate the transcription of the ACC synthase and ACC oxidase genes induced by chilling, with the translation and/or activation of the enzymes being completed at warmer temperatures as suggested by Wang and Adams (1982) and Wang (1989). We suggest that as the time under low temperature is increased, the process of translation and activation of the enzymes is probably completed, explaining the decrease in the lag period for ethylene production, upon rewarming of the fruit, as reported by Hyodo and Fukazawa (1985), Hyodo et al. (1987) and Arpaia et al. (1994a). However, more research is needed at the molecular level to confirm this hypothesis.

The warming up of cucumber exposed to a chilling temperature (2.5°C) gave an increase in ethylene production while fruit exposed to low but non chilling temperature (13°C) showed very little change in ACC level, ethylene production or ACC synthase activity (Wang and Adams, 1982). In the present study it was observed that not only 0°C but also temperatures up to 15°C can induce an earlier ethylene production and ripening of kiwifruit upon transference to 20°C.

Sfakiotakis and Dilley (1974) reported a better stimulation of ethylene production in pears after 7 days at 5° or 10°C rather than at 0° or 7.5°C. In the present study it was observed that the efficiency of the low temperature on the capacity of the fruit to produce ethylene depends mostly on the time of exposure. Fruit kept for 12 days at 0°C had a greater capacity to produce ethylene than if kept at superior temperatures. After 17 days storage, fruit at 0°C had decreased their capacity to produce ethylene while the temperature of 15°C allowed for better efficiency on ethylene production. Wang and Adams (1982) and Andersen (1986) reported that the conversion of ACC to ethylene was damaged by prolonged chilling.

Blankenship and Richardson (1985) reported, for pears, that during the chilling period required for initiation of ethylene production, the capacity to convert ACC to ethylene developed first, followed by production of ACC and ethylene. Hyodo and Fukasawa (1985) and Hyodo et al. (1987) believe that chilling induces an increase in the levels of ACC and ACC oxidase activity in kiwifruit upon rewarming. It was found in the present study that, up to 17 days storage at chilling temperatures, ACC content and ACC synthase and ACC oxidase activities were very low. Ethylene production upon rewarming was correlated with the activities of both ACC synthase and ACC oxidase as well as ACC content. Kiwifruit producing more ethylene showed higher ACC synthase and ACC oxidase activities than fruit producing less ethylene in agreement with Hyodo et al. (1987).

The warming up of kiwifruit, by itself, increased the respiration rate in 24 hours independently from ethylene production coinciding with the postulated by Arpaia et al. (1994a). When fruit started to produce ethylene, respiration increased too and was generally higher in fruit that produced more ethylene than in fruit that produced less ethylene, as a response to the endogenous ethylene (Tucker, 1993). This behaviour

showed that respiration increases due to two factors: the increase in temperature and the increase in ethylene production. Jobling et al. (1991) found that respiration of apples, when they were rewarmed, increased with time of exposure to low temperature, up to 16 days. The same authors found a rise and a subsequent decrease in internal CO_2 upon rewarming, while in this work kiwifruit respiration increased in the first 24 hours upon rewarming and then remained constant during the 10 days the experiment lasted. It seems that respiration remains at a high level till kiwifruit reaches the ethylene climacteric peak as a response to ethylene production. The decrease in respiration may occur after the ethylene production declines late in the senescence, as for some other fruit (Tucker and Grierson, 1987).

Changes in membrane permeability have been correlated to changes in the lipid composition of the membrane, either in the sterol level - the ratio of phospholipids to sterols - or the fatty acids composition of the phospholipids (Lurie and Ben-Aire, 1983). In the present study there was a positive correlation between the unsaturated/saturated fatty acid ratio and membrane permeability.

The increase in fatty acid unsaturation and membrane permeability were higher at lower temperatures as a response to chilling stress in order to prevent chilling injury as reported by Wilson and Crawford (1974a,b). The increase in unsaturation may be the cause of the increase on membrane permeability. These results are confirmed by Heureux et al. (1993). However, Spychalla and Desborough (1990) reported that higher rates of fatty acid unsaturation were related to lower membrane permeability. Whitaker (1994) reported that fatty acid unsaturation increased slightly during chilling in tomato fruit, while Parkin and Kuo (1989) found an increase only upon rewarming of cucumber fruit.

Forney (1990) reported that the increase in unsaturation of fatty acids was primarily due to the increase of palmitoleic and oleic acids since the other fatty acids had only small changes. The results of this study showed that in kiwifruit the increase in unsaturated/saturated fatty acid ratio was mostly due to a decrease in palmitic and an increase in oleic acids. The changes in those fatty acids were more pronounced at lower temperatures.

Gerasopoulos (1988) suggested that the increase in fatty acid unsaturation during chilling, which coincides with the chilling requirements for ethylene production, may activate membrane-bound enzymes such as ACC oxidase and induce ethylene production and ripening, upon rewarming, in pears. The results of our experiment indicated no clear correlation between fatty acid unsaturation or electrolyte leakage and ethylene production or ripening in kiwifruit. In the present study, it was observed that the most pronounced increase in the unsaturated/saturated fatty acid ratio and electrolyte leakage occurred in the first 5 days storage preceding the requirement for the induction of ethylene production (12 days).

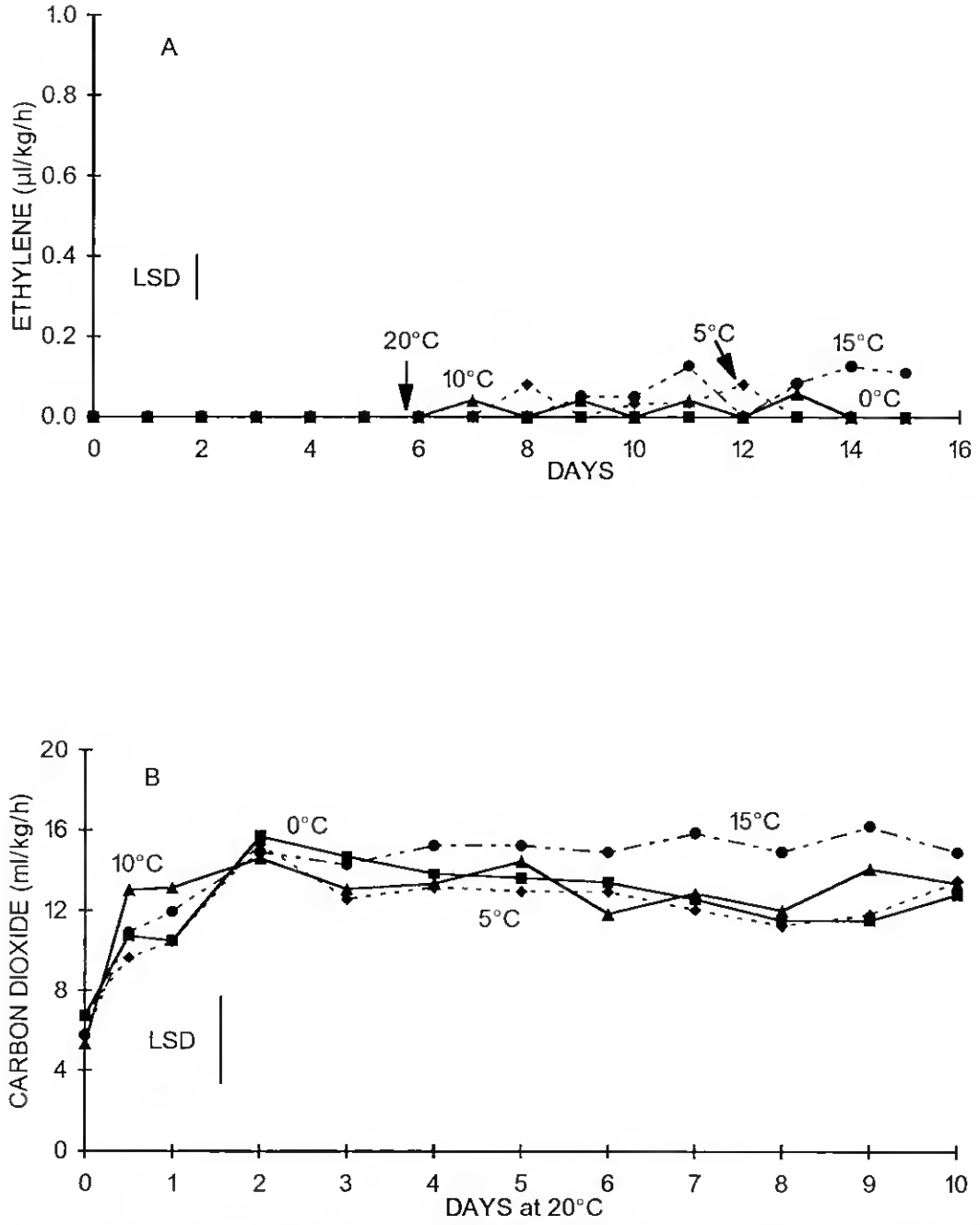


Figure 6.1. Ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, and 15°C for 5 days, then transferred to 20°C. Ethylene was measured during all the experiment, while CO_2 was measured only when fruit were rewarmed. The first arrow in A shows when fruit were transferred to 20°C.
LSD at α=0.05.

Table 6.1. ACC content and ACC synthase and ACC oxidase activities of 'Hayward' kiwifruit at harvest, after 5 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	ACC (nmol/g)	ACC synthase (units/mg)	ACC oxidase (nl/g/h)
0	ambient	0.013 c**	2.56 a	0.15 d
5	0	0.055 ab	1.56 a	0.33 d
	5	0.044 b	1.56 a	1.48 d
	10	0.053 ab	3.39 a	1.00 bc
	15	0.071 a	0.74 a	1.44 b
5+10	0->20	0.076 a	1.15 a	2.30 a
	5->20	0.055 ab	1.15 a	2.94 a
	10->20	0.042 b	1.56 a	0.69 cd
	15->20	0.065 ab	1.73 a	1.43 b

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).
1 unit/mg = 1pmol ACC/mg protein/2hours.

Table 6.2. Ripening parameters of 'Hayward' kiwifruit at harvest, after 5 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	Flesh firmness (kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0	ambient	8.45 a**	16.93 a	5.13 d	-17.63 a
5	0	8.77 a	16.97 a	5.87 bc	-18.70 ab
	5	9.27 a	16.97 a	6.10 ab	-17.29 b
	10	7.83 a	16.77 a	5.93 bc	-17.27 a
	15	8.23 a	16.73 a	5.43 cd	-18.32 ab
5+10	0->20	8.60 a	16.70 a	5.87 bc	-18.47 ab
	5->20	8.70 a	16.10 b	5.50 cd	-17.51 a
	10->20	8.40 a	16.90 a	6.53 a	-19.25 b
	15->20	8.43 a	16.80 a	5.57 bcd	-18.35 ab

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

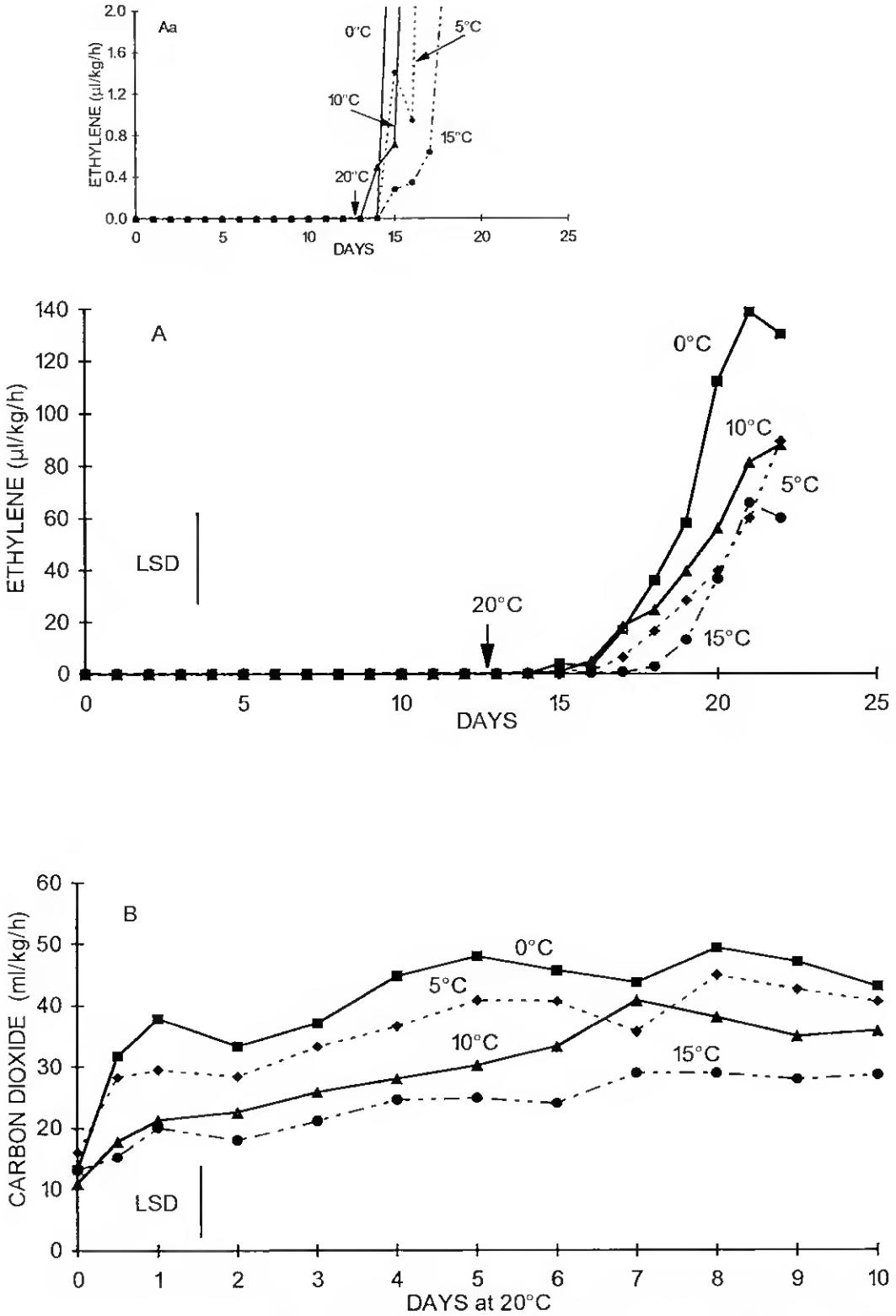


Figure 6.2. Ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, and 15°C for 12 days, then transferred to 20°C. Ethylene was measured during all the experiment, while CO_2 was measured only when fruit were rewarmed. The arrow in A shows when fruit were transferred to 20°C. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.

Table 6.3. ACC content and ACC synthase and ACC oxidase activities of 'Hayward' kiwifruit at harvest, after 12 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	ACC (nmol/g)	ACC synthase (units/mg)	ACC oxidase (nl/g/h)
0	ambient	0.013 e**	2.56 c	0.15 c
12	0	0.058 e	3.67 c	0.07 c
	5	0.063 e	2.23 c	0.81 c
	10	0.050 e	3.34 c	0.83 c
	15	0.080 e	1.11 c	1.04 c
12+10	0->20	5.027 a	43.77 a	51.24 a
	5->20	2.279 c	39.87 a	45.21 a
	10->20	3.591 b	34.30 ab	48.99 a
	15->20	1.199 d	24.62 b	31.62 b

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).
1 unit/mg = 1pmol ACC/mg protein/2hours.

Table 6.4. Ripening parameters of 'Hayward' kiwifruit at harvest, after 12 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	Flesh firmness (kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0	ambient	8.45 ab**	16.93 a	5.13 e	-17.63 cd
12	0	9.10 a	16.93 a	6.73 cd	-19.14 d
	5	8.73 a	17.03 a	7.67 c	-18.65 d
	10	6.10 c	15.63 b	7.27 c	-16.88 c
	15	7.27 bc	15.43 b	6.10 de	-18.78 d
12+10	0->20	0.27 d	0.33 d	11.53 b	-12.98 ab
	5->20	0.43 d	1.30 d	11.93 b	-13.22 ab
	10->20	0.57 d	0.90 d	12.07 b	-12.32 a
	15->20	1.23 d	2.63 c	12.30 a	-14.00 b

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

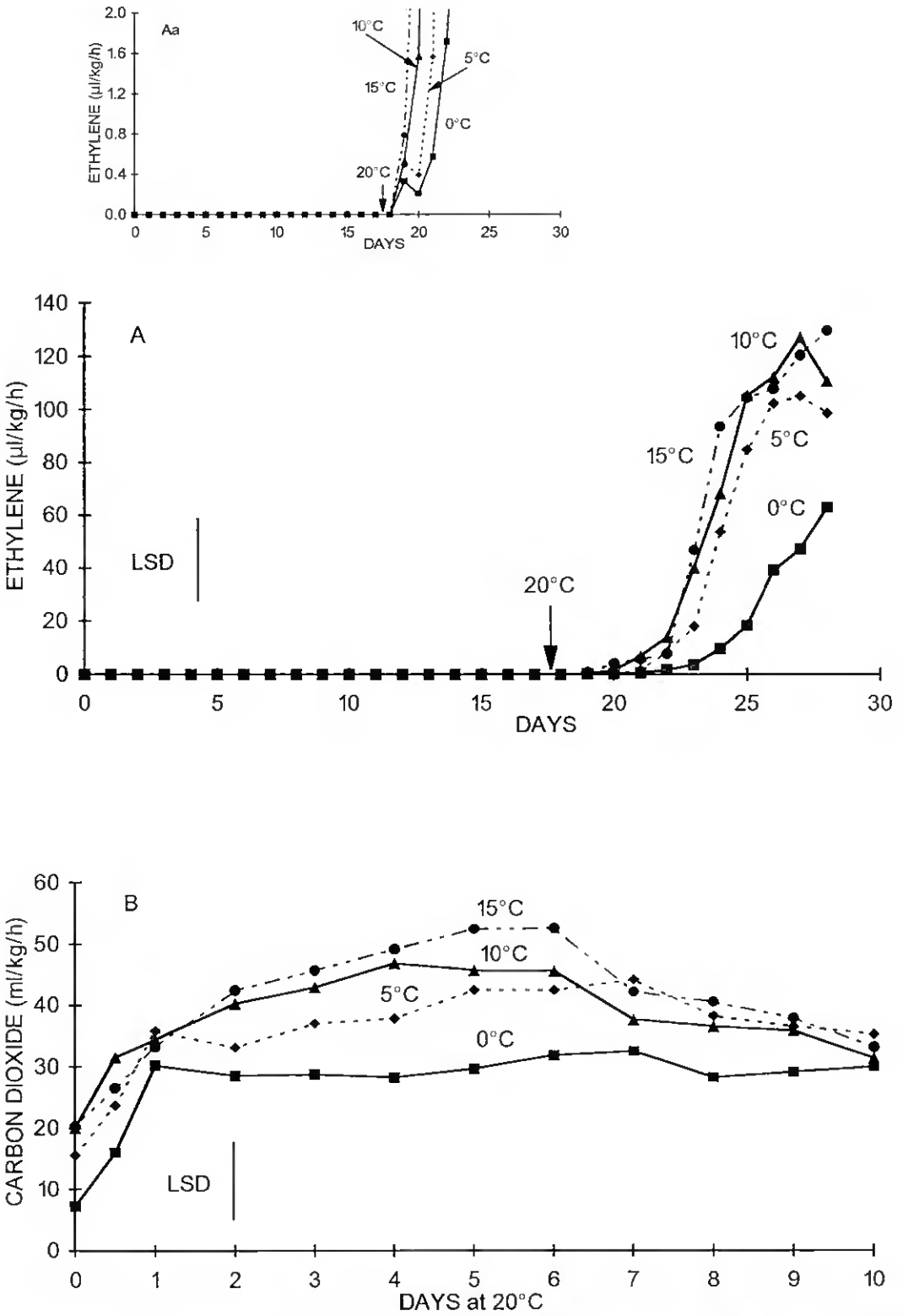


Figure 6.3. Ethylene (A) and CO_2 (B) production of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, and 15°C for 17 days, then transferred to 20°C. Ethylene was measured during all the experiment, while CO_2 was measured only when fruit were rewarmed. The arrow in A shows when fruit were transferred to 20°C. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.

Table 6.5. ACC content and ACC synthase and ACC oxidase activities of 'Hayward' kiwifruit at harvest, after 17 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	ACC (nmol/g)	ACC synthase (units/mg)	ACC oxidase (nl/g/h)
0	ambient	0.013 d**	2.56 c	0.15 c
17	0	0.050 d	2.56 c	0.65 c
	5	0.071 d	3.67 c	0.62 c
	10	0.058 d	1.11 c	0.75 c
	15	0.063 d	2.23 c	0.41 c
17+10	0->20	0.795 c	19.15 b	32.64 b
	5->20	2.360 a	31.85 a	42.56 a
	10->20	1.953 b	35.41 a	50.39 a
	15->20	2.245 a	31.07 a	49.55 a

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).
1 unit/mg = 1pmol ACC/mg protein/2hours.

Table 6.6. Ripening parameters of 'Hayward' kiwifruit at harvest, after 17 days at 0, 5, 10, and 15°C under a continuous, humidified, air stream and after transferred to 20°C for 10 more days.

Days	Temperature (°C)	Flesh firmness (kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0	ambient	8.45 a**	16.90 a	5.13 f	-17.63 c
17	0	8.27 a	17.13 a	7.73 d	-18.30 c
	5	7.97 a	16.77 a	8.23 d	-17.55 c
	10	6.13 b	15.27 b	8.27 d	-18.29 c
	15	7.90 a	16.83 a	6.23 e	-18.92 c
17+10	0->20	0.80 c	0.80 d	11.53 c	-13.45 b
	5->20	0.40 c	0.50 d	11.83 bc	-11.81 a
	10->20	0.27 c	0.27 d	12.73 a	-12.00 a
	15->20	0.30 c	0.37 d	12.60 ab	-11.89 a

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

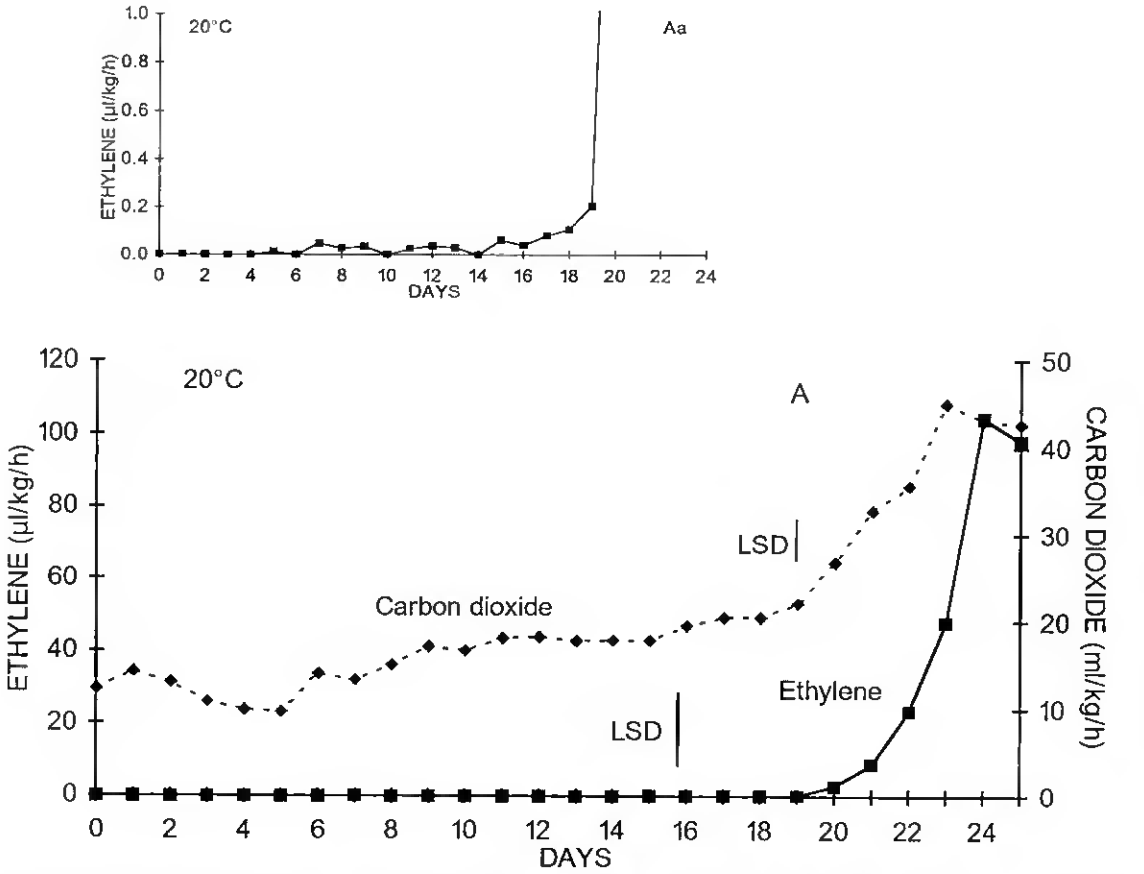


Figure 6.4. Ethylene and CO_2 production of harvested 'Hayward' kiwifruit kept at 20°C in a continuous, humidified, air stream. Aa shows a blow up graph of the threshold level for initiation of ethylene autocatalysis.
LSD at α=0.05.

Table 6.7. ACC content and ACC synthase and ACC oxidase activities of 'Hayward' kiwifruit at harvest and after 8, 15 and 26 days at 20°C under a continuous, humidified, air stream.

Days	Temperature (°C)	ACC (nmol/g)	ACC synthase (units/mg)	ACC oxidase (nl/g/h)
0	ambient	0.013 b**	2.56 b	0.15 b
8	20	0.015 b	3.67 b	1.24 b
15		0.024 b	3.67 b	1.42 b
26		4.737 a	34.98 a	41.44 a

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).
1 unit/mg = 1pmol ACC/mg protein/2hours.

Table 6.8. Ripening parameters of 'Hayward' kiwifruit at harvest and after 8, 15 and 26 days at 20°C under a continuous, humidified, air stream.

Days	Temperature (°C)	Flesh firmness (kgf)	Core firmness (kgf)	SSC (% Brix)	Flesh colour (a* value)
0	ambient	8.45 a**	16.93 a	5.13 b	-17.63 b
8	20	8.83 a	17.03 a	5.47 b	-18.86 c
15		7.17 b	16.07 b	5.43 b	-17.84 bc
26		0.40 c	0.60 c	12.33 a	-13.55 a

**Values in the same column followed by the same letter are not significantly different by Duncan's multiple range test (α=0.05).

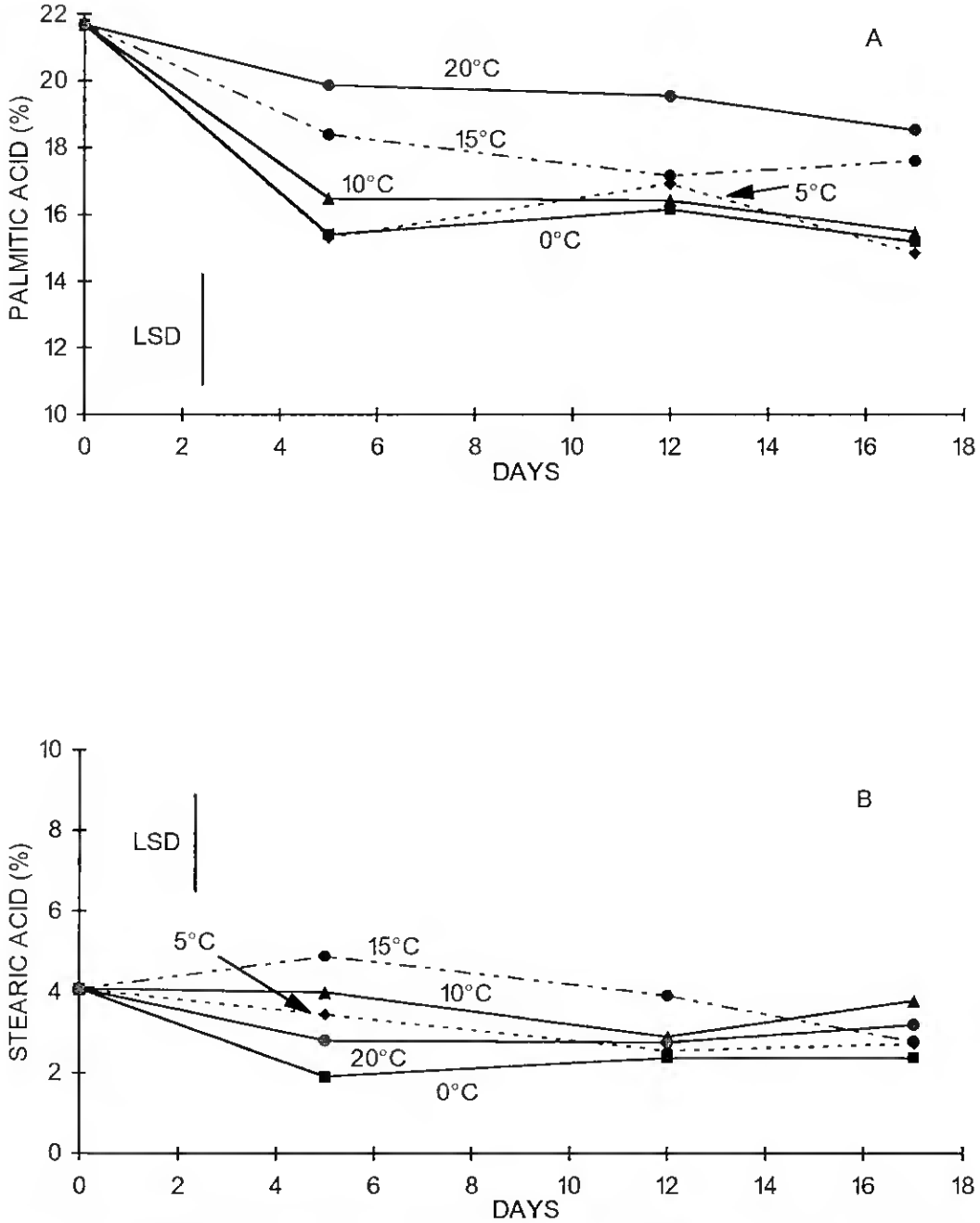


Figure 6.5. Palmitic (A) and stearic (B) acids changes of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20°C. LSD at α=0.05.

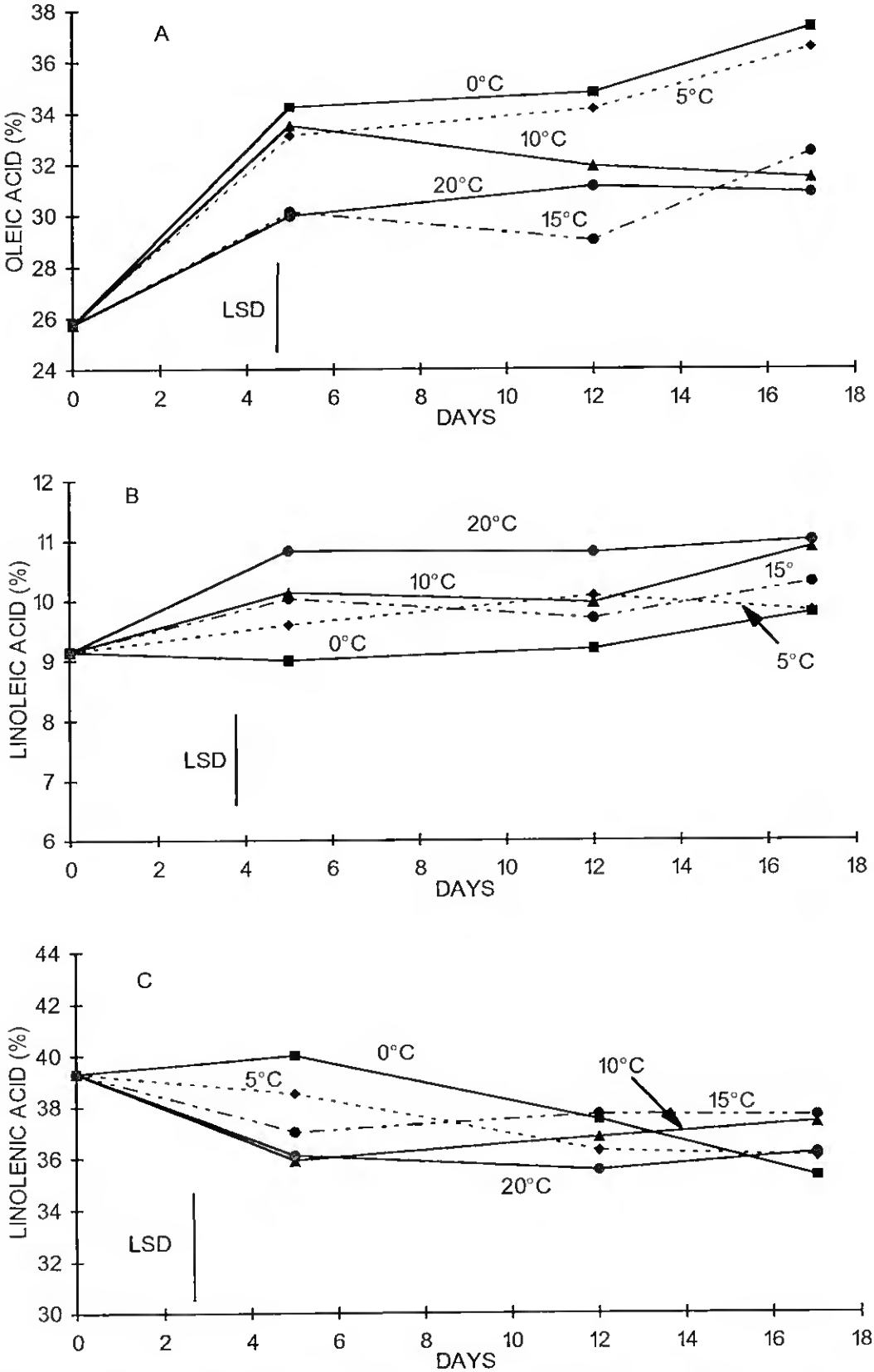
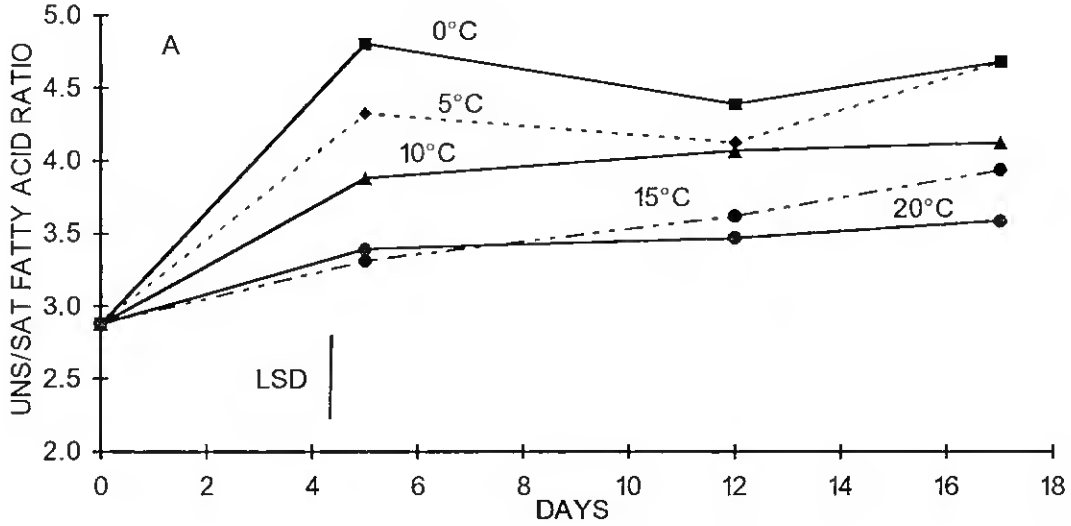


Figure 6.6. Oleic (A), linoleic (B) and linolenic (C) acids changes of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20°C.
LSD at α=0.05.



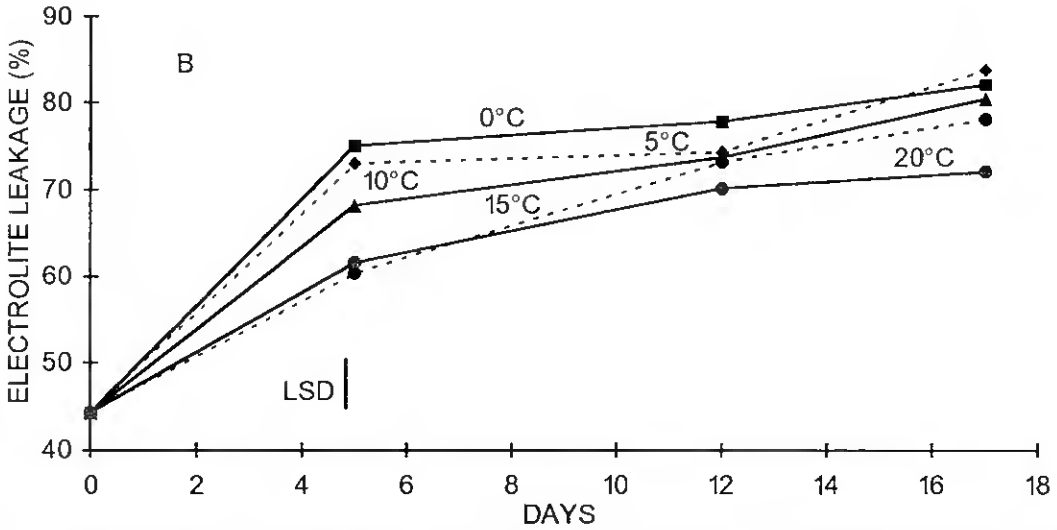


Figure 6.7. Unsaturated/saturated fatty acid ratio (A) and electrolyte leakage (B) of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20°C.
LSD at α=0.05.

CHAPTER 7. ETHYLENE BIOSYNTHESIS AND RIPENING BEHAVIOUR OF 'HAYWARD' KIWIFRUIT SUBJECTED TO CONVENTIONAL (CS), CONTROLLED ATMOSPHERE (CA) AND ULTRA LOW OXYGEN (ULO) STORAGE CONDITIONS

ABSTRACT

The effect of conventional storage (CS), controlled atmosphere (CA) and ultra low oxygen (ULO) on ethylene biosynthesis and ripening of 'Hayward' kiwifruit during and after storage at 0°C were investigated. Fruit were stored for 60, 120 and 180 days at 0°C in CS (air-control), CA ($2\%O_2+5\%CO_2$) and ULO ($0.7\%O_2+0.7\%CO_2$ and $1\%O_2+1\%CO_2$). At harvest and on removal from storage, fruit were treated with or without 130µl/l propylene for 9 days at 20°C. Measurements of ethylene and CO_2 production, ACC content, ACC synthase and ACC oxidase activities, SSC, flesh firmness, flesh colour, ethanol and acetaldehyde content were performed during the experiment.

Fruit treated with propylene at 20°C after harvest produced ethylene with a lag period of 3 days, had concomitant ACC production, ACC synthase and ACC oxidase activities, and were ripe after 3-5 days. Fruit not treated with propylene were not able to produce ethylene and to ripe during the 9 days experiment.

On removal from storage, fruit of CA had the highest firmness followed by ULO and CS. Firmness of CS stored fruit decreased faster during the first 60 days storage than in the remaining period. This effect was reduced in CA and ULO treatments. After 9 days shelf-life at 20°C, only CA and CS fruit were eating-ripe. Fruit of ULO storage needed to be treated with propylene to ripe. The SSC increased markedly during the first 60 days storage and remained almost constant thereafter in all treatments. The fading of flesh colour was lower in CA and ULO than in CS. The $0.7\%O_2+0.7\%CO_2$ treatment had the highest accumulation of ethanol and acetaldehyde followed by $1\%O_2+1\%CO_2$.

When kiwifruit were placed at 20°C, after 60, 120 and 180 days storage at 0°C, there was an induction of ethylene production with no lag period in fruit from CA and CS, independently of being treated or not with propylene. There was some accumulation of ACC and ACC synthase activity after 60 days storage for all treatments, while ACC oxidase activity increased only upon rewarming of the fruit in CA and CS. Kiwifruit removed from ULO storage showed drastically reduced capacity to produce ethylene mainly due to low ACC oxidase activity rather than reduced ACC production or ACC synthase activity.

Respiration increased upon rewarming of the fruit in all treatments. There was a tendency of the ethylene producing treatments to have slightly higher values of CO_2 production during shelf-life at 20°C.

With storage time, there was a decrease in the capacity of the warmed fruit to produce ethylene and CO_2 as well as in the activities of ACC synthase and ACC oxidase, mostly after 60 days storage.

INTRODUCTION

Kiwifruit can be air-stored for 4 to 6 months at 0°C, although extensive softening will occur (Arpaia et al.,1987). The rate of softening in storage will be accelerated if ethylene is present (Arpaia et al.,1980; McDonald, 1990). Softening of kiwifruit during cold storage has 2 distinct phases: an initial rapid rate of softening followed by a long period during which fruit firmness loss is very low (McDonald and Harman, 1982; Arpaia et al., 1987).

McDonald and Harman (1982) postulated that low oxygen (2-3%) with 3-5% CO_2 further delayed the rate of kiwifruit softening and increased storage life up to 3-4 months beyond normal air-storage life. The CA storage has been shown to reduce the rate of softening if ethylene is removed from storage rooms (McDonald and Harman, 1982; Arpaia et al., 1994b). Differences in the way the fruit soften are a result of both maturity at harvest and post-harvest treatment (MacRae et al., 1989). The CA storage may change the post-storage ripening behaviour of fruits. Arpaia et al. (1986) reported that

for firmness retention and maximisation of storage life, kiwifruit should promptly be cooled to 0°C and immediately stored at the same temperature in 2%O_2+5%CO_2, with ethylene excluded or removed to maintain its concentration below 0.05μl/l.

Most studies of CA storage of kiwifruit have been conducted on its effect on fruit flesh firmness. However, other ripening parameters as well as shelf-life of the fruit have to be considered. Storage atmospheres containing high CO_2 and low O_2 concentrations may induce abnormal metabolism which would injure fruit tissue (Burton, 1974). High concentrations of CO_2 may result in hard core (even when the fruit cortex is soft and ripe), flesh breakdown, loss of normal flavour and development of off-flavours (Harman and McDonald, 1989). The presence of ethylene in CA when combined with high CO_2 can induce some physiological disorders (Arpaia et al., 1986).

The effect of ULO on storage life, quality and shelf-life has not been extensively studied in kiwifruit. Previous studies have shown that kiwifruit removed from ULO storage did not ripe normally (Thomai and Sfakiotakis, 1997). Ripening-related changes in kiwifruit after removal from storage determine consumer acceptance. Hypoxic conditions increase ethanol and acetaldehyde content (Ke et al., 1990) and may cause injuries in plant tissues (Alpi et al., 1985). High levels of ethanol and acetaldehyde in 0.5%O_2+0%CO_2 stored kiwifruit were observed previously (Thomai and Sfakiotakis, 1997).

There is a marked reduction in ethylene production when parts of chilling-sensitive species are incubated below a growing temperature of 20° to 25°C (Field, 1981b; Wang and Adams, 1982). Ethylene production continues at 2.5°C in bean leaf tissue (Field, 1981b) and 5-10°C in avocado fruit (Metzidakis and Sfakiotakis, 1989), while in kiwifruit is almost null at temperatures bellow 11-14.8°C (Stavroulakis and Sfakiotakis, 1993).

Chilling temperatures similar to those encountered in storage will induce ethylene production and ripening upon transference to warm temperatures in kiwifruit (Hyodo and Fukazawa, 1985; Hyodo et al. 1987). The mechanism by which ethylene production is induced is not yet known. Hyodo and Fukasawa (1985) suggested that chilling stress in kiwifruit may advance the onset of ethylene production in the fruit when transferred to 21°C possibly by stimulating the formation of ACC as it happens in other fruit. Chilling

induces ethylene production upon rewarming, by stimulating ACC synthesis in cucumber and pears (Wang and Adams, 1982; Knee, 1987) or ACC oxidase activity in apples (Gaudierre and Vendrell, 1993).

Oxygen plays an important role in ethylene biosynthesis since it is a co-substrate of ACC oxidase (Pech et al., 1994). The system that converts ACC to ethylene is oxygen dependent, so in ULO conditions ACC oxidase activity should be low and an accumulation of ACC should be expected (Bufler and Bangerth, 1983). Under low-oxygen conditions, pears accumulated ACC which was converted to ethylene during post-storage ripening (Blankenship and Richardson, 1985; 1986). However, CA suppressed internal ethylene and ACC levels of apples (Bufler and Bangerth, 1983; Lau et al., 1984).

After prolonged chilling, ethylene production was reduced in cucumbers (Wang and Adams, 1982; Andersen, 1986), avocados (Eaks, 1983) and nectarines (Brecht and Kader, 1984). Differences in ethylene biosynthesis and ripening rates due to storage treatment can affect marketing decisions. This impairment of the ethylene-synthesising capacity may be responsible for the failure of chilled fruit to ripe normally (Eaks, 1983).

The scope of the present research was to study the effect of CS, CA and ULO storage on ethylene biosynthesis and ripening behaviour of 'Hayward' kiwifruit during storage at 0°C and posterior shelf-life at 20°C.

MATERIAL AND METHODS

1. Plant material and treatments

Kiwifruit (cv. Hayward) were harvested from an orchard in Pieria-North Greece with 7.1 kgf flesh Fm and 6.9 (% Brix) SSC. After selection for uniformity of size and freedom from defects, 32 fruit were used the same day for analysis of quality parameters.

A group of the remaining fruit were put in 5-litre jars through which a continuous, humidified, air stream with 130μl/l propylene or air free of propylene was passed at a

rate of 100ml/min. Each set of six jars was kept in a separate water bath at a constant temperature of 20°C. At intervals of 0, 3, 5, 7 and 9 days, 6 fruit per replication were removed from the jars and measurements of flesh firmness, SSC, ACC content, ACC synthase and ACC oxidase (*in vivo*) activities were taken. Ethylene production was measured daily. Respiration was measured four times during the first day and twice per day thereafter.

The other group of fruit were submerged in 1-butilcarbamoyl-2-benzimidone (600ppm) for 10 seconds and stored at 0°C. Atmospheres of ULO ($0.7\%O_2+0.7\%CO_2$ and $1\%O_2+1\%CO_2$) and CA ($2\%O_2+5\%CO_2$) were attained within 24h. The desired concentrations of O_2 and CO_2 were monitored by a paramagnetic and infrared gas analyser connected to a computer. The control consisted of normal atmosphere composition (CS). Ethylene scrubbers (K_2MnO_4) were placed in all storage chambers and the relative humidity was maintained at 90-95%. All experiments were set within 24 hours.

At intervals of 60, 120 and 180 days, fruit of each treatment were removed from storage. Thirty two fruit were immediately analysed and the remaining ones were put at 20°C in the same conditions as described above for freshly harvested fruit.

The experimental design was a three-factor experiment distributed in a complete randomised design with the storage atmospheres as the first factor, the propylene concentration as second and the duration of storage as third. Each treatment consisted of 4 replications.

2. Measurements

2.1. Ripening parameters and gas analysis

Ripening parameters (flesh firmness, SSC and flesh colour) and gas analysis (ethylene and CO_2 production) were measured as described in chapter 3.

2.2. ACC content, ACC synthase and ACC oxidase activities

ACC content and ACC synthase and ACC oxidase activities were measured as described in chapter 4.

2.3. Ethanol and acetaldehyde contents

Ethanol and acetaldehyde contents were measured according to Davis and Chace (1969). Five ml of thawed fruit juice were put in a 20ml test tube, closed with a rubber septum and incubated for 1 hour in a water bath at 60°C. One ml of the test tube gas phase was then injected into a Varian 3700 gas chromatograph, equipped with a glass column (2mmx1.8m) at 85°C and a flame-ionisation detector at 250°C. The column contained 5% Carbowax 20M on 60/80 Carbopack as stationary phase and N_2, at a flow rate of 20ml/min, as mobile phase.

3. Statistical analysis

Statistical analysis were performed as described in the methods of chapter 3.

RESULTS

1. Ripening behaviour during storage

1.1. Flesh firmness

Flesh firmness decreased significantly during storage in all treatments (Fig. 7.1A). On removal from storage, kiwifruit were firmer in $2\%O_2+5\%CO_2$ treatment followed by $1\%O_2+1\%CO_2$, $0.7\%O_2+0.7\%CO_2$ and air-control. Firmness of CS stored fruit decreased mostly during the first 60 days and remained almost constant thereafter. This effect was decreased in CA and ULO treatments. Firmness of the control fruit was always significantly lower than in the other treatments except at the end of the experiment where its values did not show significant differences from the fruit kept at $0.7\%O_2+0.7\%CO_2$. After 60 days storage, $2\%O_2+5\%CO_2$ and $1\%O_2+1\%CO_2$ stored

fruit did not show significant differences between them, but were significantly firmer than the other treatments. After 120 and 180 days storage, firmness was significantly higher for fruit of CA followed by ULO and CS.

1.2. Soluble solids content (SSC)

The SSC had a high significant increase during the first 60 days storage and remained almost constant thereafter (Fig. 7.1B). There were no significant differences among treatments in terms of SSC during storage.

1.3. Flesh colour

There was a significant fading of flesh green colour during storage as it is visible by the increase of the a* value (Fig. 7.1C). The loss of green colour (due to chlorophyll breakdown) was similar in all treatments except in control fruit which presented a significantly higher degreening. The loss of flesh green colour was more pronounced during the first 60 days storage in the control, while in the other treatments it was constant during all storage period.

1.4. Ethanol content

There was a higher significant accumulation of ethanol in $0.7\%O_2+0.7\%CO_2$ treatment during storage than in the other treatments (Fig. 7.2A). Ethanol content of $0.7\%O_2+0.7\%CO_2$ stored fruit started to increase from the beginning of storage, while in the other treatments increased significantly only after 60 days storage. There were no significant differences between $1\%O_2+1\%CO_2$, $2\%O_2+5\%CO_2$ and control treatments except after 180 days storage where $1\%O_2+1\%CO_2$ showed significantly higher values of ethanol than $2\%O_2+5\%CO_2$ and control.

1.5. Acetaldehyde content

On removal from 60 days storage, acetaldehyde was significantly higher in $0.7\%O_2+0.7\%CO_2$ followed by $1\%O_2+1\%CO_2$, $2\%O_2+5\%CO_2$ and control (Fig. 7.2B). Acetaldehyde remained almost constant thereafter in $0.7\%O_2+0.7\%CO_2$ stored fruit, while it significantly increased in the other treatments. There were no significant differences between CA and CS during storage. After 120 days, acetaldehyde content

was significantly higher at $0.7\%O_2+0.7\%CO_2$ than at $2\%O_2+5\%CO_2$ or the control, while at the end of the experiment there were no significant differences between treatments.

1.6. Ethylene production

Ethylene production was almost null during storage at 0°C in all treatments (Fig. 7.3A).

1.7. 1-aminocyclopropane-1-carboxilic acid (ACC) content

The ACC content of kiwifruit was almost null at harvest, but increased significantly during the first 60 days of storage in all treatments (Fig. 7.3B). The ACC content continued to increase in CS and CA till 120 days and then decreased till 180 days storage. Fruit of ULO treatments decreased their ACC content after 60 days storage with a more pronounced decrease from 120 to 180 days.

1.8. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) activity

The activity of ACC synthase was almost null at harvest, increased significantly during the first 60 days storage in all treatments and decreased slightly thereafter (Fig. 7.4A). The ACC synthase activity was always higher in CA and CS than in ULO. However, differences were significant only between CA and ULO treatments till 120 days, while at the end of the experiment CS and CA were significantly higher than ULO.

1.9. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activity

The ACC oxidase activity was almost null at harvest and remained very low during 180 days storage (Fig. 7.4B). Although values were significantly higher in $2\%O_2+5\%CO_2$ and $1\%O_2+1\%CO_2$ after 120 days and in $2\%O_2+5\%CO_2$ after 180 days than in the other treatments, they did not pass 3nl/g/h which is insignificant in terms of ACC oxidase activity in ethylene producing kiwifruit.

2. Ripening behaviour during shelf-life

2.1. Flesh firmness

Flesh firmness of kiwifruit treated with propylene at harvest decreased rapidly in the first 3 days at 20°C and had a slower decrease thereafter (Fig. 7.5). Fruit were fully ripe after 5 days. Fruit not treated with propylene did not significantly changed their flesh firmness during 9 days at 20°C.

When placed at 20°C, after 60 days storage, and treated with propylene, fruit of CA and ULO significantly decreased their firmness reaching 1kgf after 5 days (Fig. 7.6A). Control fruit had only a small decrease in firmness during shelf-life at 20°C because they were already soft on removal from storage. After 3 days shelf-life at 20°C, Fruit of CA were significantly firmer followed by ULO and CS, while after 5 days there were no significant differences in firmness among treatments, all fruit being ripe if treated with propylene.

After 60 days storage, fruit placed at 20°C in air free of propylene behaved differently than fruit treated with propylene (Fig. 7.6D). All treatments decreased significantly their firmness during shelf-life except control because it was already soft. Fruit of CA were significantly firmer than the other treatments after 3 days shelf-life at 20°C, significantly higher than CS after 5 days and significantly lower than ULO after 7 and 9 days. After 9 days shelf-life at 20°C in air free of propylene, only CA and CS fruit reached a ripe firmness value (<1kgf). Fruit stored in ULO were not able to fully ripe and did not show significant differences between them during 9 days shelf-life at 20°C if not treated with propylene.

Fruit treated with propylene after 120 days storage behaved similarly as after 60 days storage, but all treatments reached a ripe firmness value after 3 days shelf-life at 20°C and did not change significantly thereafter (Fig. 7.6B). Fruit not treated with propylene in the same conditions followed the same pattern as after 60 days storage (Fig. 7.6E).

After 180 days storage and when treated with propylene, fruit of all treatments showed a similar pattern during shelf-life as after 120 days (Fig. 7.6C). When not treated with propylene, fruit of $2\%O_2+5\%CO_2$ and $1\%O_2+1\%CO_2$ showed significantly higher

firmness than 0.7%O_2+0.7%CO_2 and control till 3 days at 20°C, but all treatments reached a firmness value of 1kgf after 7 days (Fig. 7.6F).

2.2. Soluble solids content (SSC)

The SSC of kiwifruit treated with propylene at harvest increased significantly reaching a ripe value of 14% Brix after 3 days at 20°C and continued to increase significantly reaching a value of 17% Brix after 9 days (Fig. 7.7). In air free of propylene, kiwifruit increased significantly their SSC during 9 days. However, they only reached values of 10% Brix, significantly lower than fruit treated with propylene.

The SSC increased significantly in the first 60 days storage, reaching values of 14% Brix, and remained almost constant thereafter for all treatments (Fig. 7.8). The SSC did not significantly change during 9 days shelf-life at 20°C independently of being treated or not with propylene.

2.3. Ethylene production

After 3 days exposure to propylene at 20°C, freshly harvested fruit started to show a typical increase in ethylene production reaching 180µl/kg/h after 9 days (Fig. 7.9). Without propylene, fruit did not produce ethylene during the experiment.

Ethylene production was almost null in all treatments on removal from storage (Fig. 7.10). Upon transfer to 20°C in air+130µl/l propylene, only CA and CS fruit exhibited the capacity to produce ethylene without delay and no significant differences between them (Fig. 7.10A, B and C). However, their maximum capacity to produce ethylene after 60 days storage was about 10% of that at harvest, 7% after 120 days storage and 3% after 180 days storage.

After 60, 120 and 180 days storage, fruit kept in air free of propylene at 20°C for 9 days, showed similar pattern in ethylene production as fruit treated with propylene in the same conditions, except that their values were slightly lower (Fig. 7.10D, E and F).

After removal from 60, 120 or 180 days storage, fruit kept in ULO did not increase significantly their ethylene production during shelf-life at 20°C, even when treated with propylene (Fig. 7.10).

2.4. 1-aminocyclopropane-1-carboxilic acid (ACC) content

Fruit harvested and placed at 20°C in air+130µl/l propylene started to produce ACC after a lag period of 3 days with a significant increase from day 5 to day 9 (Fig. 7.11). Fruit not treated with propylene did not increase significantly their ACC levels during 9 days at 20°C.

On removal from 60 days storage, ACC levels were of about 0.40-0.55 nmol/g in all treatments and remained almost constant during 9 days in air+130µl/l propylene at 20°C, except for CA which had a significant increase in ACC levels from day 7 to day 9 (Fig. 7.12A). However, its maximum values were about 42% of that of fruit ripened with propylene at harvest.

After 120 days storage, fruit from CA and CS showed significantly higher ACC content than ULO (Fig. 7.12B). The ACC content slightly increased in fruit from CS and CA during 9 days treatment with propylene at 20°C, while in ULO treatments it remained almost constant. There were no significant differences in ACC content between control and $2\%O_2+5\%CO_2$ and between $0.7\%O_2+0.7\%CO_2$ and $1\%O_2+1\%CO_2$.

After 180 days storage, ACC content of fruit treated with propylene for 9 days at 20°C followed the same pattern as after 120 days for all treatments, but its values were 40-50% lower than after 120 days storage (Fig. 7.12C). Fruit did not show significant changes in ACC content during 9 days shelf-life at 20°C in air+130µl/l propylene.

Fruit not treated with propylene during shelf-life followed a similar pattern to fruit treated with propylene after the same storage period (Fig. 7.12D, E and F).

2.5. 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) activity

ACC synthase activity followed the same pattern as ACC content both at harvest and during shelf-life, after 60 days storage, in fruit treated or not with propylene (Figs. 7.13 and 7.14A and D). The maximum ACC synthase activity during shelf-life was about 50% lower than at harvest and decreased slightly with storage time (Fig. 7.13 and 7.14).

After 120 days storage, ACC synthase activity was similar in all treatments (Fig. 7.14B and E). After 5 days treatment with propylene at 20°C, it increased significantly in fruit

from CA and CS and remained almost constant in ULO treatments. Fruit in air free of propylene followed the same pattern, except fruit from CS where ACC synthase activity did not significantly change during shelf-life (Fig. 7.14E).

After 180 days storage, ACC synthase activity followed a similar pattern to ACC content (Fig. 7.14C and F). However, in fruit treated with propylene at 20°C, values were significantly higher only in CA than in ULO treatments after 7 days shelf-life (Fig. 7.14C). ACC synthase activity of fruit not treated with propylene during shelf-life, differed from ACC content pattern only in that there were no significant differences after 3 days shelf-life between 1%O_2+1%CO_2 and control or 2%O_2+5%CO_2 (Fig. 7.14F).

2.6. 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activity

The ACC oxidase activity of kiwifruit was almost null at harvest but increased significantly upon exposure to propylene reaching values of 23 nl/g/h after 9 days (Fig. 7.15). Kiwifruit did not significantly increase ACC oxidase activity in air free of propylene.

The ACC oxidase activity was very low and similar in all treatments on removal from 60 days storage and increased significantly during 9 days in air+130µl/l propylene at 20°C in fruit from CS and CA treatments (Fig. 7.16A). Fruit of ULO treatments did not show a significant increase in ACC oxidase activity till 7 days shelf-life, but there was a significant increase between day 0 and day 9. Fruit in air free of propylene showed a similar pattern to fruit treated with propylene, except that ACC oxidase of ULO treatments did not significantly change during shelf-life (Fig. 7.16D). The maximum ACC oxidase activity was about 40% lower than at harvest and decreased slightly thereafter (Figs. 7.15 and 7.16).

After 120 days storage, kiwifruit ACC oxidase activity followed the same pattern as after 60 days, except that there was not a significant increase during shelf-life at 20°C in ULO treatments independently of being treated or not with propylene (Fig. 7.16B and E).

During shelf-life at 20°C, ACC oxidase of kiwifruit removed from storage after 180 days followed the same pattern as after 120 days, except that CS showed significantly

higher values than CA after 7 and 9 days shelf-life in both fruit treated or not with propylene (Fig. 7.16C and F).

2.7. Carbon dioxide production

Kiwifruit treated with propylene and kept at 20°C after harvest, increased significantly their respiration rate reaching a peak of 49 ml/kg/h after 1 day, had a significant decrease from 3.5 to 5.5 days, an increase till day 8th (coinciding with the rise in ethylene production) and a slight decrease thereafter (Fig. 7.17). Respiration of fruit not treated with propylene increased significantly from 2.5 to 5 days and then remained almost constant. Its values were always significantly lower than fruit treated with propylene.

When fruit were removed from 60, 120 and 180 days storage and placed at 20°C in air+130µl/l propylene or air free of propylene, there was a significant increase in respiration within 1 day for all treatments, and then no significant changes occurred during the remaining 8 days of shelf-life (Fig. 7.18). During shelf-life and for every removal from storage, CO_2 production was similar in fruit treated or not with propylene. After 60 days storage, the maximum CO_2 production during shelf-life at 20°C was about 65% of that at harvest and decreased slightly thereafter with storage time (Figs. 7.17 and 7.18).

After 60 days storage and 1 day shelf-life at 20°C, CO_2 production of fruit treated with propylene at 20°C reached a peak height with 35ml/kg/h in CS fruit and 25ml/kg/h in $1\%O_2+1\%CO_2$, the other treatments presented respiration rates between these values (Fig. 7.18A). There were no significant differences between treatments, except control which was significantly higher than $0.7\%O_2+0.7\%CO_2$ after 1 day shelf-life and between 5.5 and 7 days shelf-life. Fruit not treated with propylene showed similar behaviour with significantly higher respiration in control than in $0.7\%O_2+0.7\%CO_2$ only after 8.5 and 9 days at 20°C (Fig. 7.18D).

After 120 days storage and from 1 to 5 days shelf-life at 20°C, kiwifruit treated with propylene showed significantly higher values of CO_2 production in control than in $1\%O_2+1\%CO_2$ (Fig. 7.18B). Fruit in air free of propylene did not show significant differences in respiration between treatments (Fig. 7.18E).

After 180 days storage, kiwifruit placed at 20°C in air+130µl/l propylene showed significantly higher respiration in the control than in 0.7%O_2+0.7%CO_2 only after 5 days shelf-life (Fig. 7.18C). Fruit not treated with propylene showed the same differences from 7 to 9 days shelf-life (Fig. 7.18F).

DISCUSSION

The CA of 2%O_2+5%CO_2 has been proved to give good storage performance of kiwifruit (Harman and McDonald, 1989; Sfakiotakis et al., 1989; Arpaia et al., 1994b). In the present work, not only CA but also ULO reduced the rate of softening and resulted in fewer quality parameters changes than CS, except when kiwifruit were stored under 0.7%O_2+0.7%CO_2. The rapid softening of kiwifruit during the first 60 days in CS and slow thereafter was already observed by other authors (Arpaia et. al., 1984) and may be associated with the solubilization of cell wall components and starch degradation reported by Arpaia et. al. (1987; 1994a). Our results showed that CA and ULO storage played an important role in decreasing the initial rate of softening in kiwifruit. This effect decreased with storage time because fruit were becoming softer. Arpaia et al. (1987) suggested that part of the effect of CA on maintaining kiwifruit firmness during storage is due to its effect on reducing the rate of starch hydrolysis.

The 2%O_2+5%CO_2 and 1%O_2+1%CO_2 treatments were effective in prolonging storage life of kiwifruit with few changes in quality parameters as reported by McDonald (1990) Arpaia et al. (1994b) and Thomai and Sfakiotakis (1997). As observed by Arpaia et al. (1994b), the 2%O_2+5%CO_2 stored fruit became eating-ripe during shelf-life, while 1%O_2+1%CO_2 did not (unless treated with propylene) (Thomai and Sfakiotakis, 1997). In the present study, it was found that fruit from CS and CA were able to produce ethylene during shelf-life at 20°C in agreement with Arpaia et al. (1994b), while fruit from ULO were not. We suggest that the use of CA or ULO storage conditions to reduce flesh firmness loss depends on the needs of the market. When fruit are needed to be marketed immediately after storage, 2%O_2+5%CO_2 can be used since, due to their ability to produce ethylene, fruit ripened within 9 days in air at 20°C, while ULO stored fruit did not ripe. Kiwifruit previously stored in ULO were not able to produce ethylene

for the first 9 days at 20°C, so they had longer shelf-life. However, they could be ripened at our will by external ethylene application. Storage in 0.7%O_2+0.7%CO_2 for more than 120 days resulted in development of flesh breakdown, probably due to the high accumulation of ethanol, making this ULO treatment not suitable for long kiwifruit storage. Thomai and Sfakiotakis (1997) reported the accumulation of acetaldehyde and ethanol in 0.5% O_2 stored kiwifruit.

The high increase in SSC during the initial 60 days storage and the slow increase thereafter may be due to higher starch degradation during the initial period, as reported by Arpaia et. al. (1984). It appears that the pattern of SSC evolution is not greatly influenced by CA (Arpaia et al., 1984; 1986; 1994b; Harman and McDonald, 1989) or ULO treatments. All treatments reached a SSC of about 14% Brix after 60 days storage which is considered as a minimum for good consumer acceptance (Mitchell et al. 1991). The CA and ULO were more effective in reducing kiwifruit flesh colour changes than air-storage in agreement with Harman and McDonald (1983;1989) and Thomai and Sfakiotakis (1997). The great loss of flesh green colour in CS fruit is an important factor for reduced consumer acceptability. The better maintenance of quality of kiwifruit under CA or ULO may be also related with the reduction in the expression of genes involved in fruit ripening caused by low oxygen conditions, as reported by Kanellis et al. (1990).

In accordance to Arpaia et al. (1994a) the time required by kiwifruit to ripe was lower after cold storage than before storage. Kiwifruit placed at 20°C needed propylene to ripe in 3-5 days if not stored, while it ripened without propylene in 3 days if stored for 60 days under CS and 0-3 days if stored for 120 or 180 days under CS. The CA and ULO slightly retarded the ripening in shelf-life mostly after the first 60 days storage. Kiwifruit from CA were always fully ripe after 7 days shelf-life at 20°C. The CA and CS stored fruit ripened during shelf-life because they were able to produce ethylene. Fruit not treated with propylene and kept at 20°C did not ripe during 9 days at harvest or after storage in ULO, because they were not able to produce ethylene.

At harvest, ethylene production of kiwifruit kept at 20°C was strongly stimulated by 130μl/l propylene: ethylene production started after a lag period of 3 days, as was shown previously (Chapter 3 and 4) (Stavroulakis and Sfakiotakis, 1993). This was due to the increase of the activities of both ACC synthase and ACC oxidase. The ACC synthase

activity presented a lag period similar to ethylene production and ACC oxidade activity presented no lag period, as observed in chapter 4.

From the results of the present study it can be seen that during shelf-life at 20°C only fruit previously stored in CA and CS were able to produce ethylene. The same increase in ethylene production after CA and CS storage was reported by Arpaia et al. (1994b). As it was shown in chapter 6, fruit from cold storage started to produce ethylene upon rewarming with no lag period. This may be due to the induction of ACC synthase by chilling temperatures, since ACC oxidase does not need a lag period to start its activity upon rewarming.

The ULO stored kiwifruit were not able to produce ethylene even when they were rewarmed at 20°C. Nanos et al. (1992) found that ethylene production was inhibited in pears kept under very low oxygen but it resumed upon removal to air. Blankenship and Richardson (1986) found low levels of ethylene in pears on removal from low O_2 storage, and here ethylene levels increased upon transfer to air at 20°C. In the present research, ULO storage inhibited the system that produces, upon rewarming, ethylene induced by chilling in kiwifruit.

It was previously reported that ACC accumulates in pears stored in low O_2 and that it was converted to ethylene upon transfer to air at 20°C (Blankenship and Richardson, 1986). In this study, it was observed a small ACC accumulation and a corresponding ACC synthase activity in all treatments on removal from 60 days storage. This indicates that lack of ACC accumulation in storage was not the main factor limiting ethylene production as reported for apples by Jobling et al. (1991) and Gaudierre and Vendrell (1993). During shelf-life at 20°C, the ACC synthase activity and ACC levels remained constant in fruit from ULO and slightly increased in CA and CS fruit, showing a small induction of ACC synthase only in fruit stored above 2% oxygen. When the endogenous ACC concentrations are about equal, the rates of ethylene production should represent their relative ACC oxidase activities (Riov and Yang, 1982a). In this work it was observed that low ACC oxidase activity was the main factor for the lack of ability of ULO stored fruit to produce ethylene upon rewarming.

Hyodo et al. (1987) found levels of 0.6nmol/g of ACC in kiwifruit after 120 days storage in CS, while ACC oxidase did not show any activity till fruit were rewarmed. This finding is in agreement with the present study.

In the chapter 4 it was observed that 8 days storage at low temperature (10°C) were not enough to induce transcription of ACC synthase or ACC oxidase of kiwifruit not treated with propylene. There was induction of ACC oxidase only when fruit were treated with propylene but not of ACC synthase. In addition, during 17 days at temperatures from 0 to 15°C, kiwifruit did not show ACC synthase or ACC oxidase activities (chapter 6). ACC synthase and ACC oxidase are encoded by multigene families and differentially regulated (Huang et al., 1991; Olson et al., 1991; Tang et al., 1994; Barry et al., 1996). Ikoma et al. (1995) found two ACC synthase genes from kiwifruit differentially expressed. The gene KWACC2 from wounded kiwifruit mesocarp was actively transcribed in only wounded tissue, while the gene KWACC1 from ethylene-treated fruit was transcribed not only in ethylene-treated ripe fruit but also in wounded tissue.

From the results of the present study it is suggested that as the chilling period advances, the ACC synthase gene induced by chilling is completing the process of transcription, translation and protein synthesis. Hence, ACC synthase activity is present at chilling temperatures in kiwifruit, although at a slower rate than at warm temperatures. The ACC oxidase activity is not present in kiwifruit at low temperature probably due to post-transcriptional inhibition as for apples (Jobling et al., 1991; Gaudierre and Vendrell, 1993). More research is needed to clarify this point at the molecular level.

Low ACC oxidase activity in low-oxygen stored fruit may not be due solely to the requirement for O_2 to convert ACC to ethylene (Blankenship and Richardson, 1986). The same authors reported that in the presence of ample oxygen and given exogenous ACC, the low O_2-stored fruit still do not equal the ethylene production of air-stored fruit. Thus, oxygen was not limiting the conversion of ACC to ethylene during ULO storage at 0°C. They referred that warm temperatures such as those encountered during ripening seem necessary for the conversion of the pool of ACC to ethylene. However, in the present study the temperature increase did not induce ACC oxidase activity of ULO stored fruit. Wang and Adams (1980) reported that ACC synthesis is readily stimulated by chilling, whereas ACC oxidase is vulnerable to chilling injury. Upon rewarming,

kiwifruit levels of ACC and ACC oxidase activity increased in proportion to the rate of ethylene production suggesting that ACC production and ACC oxidase activity are limiting step factors affecting ethylene formation (Hyodo and Fukazawa, 1985; Hyodo et al. 1987). Bufler and Bangerth (1983) suggested that a ripening promoter may be induced before the autocatalytic ethylene production which depends on oxygen. It appears that kiwifruit exposure to less than 1% oxygen for 60 days at 0°C damages the receptor of the stimulus that induces ACC oxidase at gene transcription level, translation or enzyme activity. More research is needed to clarify this point at the molecular level. Nevertheless, at 120 and 180 days storage, the lower levels of ACC in ULO stored fruit may also be a cause for the loss of capacity to produce ethylene by those treatments.

Arpaia et al. (1994a) referred that even after 180 days storage in CS at 0°C, kiwifruit exhibit the characteristic increase in respiration and ethylene in the same way as fruit not stored but kept at 20°C after harvest. Our results are in agreement with these authors for CA and CS stored fruit. However, we observed that for CA and CS stored kiwifruit, as storage time increases the capacity to produce ethylene during shelf-life decreases, mostly in the first 60 days. After 180 days storage at 0°C, ethylene production during shelf-life at 20°C was very low with a maximum of 7µl/kg/h. Wang and Adams (1982) and Andersen (1986) reported that prolonged chilling can reduce ethylene production upon rewarming by damaging ACC oxidase. The results of the present study showed that the loss of the capacity to produce ethylene with storage time is related to decreased ACC synthase and ACC oxidase activities, in comparison with freshly harvested fruit ripened with propylene at 20°C, probably, due to a progressive decrease in the expression of their respective genes.

For all treatments, fruit respiration was low in storage and increased upon rewarming of the fruit because the rate of respiration is mostly temperature dependent (Blanke, 1991). The tendency of the CS and CA to show higher respiration was due to ethylene production by these treatments, since respiration increases with ethylene production (Arpaia et al., 1994a). It was also observed a decrease in the respiration rate of the rewarmed fruit with storage time. This decrease was lower than the decrease in ethylene production and may be associated mainly with the advance in the natural senescence process.

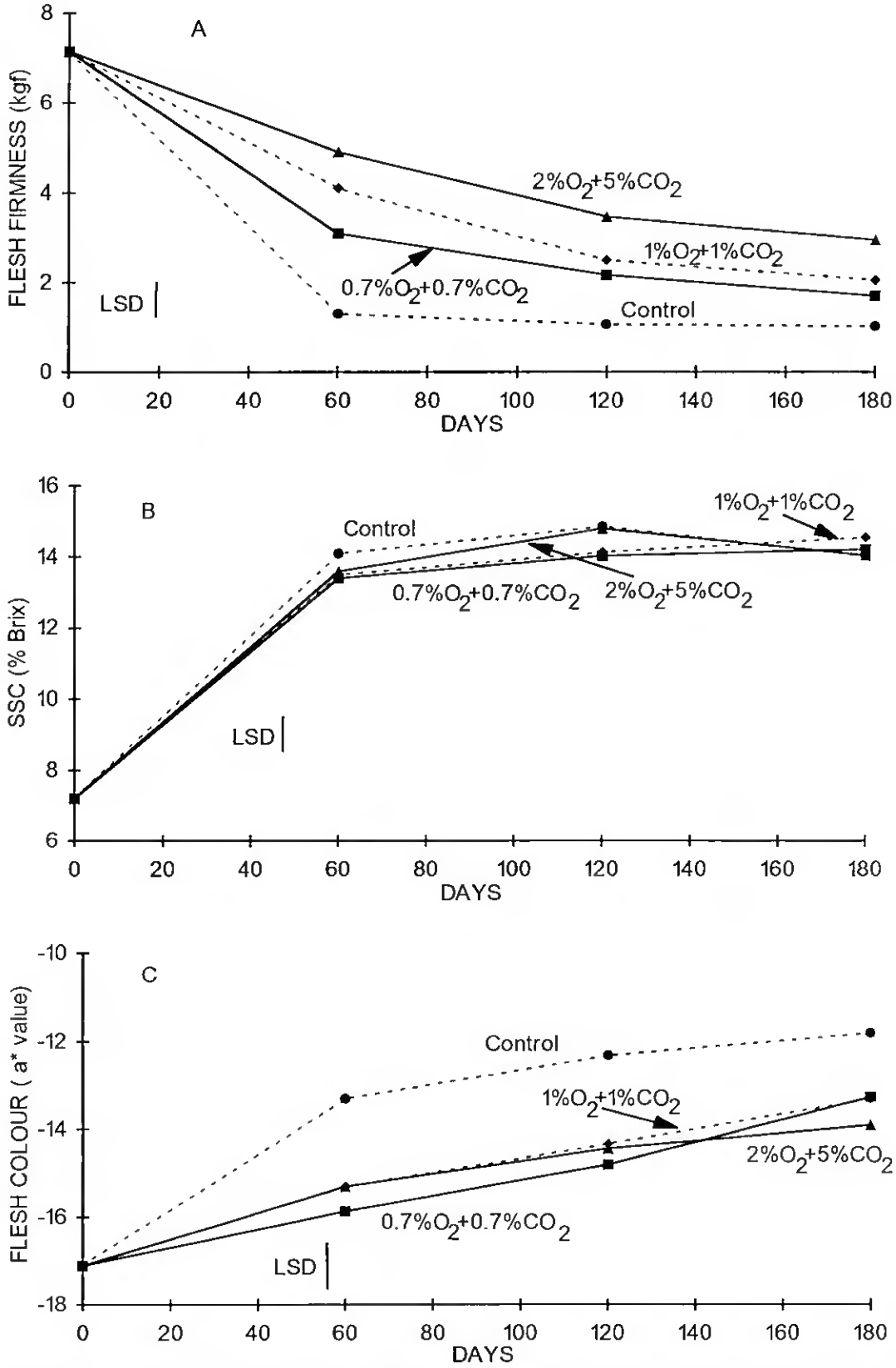


Figure 7.1. Flesh firmness (A), SSC (B) and flesh colour (C) of 'Hayward' kiwifruit during storage at 0°C in ULO ($0.7\%O_2+0.7\%CO_2$; $1\%O_2+1\%CO_2$), CA ($2\%O_2+5\%CO_2$) and CS (control).
LSD at $\alpha=0.05$.

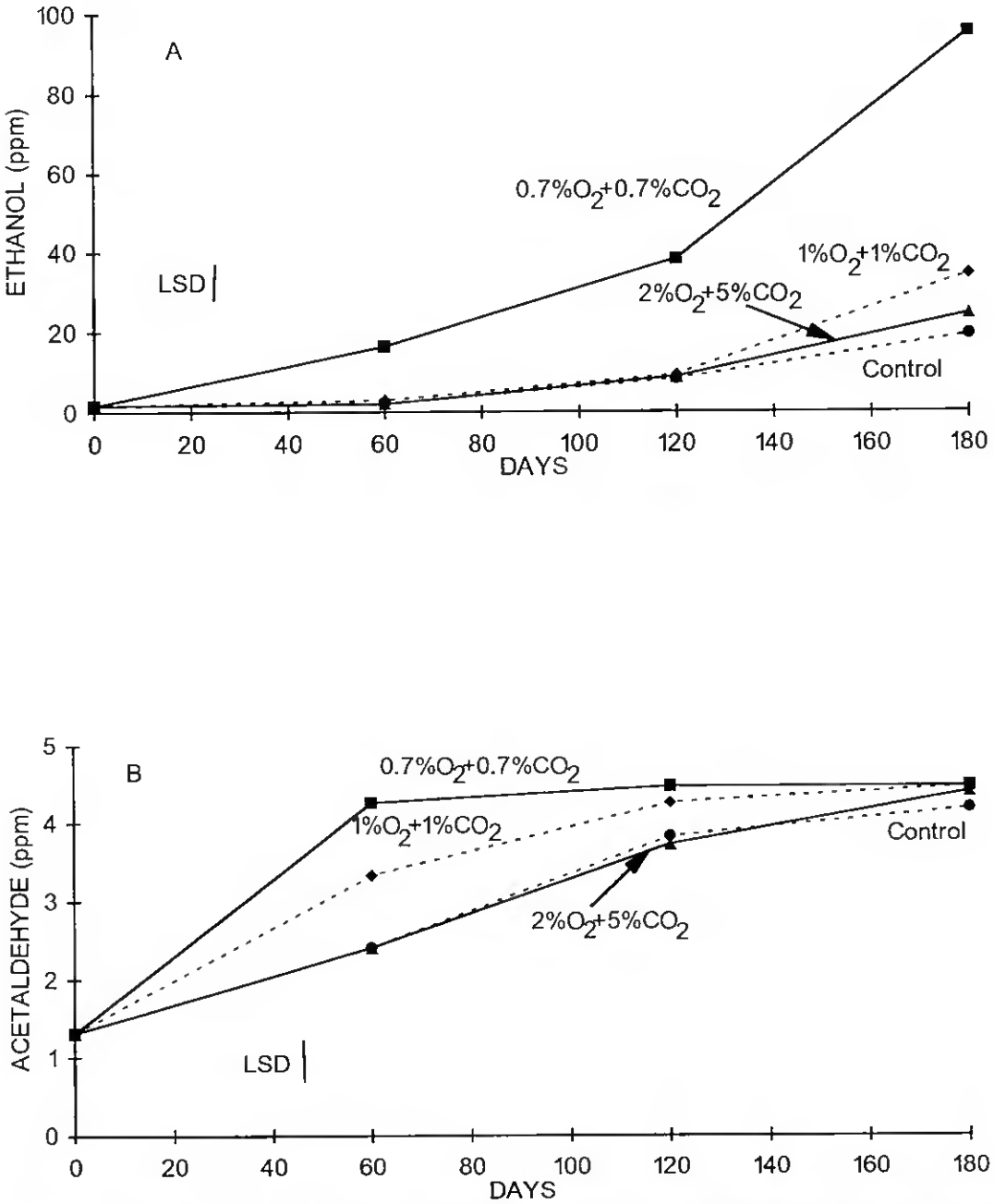


Figure 7.2. Ethanol (A) and acetaldehyde (B) content of 'Hayward' kiwifruit during storage at 0°C in ULO ($0.7\%O_2+0.7\%CO_2$; $1\%O_2+1\%CO_2$), CA ($2\%O_2+5\%CO_2$) and CS (control).
LSD at $\alpha=0.05$.

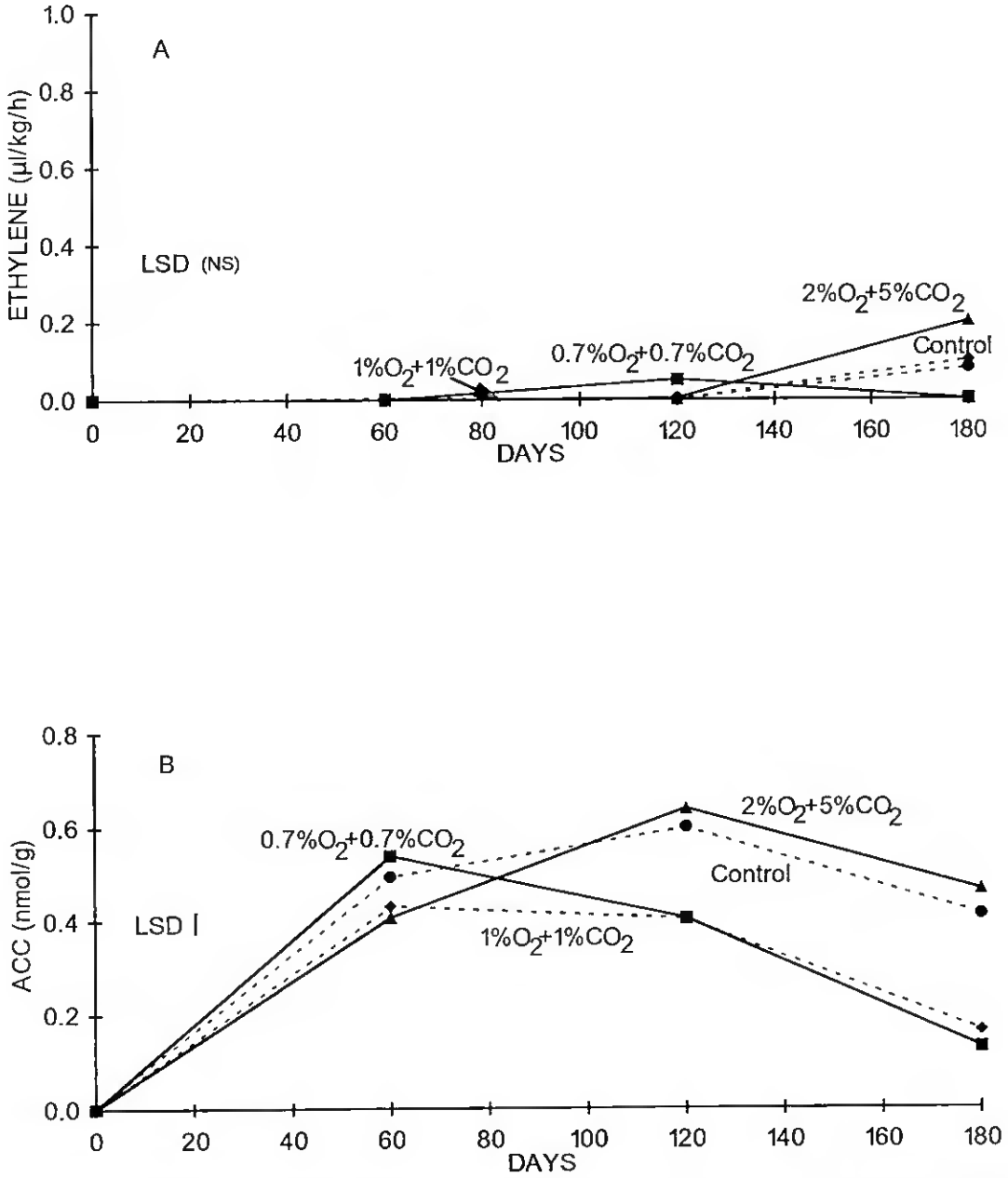
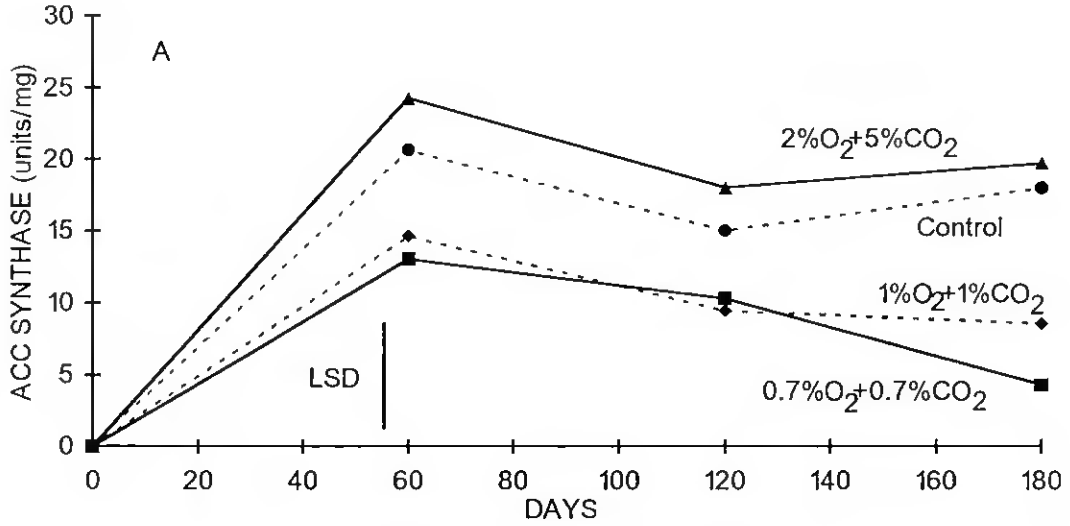


Figure 7.3. Ethylene production (A) and ACC content (B) of 'Hayward' kiwifruit during storage at 0°C in ULO ($0.7\%O_2+0.7\%CO_2$; $1\%O_2+1\%CO_2$), CA ($2\%O_2+5\%CO_2$) and CS (control).
LSD at $\alpha=0.05$.



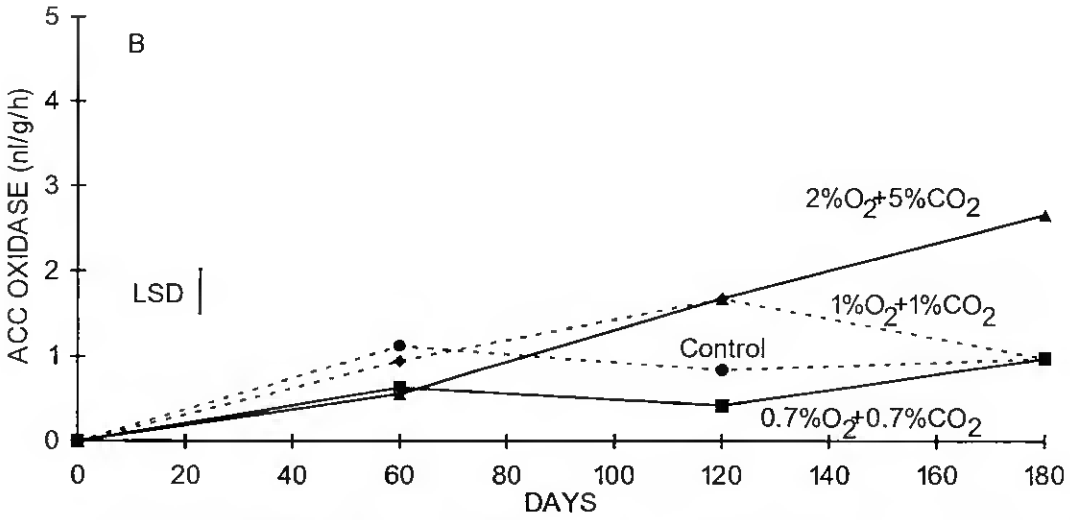


Figure 7.4. ACC synthase (A) and ACC oxidase (B) activities of 'Hayward' kiwifruit during storage at 0°C in ULO ($0.7\%O_2+0.7\%CO_2$; $1\%O_2+1\%CO_2$), CA ($2\%O_2+5\%CO_2$) and CS (control).
1 unit/mg = 1pmol ACC/mg protein/2hours.
LSD at $\alpha=0.05$

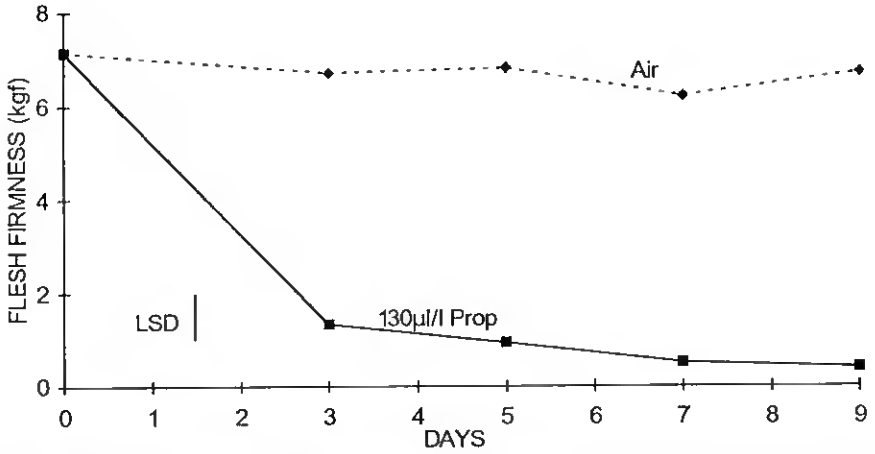


Figure 7.5. Flesh firmness of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

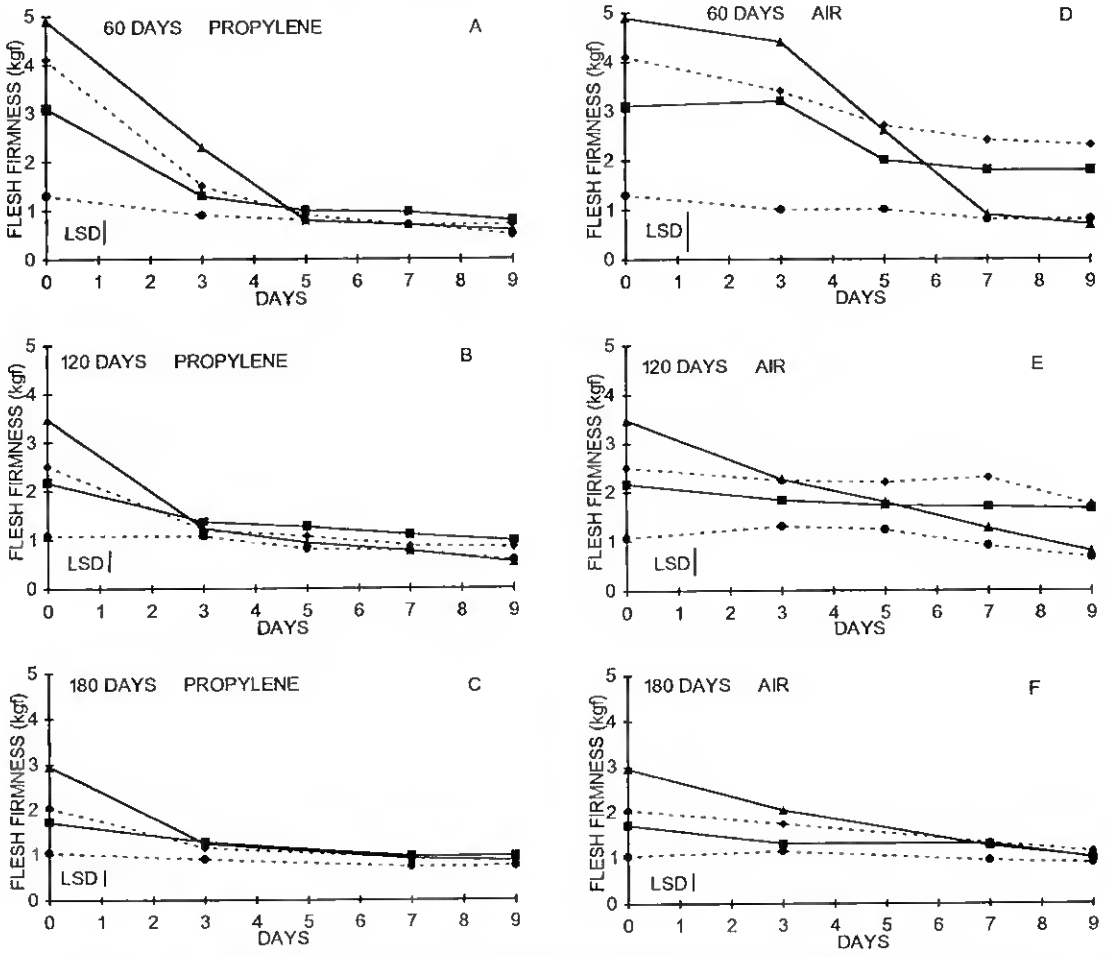


Figure 7.6. Flesh firmness of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO ($0.7\%O_2+0.7\%CO_2$; $1\%O_2+1\%CO_2$), CA ($2\%O_2+5\%CO_2$) and CS (control). —■— ($0.7\%O_2+0.7\%CO_2$); ---◆--- ($1\%O_2+1\%CO_2$); —▲— ($2\%O_2+5\%CO_2$) ; ---●--- (control). LSD at α=0.05.

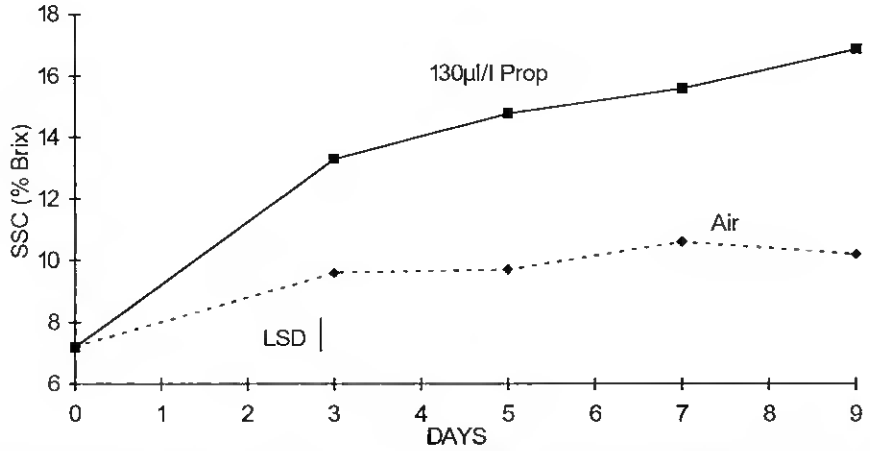


Figure 7.7. SSC of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

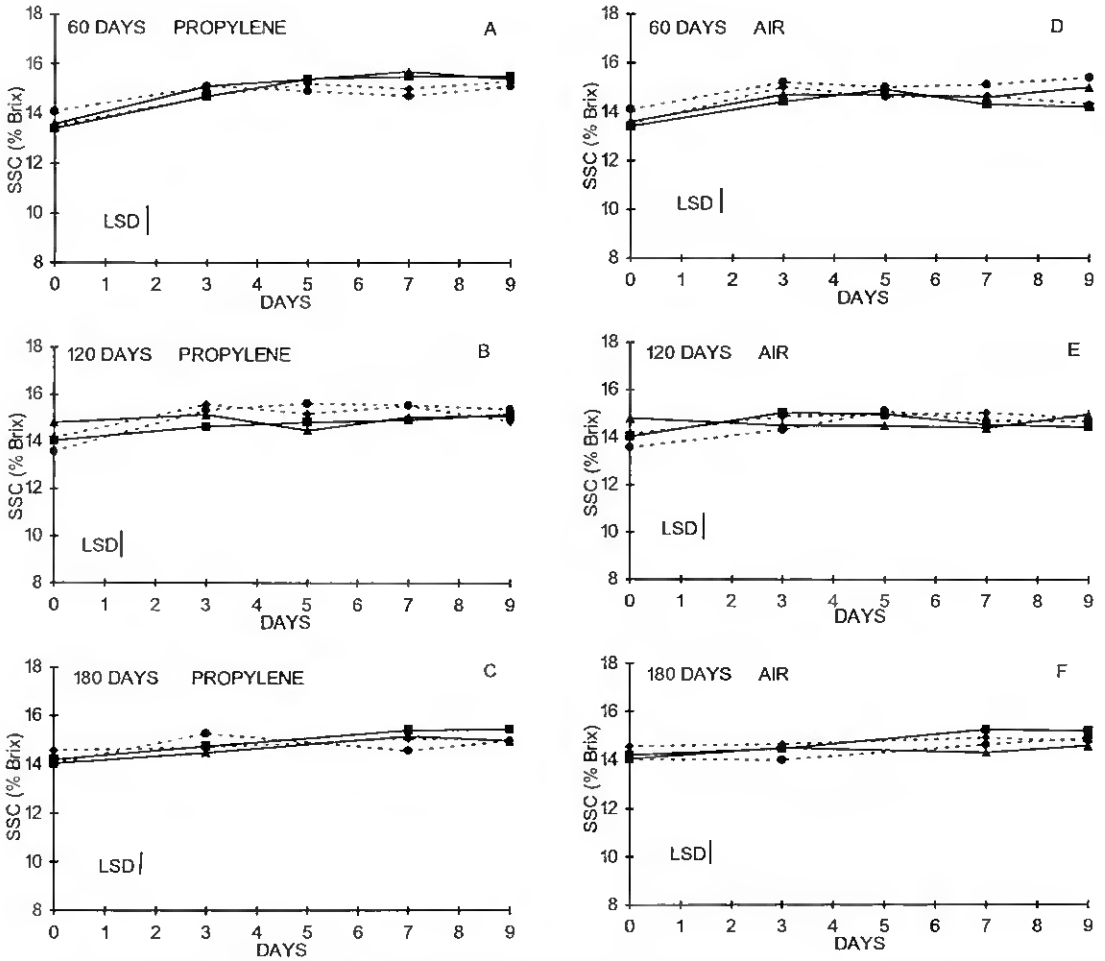


Figure 7.8. SSC of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control). LSD at α=0.05.

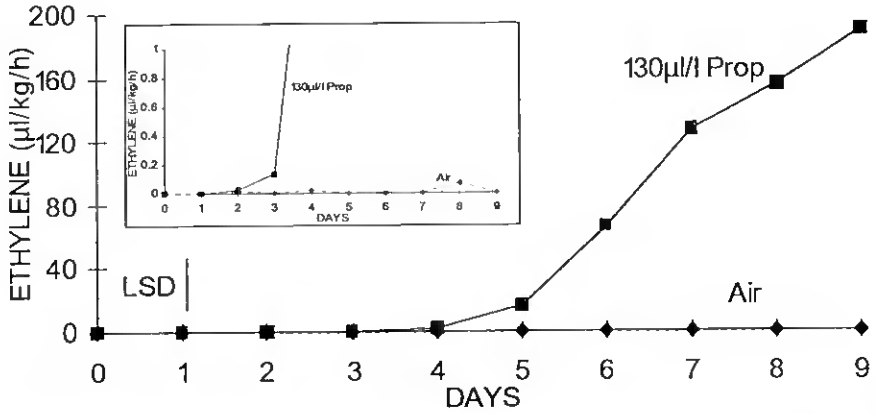


Figure 7.9. Ethylene production of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

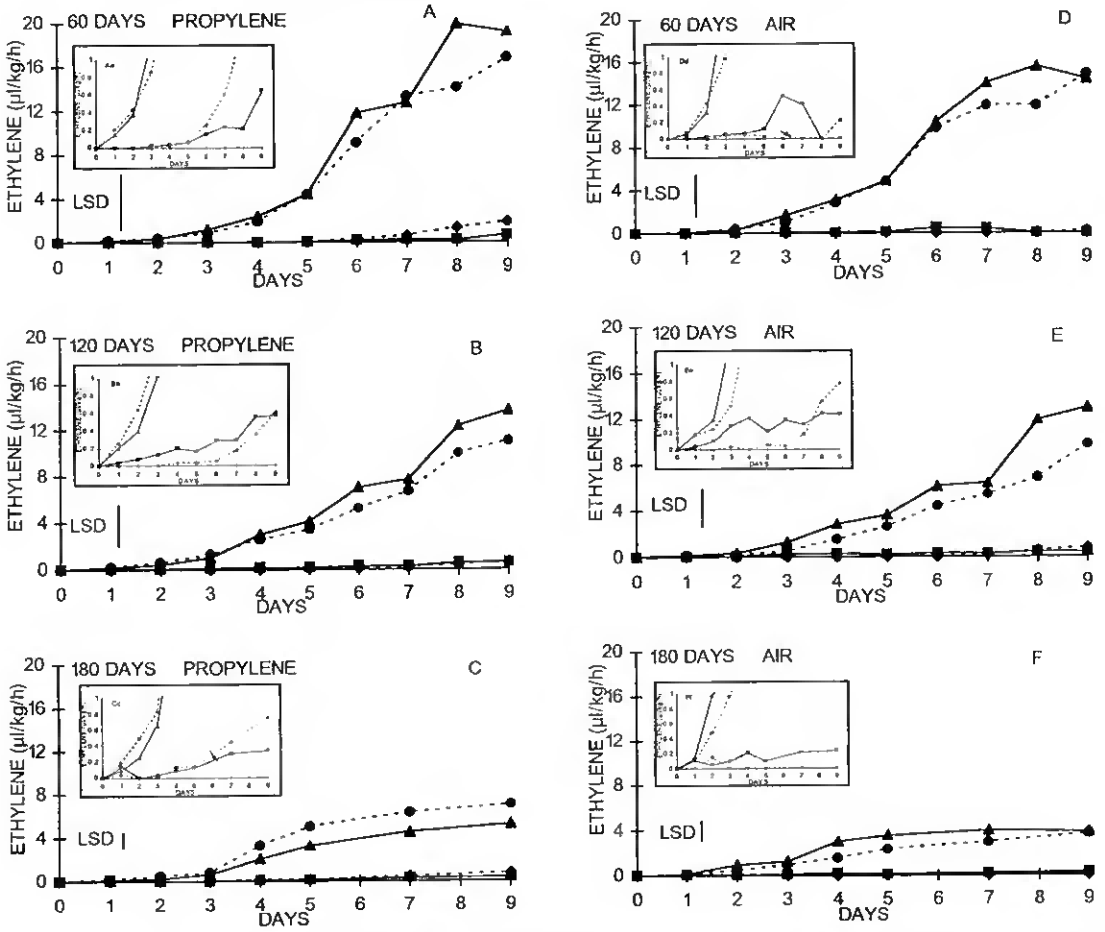


Figure 7.10. Ethylene production of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control). Graphs in frames show a blow up of the threshold level for initiation of ethylene autocatalysis of the respective graph. LSD at α=0.05.

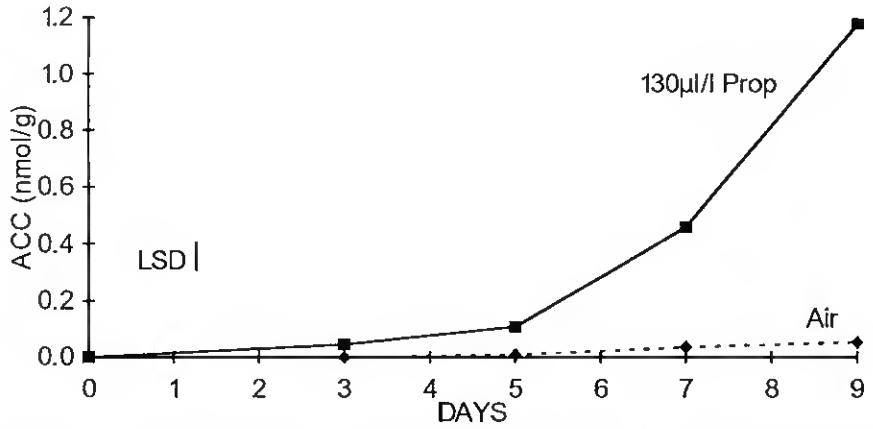


Figure 7.11. ACC content of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

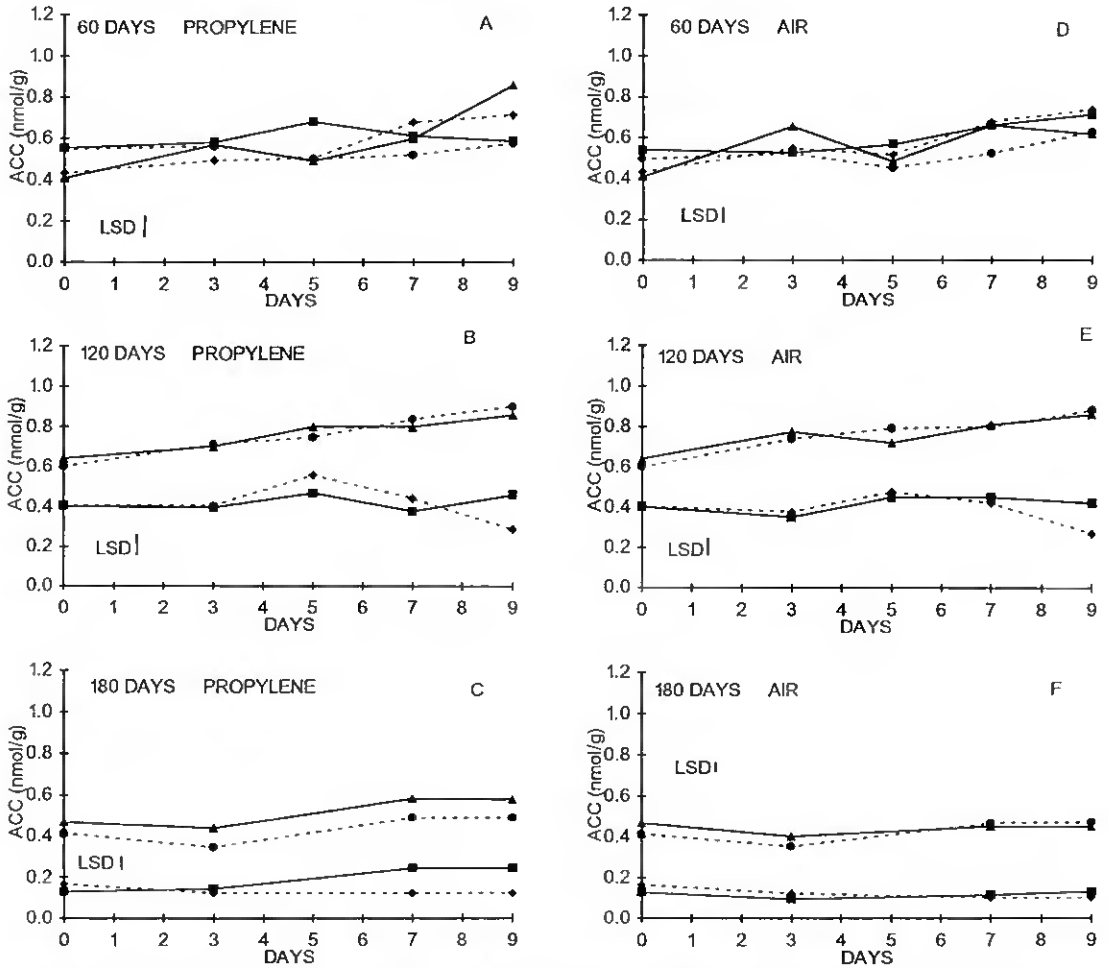


Figure 7.12. ACC content of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control). LSD at α=0.05.

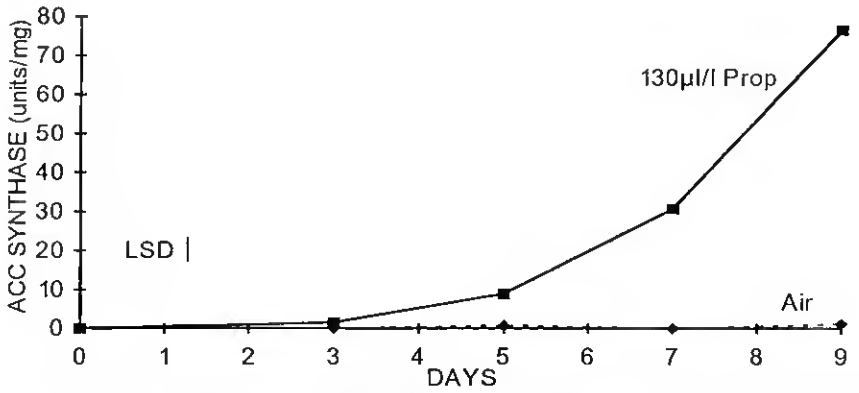


Figure 7.13. ACC synthase activity of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene.
LSD at α=0.05; 1 unit/mg = 1pmol ACC/mg protein/2hours.

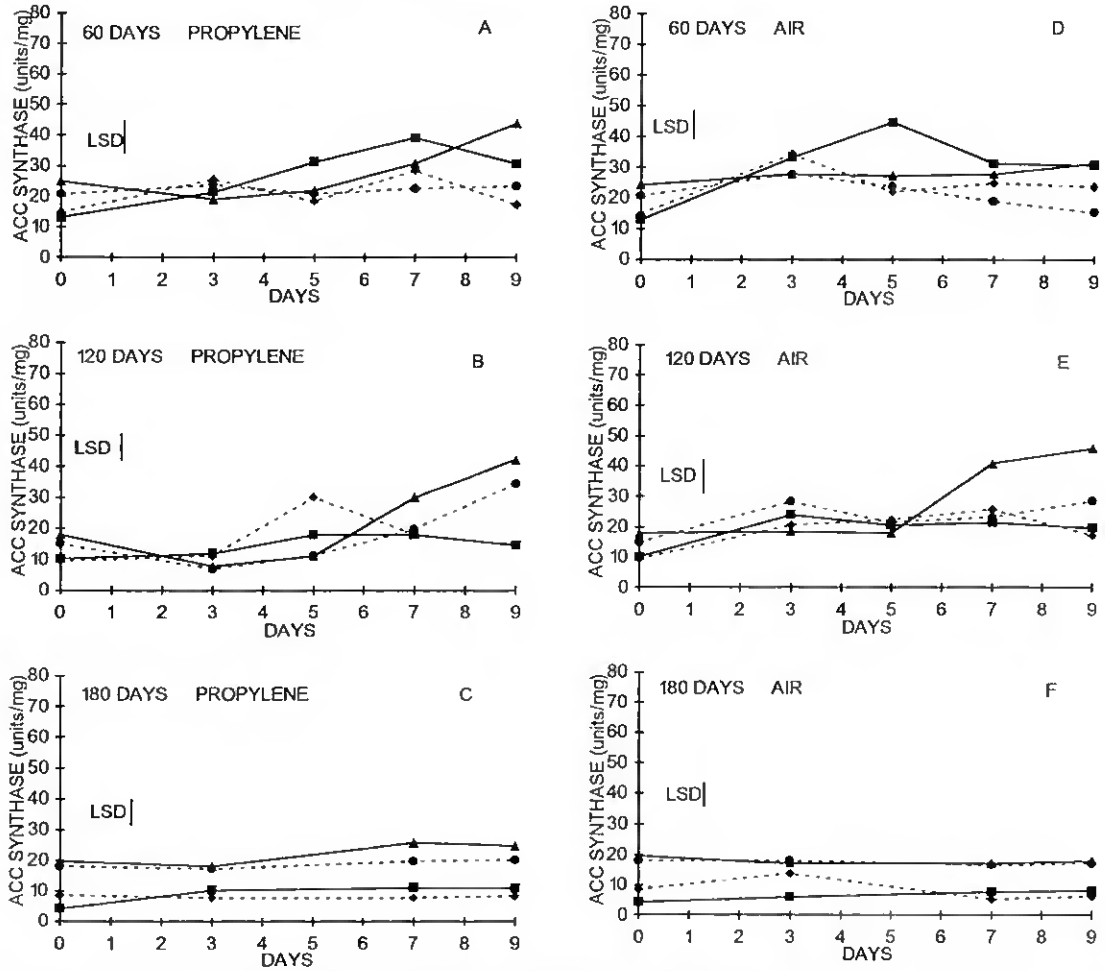


Figure 7.14. ACC synthase activity of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control).
LSD at α=0.05; 1 unit/mg = 1pmol ACC/mg protein/2hours.

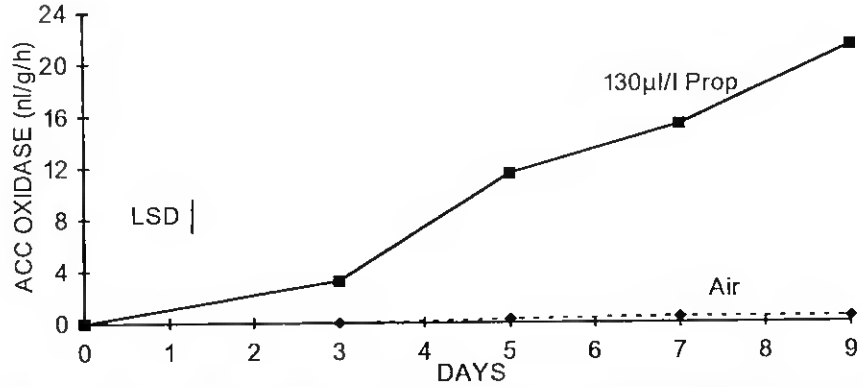


Figure 7.15. ACC oxidase activity of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

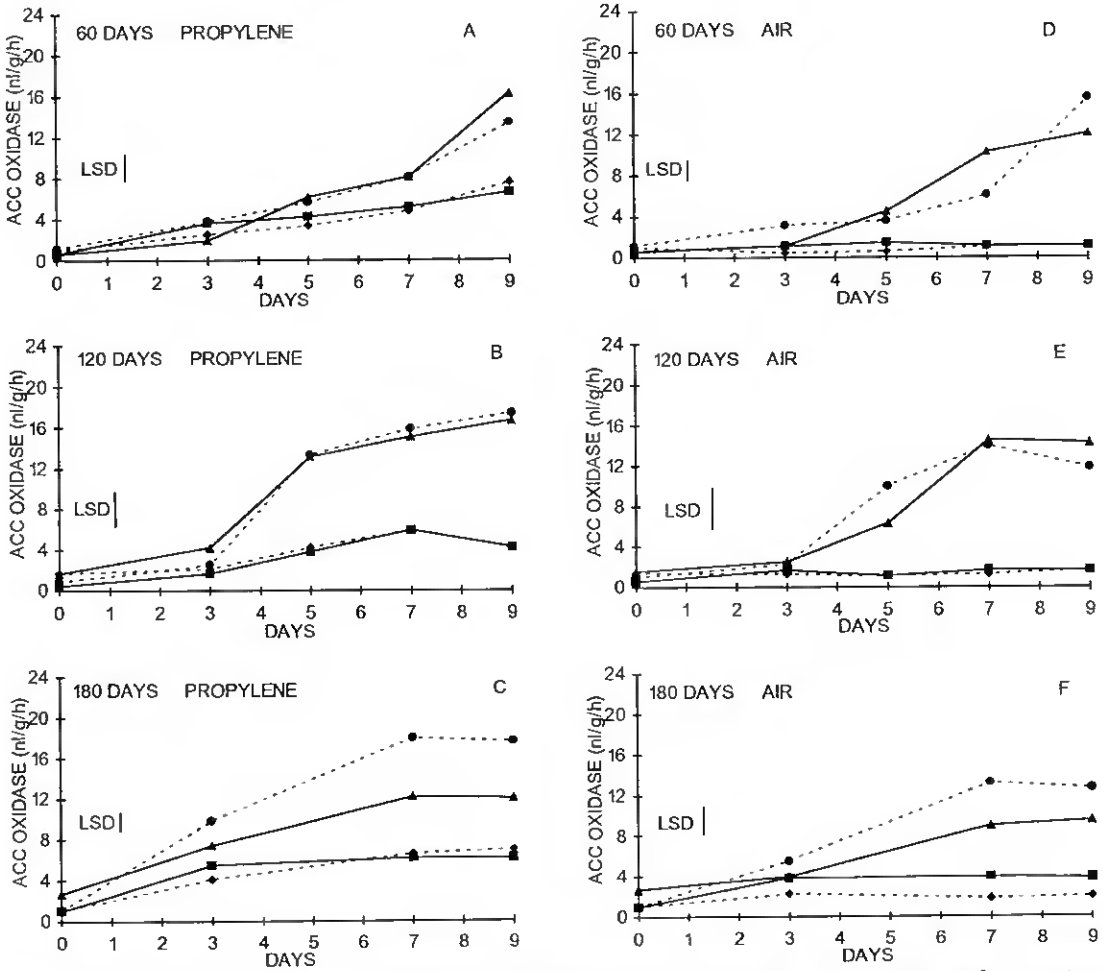


Figure 7.16. ACC oxidase activity of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control). LSD at α=0.05.

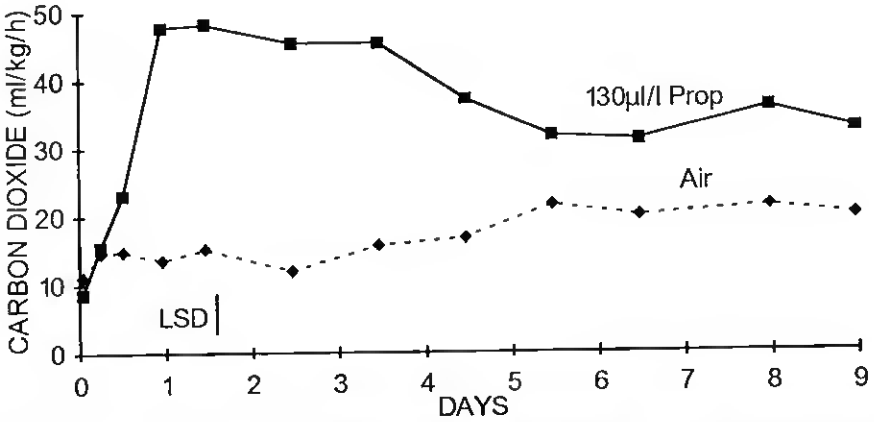


Figure 7.17. Carbon dioxide production of harvested 'Hayward' kiwifruit kept at 20°C under a continuous, humidified, air stream with 130µl/l propylene or air free of propylene. LSD at α=0.05.

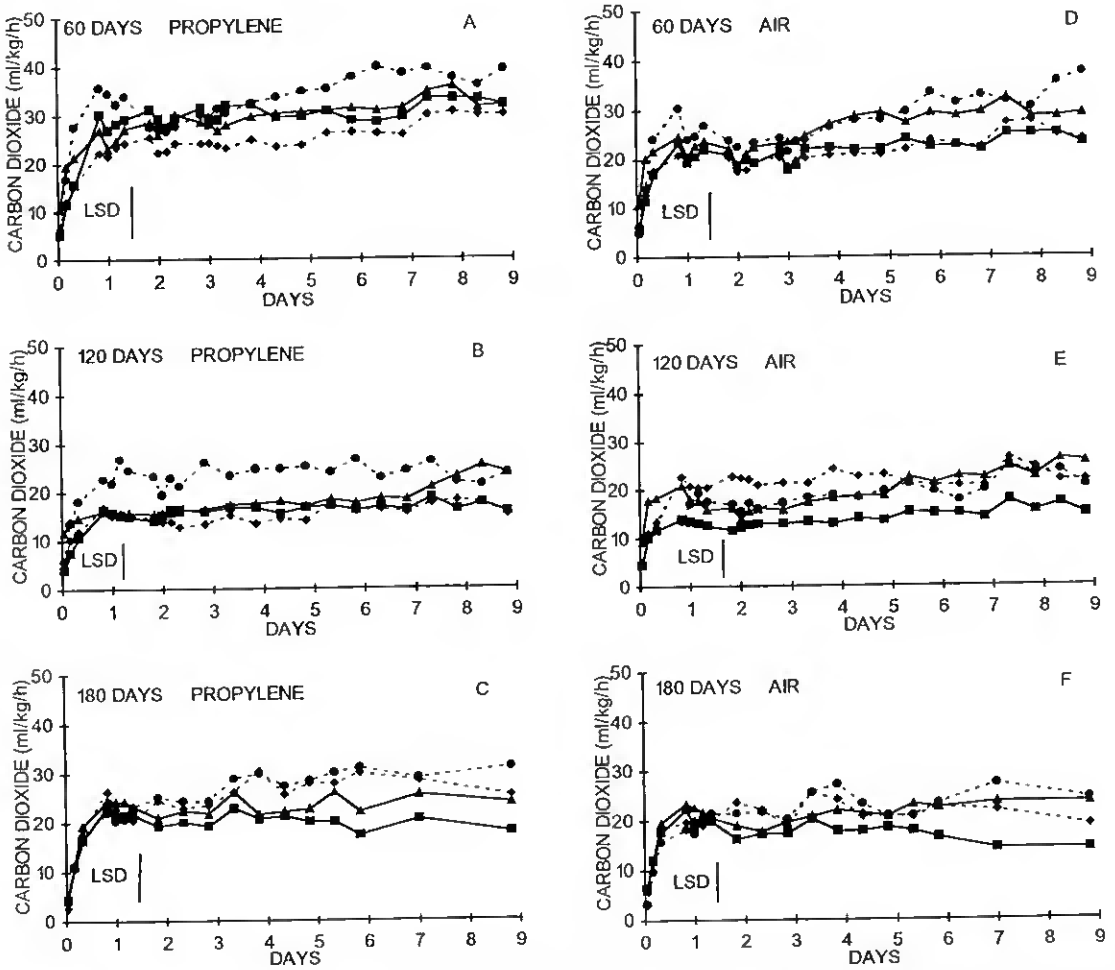


Figure 7.18. Carbon dioxide production of 'Hayward' kiwifruit placed at 20°C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or air free of propylene (D, E and F) after 60, 120 and 180 days, respectively, storage at 0°C in ULO (0.7%O_2+0.7%CO_2; 1%O_2+1%CO_2), CA (2%O_2+5%CO_2) and CS (control). —■— (0.7%O_2+0.7%CO_2); ---◆--- (1%O_2+1%CO_2); —▲— (2%O_2+5%CO_2) ; ---●--- (control). LSD at α=0.05.

CHAPTER 8. GENERAL DISCUSSION

Is kiwifruit a climacteric fruit?

Kiwifruit has been classified as a climacteric fruit (Arpaia et al., 1994a). All climacteric fruit are characterized by transient increases in both ethylene synthesis and respiration at an early stage of ripening (Tucker and Grierson, 1987). The present research confirm that kiwifruit cv. 'Hayward' behaves as a climacteric fruit by starting autocatalysis of ethylene production, respiration climacteric and ripening in approximately 19 days after harvest when placed at 20°C in air free of propylene (Fig. 3.1). If we consider the respiration rise as an indicator of the commencement of ripening (Yang et al., 1986), we may conclude that kiwifruit belongs to that group of climacteric fruit which shows the respiration rise coincidentally to the rise in ethylene production (Tucker, 1993).

The application of external ethylene to a climacteric fruit, providing it is mature enough, will advance the onset of the climacteric, this effect being proportional to the concentration of applied ethylene (Tucker and Grierson, 1987). We observed that the application of increasing propylene concentrations to kiwifruit at 20°C advanced the respiration climacteric and the autocatalysis of ethylene production in the same way as for other climacteric fruit (Fig. 3.4). However, the application of propylene changed the climacteric pattern: the rise in respiration rate and ripening associated changes started after 4 to 10 hours, while ethylene burst initiated late in the ripening process, after a lag period of 68-79 hours, just preceding fruit senescence, making kiwifruit different from most climacteric fruit as reported by Wittaker et al. (1997).

The removal of propylene after 24 hours exposure did not affect autocatalysis of ethylene production, but did decrease respiration which rose again when autocatalysis of ethylene started (Fig. 3.2). This effect shows that, after 24 hours, kiwifruit had not shifted yet from system I to system II of ethylene production postulated by McMurchie et al. (1972).

Stavroulakis and Sfakiotakis (1993) observed that at temperatures below a critical range (11-14.8°C) kiwifruit does not produce ethylene even when treated with 130µl/l propylene. In the present study, fruit at 10°C did not show autocatalysis of ethylene production, but propylene treatment induced an immediate rise in CO_2 production being the respiration rate dependent on the concentration of the applied propylene (Fig. 3.5). Removal of propylene at 10°C decreased respiration rate of kiwifruit which recovered upon reexposure to propylene (Fig. 3.3). This behaviour is characteristic of a non-climacteric fruit (Tucker, 1993).

Yano and Hasegawa (1993a) already referred that kiwifruit seems different from the other climacteric fruit, showing a long lag period for ethylene production unless external ethylene is applied or a diseased fruit is packed together with sound fruit. From the above it is shown that kiwifruit, generally considered as climacteric fruit, is an unique fruit which behaves like a climacteric fruit in what concerns respiration and ethylene production at ambient temperature, while at temperature as low as 10°C it behaves like a non-climacteric fruit.

The results of this study are also compatible with the concept of 2 systems for ethylene production: system I, that is present in preclimacteric and non-climacteric fruit, and system II present only in autocatalytically ethylene producing climacteric fruit (McMurchie et al., 1972). Temperatures as low as 10°C inhibit, in kiwifruit, the conversion of system I to system II in terms of autocatalysis of ethylene production induced by propylene.

This behaviour of kiwifruit with respect to respiration and ethylene production offers certain advantages in handling operations. After harvest, by keeping the fruit at low temperatures there is no accumulation of ethylene production in storage rooms, thus the post-harvest life of the fruit is prolonged, providing there are not diseased fruit or other sources of ethylene nearby.

Why kiwifruit shifts from climacteric to non-climacteric at 10°C?

Propylene induced the transcription of the ACC oxidase and ACC synthase genes to mRNA at 20°C as observed by Whittaker et al. (1997), while at 10°C only ACC oxidase genes were transcribed (Fig. 4.4).

It was also observed that ACC oxidase activity was very low at 10°C (Fig. 4.3C). Since there is transcription of the ACC oxidase gene at this temperature, when propylene is applied, the inhibitory effect of low temperature can be at the translational level or in the function of the enzyme. However, Stavroulakis and Sfakiotakis (1993) observed that the inhibition of ACC production was more pronounced than ACC oxidase activity when temperatures were decreased gradually around the critical range for ethylene production in kiwifruit (11-14.8°C).

From the results of the present study, it is suggested that the inhibition of the transcription of the ACC synthase genes induced by propylene at temperatures as low as 10°C was the main reason for the non-climacteric pattern of kiwifruit at this temperature.

More research is needed at the molecular level to clarify how progressively decreasing temperature is affecting both ACC synthase and ACC oxidase at the transcriptional and translational level and their activity.

Thermoregulation of the propylene induced ethylene production and ripening

Kiwifruit ripened and started autocatalysis of ethylene production when treated with propylene at 20-34°C. Ethylene production was strongly inhibited at 38°C, and at temperatures above 40°C or bellow 10°C autocatalysis did not occur (Figs.4.1, 4.2A and 5.4A). The time required for the fruit to reach eating-ripe values of firmness (≈0.7kgf) and SSC (≈14% Brix) decreased as temperature increased till 34°C. Ripening was not uniform at 38°C, the core being hard when flesh was already eating-ripe, and at temperatures above 40°C ripening was inhibited culminating with fruit breakdown at 45°C.

The inhibition of the propylene-induced ethylene production at high and low temperatures is due to reduced ACC synthase and ACC oxidase activities. However, at high temperatures the first enzyme to be affected is ACC oxidase since there was a considerable accumulation of ACC and ACC synthase activity at 38°C (Figs. 8.1, 5.5A and 5.6A). At low temperatures ACC synthase is the most affected as we discussed above.

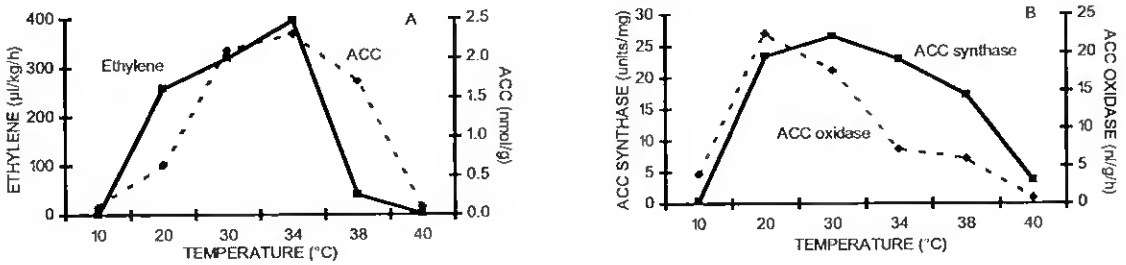


Figure 8.1. The effect of temperature on ethylene production and ACC content (A) and activities of ACC synthase and ACC oxidase (B) of 'Hayward' kiwifruit kept in an atmosphere containing 130µl/l propylene.
Values were taken four days after the initiation of ethylene autocatalysis.
1 unit/mg = 1pmol ACC/mg protein/2hours.

The temporal separation of ethylene sensitivity and climacteric ethylene production in kiwifruit treated with propylene allows us to distinguish between the responses of the ethylene biosynthetic genes to exogenous ethylene and their behaviour during ethylene biosynthesis. Whittaker et al. (1997) found transcription of ACC oxidase immediately following treatment with exogenous ethylene, while ACC synthase transcription occurred later, coincidentally with the climacteric ethylene production.

It was observed in our study that in kiwifruit the lag period for ethylene production decreased with temperature increase from 20 to 34°C as well as with increasing propylene concentrations (Figs. 3.4Aa and 5.4Aa). More research is needed at the molecular level to clarify the response of both ACC synthase and ACC oxidase to propylene concentration at different temperatures.

Respiration of kiwifruit increased with temperature up to 45°C independently of being treated or not with propylene (Figs. 5.8 and 5.9). The respiration climacteric peak was

higher at 45°C than at lower temperatures (142ml/kg/h), but the respiration rate decreased immediately, reaching values of about 20ml/kg/h in 50 hours, because the physiological processes stopped at this temperature.

Chilling induced ethylene production and ripening

From the results of the present study it is shown that temperatures up to 15°C advance the onset of ethylene production in kiwifruit when transferred to 20°C. Five days storage of kiwifruit at low temperature were not enough to induce autocatalytic ethylene production and ripening upon transference to 20°C (Fig. 6.1A). A period of 12 days at temperatures from 0 to 15°C satisfied the requirement of kiwifruit to autocatalyticaly produce ethylene immediately upon transference to 20°C (Fig. 6.2A). Fruit stored for 12 days at 0°C were the most efficient to produce ethylene upon rewarming.

Upon rewarming, ethylene production of chilled fruit was accompanied by a burst in CO_2 production and ripening, as it happened for kiwifruit ripened without propylene at harvest (Figs. 6.2B; 6.4 and Tables 6.4; 6.8). In a similar manner, the induction of ethylene production was caused by the induction of both ACC synthase and ACC oxidase activities which increased their activity only upon rewarming of the fruit (Table 6.3). The only difference between kiwifruit ripened at ambient temperature and fruit subjected to chilling was that the later advanced the ripening process upon rewarming as reported by Arpaia et al. (1994a).

Maximum ethylene production at 20°C occurred after 12 days storage at 0°C reaching a peak value of 140μl/kg/h. Longer storage periods gave decreased capacity of kiwifruit to produce ethylene upon rewarming: after 180 days storage at 0°C, ethylene production during shelf-life at 20°C did not pass 7μl/kg/h (Fig. 8.2). Andersen (1986) reported that prolonged chilling damages ACC oxidase. In the present research it was found that the decrease in the capacity of kiwifruit to produce ethylene with storage time at 0°C was accompanied by a decrease not only in ACC oxidase activity, but in ACC levels and ACC synthase activity, as well.

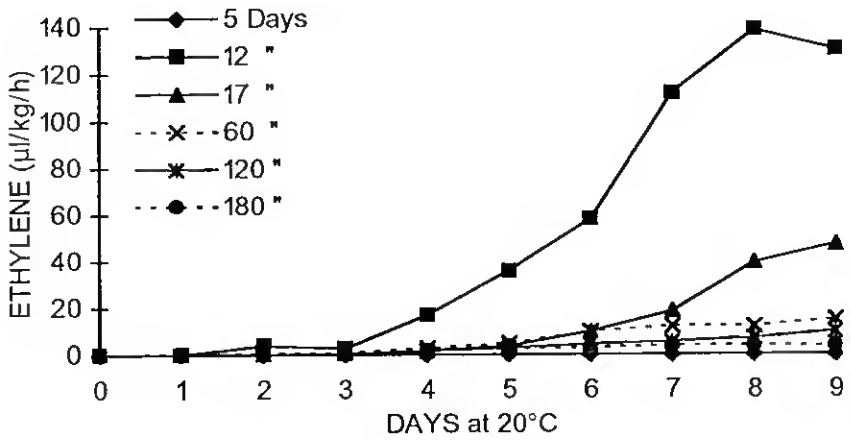


Figure 8.2. Ethylene production of harvested 'Hayward' kiwifruit at 20°C pre-stored at 0°C for 5, 12, 17, 60, 120 and 180 days.

Kiwifruit fatty acids include as major components linolenic acid (34-42%), oleic acid (26-37%), palmitic acid (15-22%), linoleic acid (8-11%) and stearic acid (1-5%) (Figs. 6.6 and 6.7). Fatty acid unsaturated/saturated ratio as well as membrane permeability increased with storage time and showed higher values at lower storage temperatures (Fig. 6.5). Their increase was more pronounced in the beginning of the storage period probably in order to help the fruit to adapt to stress conditions. Although there was a positive correlation between the unsaturated/saturated fatty acid ratio and membrane permeability, we found no clear correlation between unsaturated/saturated fatty acid ratio or membrane permeability with induction of ethylene production and ripening by low temperatures.

Controlled atmosphere storage

During the first 60 days storage in air at 0°C, kiwifruit ripening occurs at an initial high rate followed by a period of slow ripening that can be up to 120 days or more (Arpaia et al., 1984). The use of CA or ULO storage delayed the initial fast ripening rate by decreasing the rate of softening and the fading of flesh green color, but did not affect SSC increase (Fig. 7.1). The treatments 2%O_2+5%CO_2 and 1%O_2+1%CO_2 were the most efficient in extending kiwifruit storage life with fewer quality parameters changes. The 0.7%O_2+0.7%CO_2 treatment was not effective because resulted in an early

accumulation of acetaldehydes and a generally greater ethanol accumulation, which resulted in flesh breakdown after 120 days storage (Fig. 7.2).

When fruit are to be marketed immediately after storage, $2\%O_2+5\%CO_2$ can be used since fruit softened within 9 days shelf-life. Fruit stored at $1\%O_2+1\%CO_2$ can be used when longer shelf-life is expected and can be softened at our will by external ethylene application. Long storage periods (180 days) induce firmness values close to eating-ripe fruit and so decrease the importance of the above mentioned benefits of CA and ULO on firmness, but may still be important in keeping green color of the flesh in comparison with CS storage.

Kiwifruit CS and CA did not affect the chilling induction of ethylene production. Fruit rewarmed produced ethylene and ripened at a similar rate when treated or not with propylene (Fig. 7.6 and 7.10). The use of ULO storage may damage the ethylene pathway system since rewarmed fruit did not produce ethylene during 9 days shelf-life, even when treated with propylene, and ripened only upon ethylene exposure (Fig. 7.6 and 7.10).

Similarly with other fruit (Gaudiere and Vendrel, 1993), kiwifruit accumulated ACC and showed ACC synthase activity after 60 days storage in CS, CA and ULO (Figs. 7.3B and 7.4A). The ACC oxidase activity was very low during storage as for other fruit (Gaudiere and Vendrel, 1993), and increased upon rewarming only in fruit from CS and CA storage, not in fruit from ULO, independently of being treated or not with propylene (Fig. 7.16). The low ACC oxidase activity was the main reason for the inability of kiwifruit to produce ethylene after 60 days ULO storage.

There was a lower ACC synthase activity in fruit of the ULO treatments than in CS or CA only after 120 days storage, suggesting that ULO damages faster ACC oxidase than ACC synthase and this process is irreversible. However, more research is needed to clarify this point.

Future research

The results of the present research suggest that the enzymes of ethylene biosynthetic pathway in kiwifruit respond differently to different stimuli. It is known that ACC synthase and ACC oxidase are encoded by multigene families and differentially regulated (Ikoma et al., 1995; Barry et al., 1996). When kiwifruit are ripened with propylene after harvest, the activity of the ACC synthase takes about 4 days to start probably because it is needed some time for the perception of the stimuli by the ACC synthase gene induced by propylene. More research is needed at the molecular level to clarify when, after the commencement of propylene treatment, transcription and translation occur. In a similar manner it is important to clarify why higher temperatures or propylene concentrations decrease the lag period for ethylene autocatalysis.

As we observed in our study, the non-climacteric behaviour of kiwifruit at 10°C was mainly due to the inhibition of the transcription of the ACC synthase gene induced by propylene at this temperature. In addition, we found no transcription of the ACC synthase or ACC oxidase genes, induced by low temperature for up to 8 days at 10°C. After 12 days at low temperature, we found no ACC synthase or ACC oxidase activities but they started upon rewarming of the fruit with no lag period. After 60 days storage, kiwifruit showed ACC synthase activity during cold treatment being the inhibition of ethylene production at such temperatures due to low ACC oxidase activity. It seems that low temperatures inhibit the transcription of the ACC synthase genes induced by propylene but not the ACC oxidase ones. During cold treatment up to 12 days there is induction of the ACC synthase and ACC oxidase genes induced by chilling but translation and/or activation of the enzymes initiates only upon rewarming of the fruit (Wang, 1989). We suggest that as time at low temperatures increases, the process of translation and activation of ACC synthase is being completed, explaining the activity of this enzyme at low temperatures (Gaudierre and Vendrell, 1993). The ACC oxidase may undergo a similar process but its activation occurs only upon rewarming of the fruit (Gaudierre and Vendrell, 1993). More research is needed at the molecular level to support these hypothesis.

The decrease in the kiwifruit ACC synthase and ACC oxidase activity during shelf-life after prolonged storage, may be due to progressive decline of all physiological

processes with senescence. More research is needed to clarify how the process occurs and affects the enzymes of the ethylene biosynthetic pathway.

The mechanism by which ULO inhibits ACC oxidase activity upon rewarming was also not clarified. It is necessary to search if it occurs at the transcriptional or translational level and why it is irreversible.

CHAPTER 9. CONCLUSIONS

From the results of this study it can be concluded that kiwifruit respond differently to different stimuli for ethylene production:

1- Kiwifruit behaves as a climacteric fruit at temperatures from 20 to 34°C while at temperatures ≤ 10°C it behaves as a non-climacteric fruit.

2- The main reasons for the inhibition of the propylene-induced autocatalytic ethylene production in kiwifruit at low temperatures (≤10°C) are primarily the suppression of the propylene-induced ACC synthase gene expression and the possible post-transcriptional modification of ACC oxidase.

3- Kiwifruit senses immediately propylene for ripening at room temperature, while autocatalysis of ethylene production occurs later due to a time requirement for ACC synthase induction.

4- Increased temperature (from 20 to 34°C) reduces the lag period for autocatalysis of ethylene production induced by propylene.

5- High temperature stress (>38°C) inhibits ripening by strongly reduce ethylene production and sensitivity while respiration proceeds until the breakdown of the tissues at 45°C. The ACC oxidase activity is the first to be affected at high temperatures followed by ACC synthase.

6- At 20°C, mature unripe kiwifruit, in air free of propylene, start to produce ethylene, with a concomitant increase in ACC synthase and ACC oxidase activities, and ripe in about 19 days post-harvest.

7- Exposing kiwifruit to temperatures from 0 to 15°C for 12 days advances ethylene biosynthesis and ripening in comparison with fruit continuously at 20°C, by stimulating ACC synthase and ACC oxidase activities immediately upon rewarming of the fruit.

8- Storage for more than 60 days induces kiwifruit ACC synthase activity and ACC accumulation at 0°C, but not ACC oxidase which increases only upon rewarming of

the fruit. This increase in ACC synthase activity is probably due to the activation of the chilling-induced ACC synthase genes. However, prolonged storage progressively reduces the capacity of kiwifruit to produce ethylene after rewarming, by progressively decreasing ACC synthase and ACC oxidase activities.

9- The ULO storage irreversibly inhibits the induction by chilling of ethylene production upon rewarming, mostly by destroying the system that converts ACC to ethylene.

10- Some practical implications from these studies are that ripening can be advanced by applying ethylene to mature unripe kiwifruit or subjecting those fruit to 0-15°C for 12 days. In addition, due to the non-climacteric behaviour of kiwifruit at temperatures below 10°C, their storage can be extended for up to 6 months at low temperature (0°C), providing that sources of ethylene are removed from the storage chambers. When low temperature is combined with CA ($2\%O_2+5\%CO_2$) or ULO ($1\%O_2+1\%CO_2$), storage can be further extended with fewer changes in quality parameters. According to market demand, kiwifruit can be removed from the storage chambers and ripened at room temperature in 3-5 days, or in the case of ULO storage by the application of external ethylene.

REFERENCES

Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, New York, 302 pp.

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

Acaster, M. A. and H. Kende. 1983. Properties and partial purification of 1-aminocyclopropane-1-carboxylate synthase. Plant Physiol. 72: 139-145.

Adams, D. O. and S. F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxilic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA. 76: 170-174.

Ahmed, E. A. and J. M. Labavitch. 1980. Cell wall metabolism in ripening fruit. I. Cell wall changes in ripening 'Barlet' pears. Plant Physiol. 65: 1009-1013.

Alpi, A., P. Perata and H. Beevers. 1985. Physiological responses of cereal seedlings to ethanol. J. Plant Physiol. 119: 77-85.

Andersen, C. R. 1986. Rapid synthesis of ACC and ethylene by chilling-stressed cucumber fruit. HortScience 21: 858.

Andersen, C. R. and M. W. Kent. 1983. Chilling injury of cucumber fruit. Pant Physiol. 72 (Suppl.): 95.

AOAC. 1984. Official methods of analysis. Assoc. Official Analytical Chemists, Washington DC.

Apelbaum, A., A. C. Burgoon, J. D. Anderson, T. Solomos and M. Lieberman. 1981. Some characteristics of the system converting 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant Physiol. 67: 80-84.

Apelbaum, A. and S. F. Yang. 1981. Biosynthesis of stress ethylene induced by water deficit. Plant Physiol. 68: 594-596.

Arpaia, M. L., J. M. Labavitch, C. Greve and A. A. Kader. 1987. Changes in the cell wall components of kiwifruit during storage in air or controlled atmosphere. J. Amer. Soc. Hort. Sci. 112: 474-481.

Arpaia, M. L., F. G. Mitchell and A. A. Kader. 1994a. Postharvest physiology and causes of deterioration. ***In*** (J. K. Hasey, R. S. Johnson, J. A. Grant and W. O. Reil eds.). Kiwifruit growing and handling. ANR Publications, University of California, USA, pp 88-93.

Arpaia, M. L., F. G. Mitchell, A. A. Kader and G. Mayer. 1986. Ethylene and temperature effects on softening and white core inclusions of kiwifruit stored in air or controlled atmospheres. J. Amer. Soc. Hort. Sci. 111: 147-153.

Arpaia, M. L., F. G. Mitchell and G. Mayer. 1980. The effect of ethylene and high carbon dioxide on the storage of kiwifruit. HortScience 15: 423.

Arpaia, M. L., F. G. Mitchell and G. Mayer. 1994b. Cooling, storage, transportation and distribution. ***In*** (J. K. Hasey, R. S. Johnson, J. A. Grant and W. O. Reil eds.). Kiwifruit growing and handling. ANR Publications, University of California, USA, pp 108-115.

Arpaia, M. L., F. G. Mitchell, G. Mayer and A. A. Kader. 1984. Effects of delays in establishing controlled atmospheres on kiwifruit softening during and following storage. J. Amer. Soc. Hort. Sci. 109: 768-770.

Balague, C., C. F. Watson, A. J. Turner, P. Rouge, S. Picton, J. C. Pech and D. Grierson. 1993. Isolation of a ripening and wound induced cDNA from *cucumis melo* L., with homology to the ethylene-forming enzyme. Eur. J. Biochem. 212: 27-34.

Barry C. S., B. Blume, M. Bouzayen, W. Cooper, A. J. Hamilton and D. Grierson. 1996. Differential expression of the 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. Plant J. 9: 525-535.

Biale, J. B. And R. E. Yang.1981. Respiration and ripening on fruits-retrospect and prospect. ***In*** J. Friend and M. J. C. Rhodes (eds.). Recent advances in the biochemistry of fruit and vegetables. Academic Press, London, pp. 1-39.

Biggs, M. S., W. R. Woodson and A. K. Handa. 1988. Biochemical basis of high-temperature inhibition of ethylene biosynthesis in ripening tomato fruit. Physiol. Plant. 72: 572-578.

Blanke, M. M. 1991. Respiration of apple and avocado fruit. Postharvest News and Information 2: 429-436.

Blankenship, S. M. and D. G. Richardson. 1985. Development of ethylene biosynthesis and ethylene-induced ripening in D' Anjou pears during the cold requirement for ripening. J. Am. Soc. Hort. Sci. 110: 520-523.

Blankenship, S. M. and D. G. Richardson. 1986. ACC and ethylene levels in "d'Anjou" pears in air and controlled-atmosphere storage. HortScience 21: 1020-1022.

Bleecker, A. B., W. H. Kenyon, S. C. Somerville and H. Kende. 1986. Use of monoclonal antibodies in the purification and caracterization of 1-aminocyclopropane-1-carboxylate synthase, an enzyme in ethylene biosynthesis. Proc. Natl. Acad. Sci. USA. 83: 7755-7759.

Bleecker, A. B., G. Robison, H. Kende. 1988. Studies on the regulation of 1-aminocyclopropane-1-carboxylate synthase in tomato using monoclonal antibodies. Planta 173: 385-390.

Boller, T., R. C. Herner and H. Kende. 1979. Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145: 293-303.

Boller, T. and H. Kende. 1980. Regulation of wound ethylene synthesis in plants. Nature 286: 259-260.

Botella, J. R., R. N. Arteca and J. A. Frangos. 1995. A mechanical strain-induced , 1-aminocyclopropane-1-carboxylic acid synthase gene. Proc. Natl. Acad. Sci. USA. 92(5): 1595-1598.

Botella, J. R., C. D. Schlagnhaufer, R. N. Arteca, and A. T. Phillips. 1992. Identification and characterization of three putative genes for 1-aminocyclopropane-1-carboxylate synthase from etiolated mung been hypocotyl segments. Plant Mol. Biol. 18: 793-797.

Bousquet, J. F. and K. V. Thimann. 1984. Lipid peroxidation forms ethylene from 1-aminocyclopropane-1-carboxylic acid and may operate in leaf senescense. Proc. Natl. Acad. Sci. USA. 81: 1724-1727.

Bouzayen, M., G. Felix, A. Latche, J. C. Pech and T. Boller. 1991. Iron: an essential cofactor for the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Planta 184: 244-247.

Bouzayen, M. A. Hamilton, S. Picton, S. Barton and D. Grierson. 1992. Identification of genes for the ethylene-forming enzyme and inhibition of ethylene synthesis in transgenic plant using antisense genes. Biochem. Sci. Transaction 20: 76-79.

Bouzayen, M., A. Latche and J. C. Pech. 1990. Subcellular localization of the sites of conversion of 1-aminocyclopropane-1-carboxylic acid into ethylene in plant cells. Planta 180: 175-180.

Brackmann, A. J. Streif and F. Bangerth. 1993. Relationship between a reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. J. Amer. Soc. Hort. Sci. 118: 243-247.

Bradford, K. J. and S. F. Yang. 1980. Xilem transport of Enhanced 1-aminocyclopropane-1-carboxylic acid, an ethylene percursor, in waterlogged tomato plants. Plant Physiol. 65: 322-326.

Brady, C. J. 1987. Fruit ripening. Ann. Rev. Plant Physiol. 38: 155-178.

Brecht, J. K. and A. A. Kader. 1984. Regulation of ethylene production by slow ripening nectarine fruit as influenced by ethylene and low temperature. J. Amer. Soc. Hort. Sci. 109: 869-872.

Bufler, G. 1984. Ethylene-Enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening apples. Plant Phisiol. 75: 192-195.

Buffler, G. 1986. Ethylene--promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. Plant Physiol. 80: 539-543.

Bufler, G. and F. Bangerth. 1983. Effects of propylene and oxygen on the ethylene production systems of apples. Physiol. Plant. 58: 486-492.

Bufler, G., Y. Mor, M. S. Reid and S. F. Yang. 1980. Changes in 1-aminocyclopropane-1-carboxylic acid content of cut carnation flowers in relation to their senescence. Planta. 150: 439-442.

Burg, S. P. 1962. The physiology of ethylene formation. Annu. Rev. Plant Physiol. 13: 265-302.

Burg, S. P. 1973. Ethylene in plant growth. Proc. Natl. Acad. Sci. USA. 70: 591-597.

Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144-152.

Burton, W. G. 1974. Some biophysical principles underlying the controlled atmosphere storage of plant material. Ann. Appl. Biol.78: 149-168.

Burton, W. G. 1982. Postharvest Physiology of Food Crops. Longman, Harlow, U.K, 339 pp.

Cameron, A. C., C. A. L. Fenton, Y. B. Yu, D. O. Adams and S. F. Yang, 1979. Increased production of ethylene by plant tissues tissues treated with 1-aminocyclopropane-1-carboxilic acid. Hortscience 14: 178-180.

Chalutz, E., A. K. Mattoo, T. Solomos and J. D. Anderson. 1984. Enhancement by ethylene of cellulysin-induced ethylene production by tobacco leaf discs. Plant Physiol. 74: 99-103.

Chappell, J., K. Hahlbrock and T. Boller. 1984. Rapid induction of ethylene biosynthesis in cultured parsley cells by fungal elicitor and its relationship to the induction of phenylalanine ammonia-lyase. Planta 161: 475-480.

Chen, P. M., W. M. Melenthin and S. B. Kelly. 1983. Fruit qualities of "Bosc" pears stored in low oxygen or air as influenced by maturity. Scientia Hort. 21: 45-52.

Chen, T. S. and R.L. Shewfelt. 1988. Effect of chilling exposure of tomatoes during subsequent ripening. J. Food Sci. 53: 1160-1162.

Chou, T. C., A. W. Coulter, J. B. Lombardini, J. R. Sufrin and P. Talalay. 1977. The enzymatic synthesis of adenosil-methionine, mechanism and inhibition. ***In*** F. Salvatore et. al. (eds). The biochemistry of adenosyl-methionine. Columbia Univ. Press, New York, pp. 18-36.

Chou, M. C. And C. H. Kao. 1992. Stimulation of 1-aminocyclopropane-1-carboxilic acid-dependent ethylene production in detached rice leaves by methyl jasmonate. Plant Sci. 83: 137-141.

Christensen, H. N. And G. A. Palmer. 1967. Enzyme kinetics. W. S. Saunders Company, London.

Church, G. M. and W. Gilbert.1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA. 81: 1991-1995.

Czarnecka, E., L. Edelman, F. Schoffl and J. Key. 1984. Comparative analysis of physical stress responses in soybean seedlings using cloned heat shock cDNAs. Plant Mol. Biol. 3: 45-58.

Davies, P. L. and W. G. Chace. 1969. Determination of alcohol in citrus juice by gas chromatographic analysis of headspace. Hortscience 4:117-119.

Davies, K. M. and D. Grierson. 1989. Identification of cDNA clones for tomato mRNAs that accumulate during fruit ripening and leaf senescence in response to ethylene. Planta. 179: 73-80.

Dhindsa, R. S., P. Plumb-Dhindsa and T. A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32: 93-101.

Dilley, D. R., J. Kuai, L. Poneleit, Y. Zhu, Y. Pekker, I. D. Wilson, D. M. Burmeister, C. Gran and Alex Bowers. 1993. Purification and characterization of ACC oxidase and its expression during ripening in apple fruit. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 46-52.

Dong, J. G., W. T. Kim, W. K. Yip, G. A. Thompson, A. B. Bennet and S. F. Yang. 1991a. Cloning of a cDNA encoding 1-aminocyclopropane-1-carboxylate synthase and expression of its mRNA in ripening apple fruit. Planta 185: 38-45.

Dong, J. G., D. Olson, A. Silverstone and S. F. Yang. 1992. Sequence of a cDNA coding for a 1-aminocyclopropane-1-carboxylate oxidase homolog from apple fruit. Plant Physiol. 98: 1530-1531.

Dong, J. C., W. K. Yip and S. F. Yang. 1991b. Monoclonal antibodies against apple 1-aminocyclopropane-1-carboxylate synthase. Plant Cell Physiol. 32: 25-31.

Dopico, B., A. L. Lowe, I. D. Wilson, C. Merodio and D. Grierson. 1993. Cloning and characterization of avocado fruit mRNAs and their expression during ripening and low-temperature storage. Plant. Mol. Biol. Int. J. Mol. Biol. Biochem. Genet. Eng. 21: 437-449.

Eaks, I. L. 1978. Ripening, respiration, and ethylene production of 'Hass' avocado fruits at 20° to 40°C. J. Amer. Soc. Hort. Sci. 103: 576-578.

Eaks, I. L. 1983. Effects of chilling on respiration and ethylene production of 'Hass' avocado fruit at 20°C. HortScience 18: 235-237.

Erdmann, H., R. J. Griesbach, R. H. Lawson and A. K. Mattoo. 1989. 1-aminocyclopropane-1-carboxylic acid-dependent ethylene production during re-formation of vacuoles in evacuolated protoplasts of *Petunia hybrida*. Planta 179: 196-202.

Felix, G., D. G. Grosskopf, M. Regenass, C. W. Basse and T. Boller. 1991. Elicitor-induced ethylene biosynthesis in tomato cells. Characterization and use as a bioassay for elicitor action. Plant Phisiol. 97: 19-25.

Ferguson, A. R. 1990. Botanical nomenclature: *Actinidia chinensis*, *Actinidia deliciosa* and *Actinidia setosa*. ***In*** Ray Richards, in association with the New Zealand Soc. Hort. Sci. (eds.). Kiwifruit science and management. Auckland, New Zealand, pp 36-57.

Fernandez-Maculet, J. C. and S. F. Yang. 1992. Extraction and partial characterization of the ethylene-forming enzyme from apple fruit. Plant Physiol. 99: 751-754.

Field, R. J. 1981a. A relationship between membrane permeability and ethylene production at high temperature in leaf tissue of *Phaseolus vulgaris* L. Ann. Bot. 48: 33-39.

Field, R. J. 1981b. The effect of low temperature on ethylene production by leaf tissues of *Phaseolus vulgaris* L. Ann. Bot. 47: 215-224.

Field, R. J. 1984. The role of 1-aminocyclopropane-1-carboxylic acid in the control of low temperature induced ethylene production in leaf tissue of *Phaseolus vulgaris* L. Ann. Bot. 54: 61-67.

Field, R. J. 1985. The effects of temperature on ethylene production by plant tissues. ***In*** J. A. Roberts and G. A. Tucker (eds.). Ethylene and plant development. Butterworths, London, pp 47-69.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Forney, C. F. 1990. Ripening and solar exposure alter polar lipid fatty acid composition of 'Honey Dew' muskmelons. HortScience 25:1260-1264.

Frankel, E. N. 1980. Lipid oxidation. Prog. Lipid Res. 19: 1-22.

Fuhrer, J. 1982. Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus vulgaris* L.). Plant Physiol. 70: 162-167.

Fukuda, H., T. Ogawa, K. Ishihara, T.Fujii, K. Nagahama, T.Omata, Y. Inoue, S. Tanase and Y. Morino. 1992. Molecular cloning in escherichia coli, expression, and nucleotide sequence of the gene for the ethylene-forming enzyme of *Pseudomonas syringae* pv. *phaseolicola* PK2. Biochem. Biophys. Res. Comm. 188: 826-832.

Galliard, T. 1978. Lipolytic and lipoxygenase enzymes in plants and their action in wounded tissues. ***In*** (G. Kahl ed.) Biochemistry of wounded plant tissue. Walter de Gruyter and Co., Berlin New York, pp 155-201.

Gardner, H. W. 1995. Biological roles and biochemistry of the lipoxygenase pathway. HortScience 30: 197-204.

Gaudierre, C. L. and M. Vendrell. 1993. Cold-induced climacteric rise of ethylene metabolism in Granny Smith apples. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 136-141.

Gerasopoulos, D. 1988. Differential storage temperature and duration effects on ethylene synthesis and firmness of "Anjou" pears. PhD Dissertation, Oregon State University, USA.

Given, N. K. 1993. Kiwifruit. ***In*** G. B. Seymor, J.E. Taylor and G. A. Tucker (eds). Biochemistry of fruit ripening. Chapman & Hall, London, pp 235-254.

Graham, D. and B. D. Patterson. 1982. Responses of plants to low, nonfreezing temperatures: proteins, metabolism and acclimation. Ann. Rev. Plant Physiol. 33: 317-372.

Grierson, D., M. Maunders, A. Slater, J. Ray, C. Bird, W. Shuch, M. Holdworth, G. Tucker and J. Knapp. 1986. Gene expression during tomato ripening. Philos. Trans. R. Soc. London. B314: 399-410.

Goodwin, T. W. 1976. Chemistry and biochemistry of plant pigments. Vol. 1, Academic Press, London, 870 pp.

Guy, M. 1990. Vacuolar ethylene formation does not depend on membrane potential. Physiol. Plant. 79: 526-530.

Guy, M. and H. Kende. 1984. Conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by isolated vacuoles of *Pisum sativum* L. Planta 160: 281-287.

Hamilton, A. J., M. Bouzayen and D. Grierson. 1991. Identification of a tomato gene for the ethylene forming enzyme by expression in yeast. Proc. Nat. Acad. Sci. USA. 88: 7434-7437.

Hamilton, A. J., G. W. Lycet and D. Grierson. 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 346: 284-287.

Harman, J. 1981. Kiwifruit maturity. The Orchardist of N.Z. 54: 126-127.

Harman, J. E. and B. McDonald. 1983. Controlled atmosphere storage of kiwifruit. Effects on storage life and fruit quality. Acta Hort. 138: 195-201.

Harman, J. E. and B. McDonald. 1989. Controlled atmosphere storage of kiwifruit. Effect on fruit quality and composition. Scientia Hort. 37: 303-315.

Henskens, J. A. M., G. J. A. Rouwendal, A. Ten Have and E. J. Woltering. 1994. Molecular cloning of two different ACC synthase PCR fragments in carnation flowers and organ-specific expression of the corresponding genes. Plant Mol. Biol. 26: 453-458.

Heureux, G. P., M. Bergevin, J. E. Thompson and C. Willemot. 1993. Molecular species profile of membrane lipids of tomato pericarp during chilling. Acta Hort. 343: 286-287.

Hoffman, N. E. and S. F. Yang. 1980. Changes of 1-aminocyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates. J. Amer. Soc. Hort. Sci. 105: 492-495.

Hoffman, N. E. and S. F. Yang. 1982. Enhancement of wound-induced ethylene synthesis by ethylenein preclimacteric cantaloup. Plant Physiol. 69: 317-322.

Hogsett, W. E., R. M. Raba and D. T. Tingey. 1981. Biosynthesis of stress ethylene in soybeen seedlings: similarities to endogenous ethylene biosynthesis. Physiol. Plant. 53: 307-314.

Holdsworth, M. J., C. R. Bird, J. Ray, W. Schuch and D. Grierson. 1987. Structure and expression of an ethylene related mRNA from tomato. Nucleic Acids Res. 15: 731-739.

Horiuchi, Y and H. Imaseki. 1986. High temperature sensitivity of auxin-induced ethylene production in mung bean hypocotyl sections. Plant Cell Physiol. 27: 453-461.

Huang, P. L., J. E. Parks, W. H. Rottmann and A. Theologis. 1991. Two genes encoding 1-aminocyclopropane-1-carboxylate synthase in zicchini (*Cucurbita pepo*) are clustered and similar but differentially regulated. Proc. Natl. Acad. Sci. USA. 88: 7021-7025.

Hyodo, H., S. Aizawa and S. Ao. 1987. Ethylene formation during ripening of kiwifruit. J. Japan. Soc. Hort. Sci. 56: 352-355.

Hyodo, H., and R. Fukazawa. 1985. Ethylene production in kiwifruit. J. Japan. Soc. Hort. Sci. 54: 209-215.

Hyodo, H. and T. Nishino. 1981. Wound-induced ethylene formation in albedo tissue of citrus fruit. Plant Physiol. 67: 421-423.

Hyodo, H., K. Tanaka and K. Watanabe. 1983. Wound-induced ethylene production and 1-aminocyclopropane-1-carboxylic acid synthase in mesocarp tissue of winter squash fruit. Plant Cell Physiol. 24: 963-969.

Ikoma, Y., M. Yano and K. Ogawa. 1995. Cloning and expression of genes encoding ACC synthase in kiwifruit. Acta Hort. 398: 179-186.

Imaseki, H. and A. Watanabe. 1978. Inhibition of ethylene production by osmotic shoch. Further evidence for membrane control of ethylene production. Plant cell Physiol. 19: 345-348.

Inaba, M. and K. Chachin. 1988. Influence of and recovery from high-temperature stress on harvested mature green tomatoes. HortScience 23: 190-192.

Inaba, M. and K. Chachin. 1989. High-temperature stress and mitochondrial activity of harvested mature-green tomatoes. J. Amer. Soc. Hort. Sci. 114: 809-814.

Izzo, R., A. Scartazza, A. Masia, L. Galleschi, M. F. Quartacci and F. Navari-Izzo. 1995. Lipid evolution during development and ripening of peach fruits. Phytochemistry 39: 1329-1334.

Jobling, J., W. B. McGlasson and D. R. Dilley. 1991. Induction of ethylene synthesising competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1: 111-118.

Kacperska, A. and M. Kubacka-Zebalska. 1985. Is lipoxygenase involved in the formation of ethylene from ACC? Physiol. Plant. 64: 333-338.

Kanelis, A. K., T. Solomuos and Roubelakis-Angelakis K. 1990. Suppression of celulase and polygalacturonase and induction of alcohol dehydrogenase isoenzymes in avocado fruit mesocarp subjected to low oxygen stress. Plant Physiol. 96: 269-274.

Ke, D., H. van Gorsel and A. A. Kader. 1990. Physiological and quality responses of 'Bartlett' pears to reduced O_2 and enhanced CO_2 levels and storage temperature. J. Amer. Soc. Hort. Sci. 115: 435-439.

Kende, H. 1989. Enzymes of ethylene biosynthesis. Plant Physiol. 91: 1-4.

Kende, H. 1993. Ethylene biosynthesis. Annu. Rev. Plant Mol. Biol. 44: 283-307.

Kende, H. and T. Boller. 1981. Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit. Planta 151:476-481.

Key, J. C. Lin and C. Chen. 1981. Heat shock proteins of higher plants. Proc. Nat. Acad. Sci. USA. 78: 3526-3530.

Kim, W. T., A. Silverstone, W. K. Yip, J.G. Dong and S. F. Yang. 1992. Induction of 1-aminocyclopropane-1-carboxylate synthase mRNA by auxin in mung bean hypocotyls and cultured apple shoots. Plant Phisiol. 98: 465-471.

Kim, W. T. and S. F. Yang. 1994. Structure and expression of the cDNAs encoding 1-aminocyclopropane-1-carboxylate oxidase homologues isolated from excised mung bean hypocotyls. Planta 194: 223-229.

Klein, J. D. and S. Lurie. 1990. Prestorage heat treatment as a means of improving poststorage quality of apples. J. Amer. Soc. Hort. Sci. 115: 265-269.

Knee, M. 1987. Development of ethylene biosynthesis in pear fruit at -1°C. J. Exp. Bot. 38: 1724-1733.

Konze, J. R., J. F. Jones, T. Boller and H. Kende. 1980. Effect of 1-aminocyclopropane-1-carboxylic acid on the production of ethylene in senescing flowers of *Ipomoea tricolor* Cav. Plant Physiol. 66: 566-571.

Konze J. R. and G. M. K. Kwiatkowski. 1981. Ethylene formation after wounding is controlled by the regulation of 1-aminocyclopropane-1-carboxylate synthase synthesis. Planta 151: 327-332.

Krishman, M., H. T. Ngu Yen and J. J. Burke. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol. 90: 140-145.

Kuai, J. and D. R. Dilley. 1992. Extraction, partial purification and characterization of 1-aminocyclopropane-1-carboxylic acid oxidase from apple fruit. Postharvest Biol. Technol. 1: 203-211.

Lallu, N. A. N. Searle and E. A. MacRae. 1989. Investigation into ripening and handling strategy for early season kiwifruit. J. Sci. Food Agric. 47: 387-400.

Larrigaudiere, C. and M. Vendrell. 1993. Cold-induced climacteric rise of ethylene metabolism in 'Granny Smith' apples. ***In*** J. C. Pech and C. Balague (eds.). Cellular

and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 136-141.

Lau, O. L., Y. Liu and S. F. Yang. 1984. Influence of storage atmospheres and procedures on 1-aminocyclopropane-1-carboxylic acid concentration in relation to flesh firmness in 'Golden Delicious' apple. HortScience 19: 425-426.

Lee, S. K. and R. E. Young. 1984. Temperature sensitivity of avocado fruit in relation to C_2H_4 treatment. J. Amer. Soc. Hort. Sci. 109: 689-692.

Lehninger, A. L. 1982. Principles of biochemistry. Worth Publishers, Inc. New York, pp 734-752.

Lelievre, J. M., L. Tichit, L. Fillion, C. Larrigaudiere, M. Vendrell and J. C. Pech. 1995. Cold-induced accumulation of 1-aminocyclopropane-1-carboxylate oxidase protein in Granny Smith apples. Postharvest Biol. Technol. 5: 11-17.

Lester, G., J. Dunlap and S. Lingle. 1988. Effect of postharvest heating on electrolite leakage and fresh weight loss from stored muskmelon fruit. HortScience 23: 407.

Liang, X., S. Abel, J. A. Keller, N. F. Shen and A. theologis. 1992. The 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA. 89: 11046-11050.

Lieberman, M. 1979. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30: 533-591.

Lipton, W. J. and C. Y. Wang. 1987. Chilling exposure and ethylene treatment change the level of ACC in 'Honey Dew' melons. J. Amer. Soc. Hort. Sci. 112: 109-112.

Liu, Y., N. E. Hoffman and S. F. Yang. 1985. Promotion by ethylene of the capability to convert 1-aminocyclopropane-1-carboxylic acid to ethylene in preclimacteric tomato and cantaloup fruits. Plant Physiol. 77: 407-411.

Lizada, M. C. C. and S. F. Yang. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem. 100: 140-145.

Lorbeth, R., C. Dammann, M. Ebneth, S. Amati and J.J. Sanches-Serrano. 1992. Promotor elements involved in environmental and developmental control of potato proteinase inhibitor II expression. Plant J. 2: 477-486.

Lurie, S. And R. Ben-Aire. 1983. Microsomal membrane changes during the ripening of apple fruit. Plant Physiol. 73: 636-638.

Lurie, S. and S. Ben-Yehoshua. 1986. Changes in membrane properties and abscisic acid during senescence of harvested bell pepper fruit. J. Amer. Soc. Hort. Sci. 111: 886-889.

Lurie, S. and J. D. Klein. 1990. Heat treatment of ripening apples: differential effects on physiology and biochemistry. Physiol. Plant. 78: 181-186.

Lurie, S. and J. D. Klein. 1991. Acquisition of low temperature tolerance in tomatoes by exposure to high-temperature stress. J. Amer. Soc. Hort. Sci. 116: 1007-1012.

MacDiarmid, C. W. B. and R. C. Gardner. 1993. A cDNA sequence from kiwifruit homologous to 1-aminocyclopropane-1-carboxylic acid oxidase. Plant. Physiol. 101: 691-692.

MacRae, E. A., N. Lallu, A. N. Searle and J. H. Bowen. 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) Affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49: 413-430.

Mansour, R., A. Latche, V. Vaillant, J. C. Peach and M. S. Reid. 1986. Metabolism of 1-aminocyclopropane-1-carboxylic acid in ripening apple fruit. Physiol. Plant. 66: 495-502.

Marcellin, P. 1986. Conservation des fruits en atmosphere controle. situation actuelle. Revue Generale du Froid 76: 155-159.

Mason, H. S., D. B. Dewald, R. A. Cleelman and J. E. Mullet. 1992. Coregulation of soybean vegetative storage protein geen expression by methyl jasmonate and soluble sugars. Plant Physiol. 98: 859-867.

Mattoo, A. K., J. E. Baker and H. E. Moline. 1986. Induction by copper ions of ethylene production in *Spirodela oligorrhiza*: Evidence for a pathway independent of 1-aminocyclopropane-1-carboxilic acid. J. Plant Physiol. 123: 193-202.

Mattoo, A. K., N. Li and D. Liu. 1993. Tomato ACC synthase: Regulation of gene expression and importance of the C-terminal region in enzyme activity. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 223-231.

Maunders, M. J., M. J. Holdsworth, A. Slater, J. E. Knapp, C. R. Birth, W. Schuch and G. Grierson. 1987. Ethylene stimulates the accumulation of ripening-related mRNAs in tomato. Plant Cell Environ. 10: 177-184.

Maxie, E. C., F. G. Mitchell and N. F. Sommer. 1974. Effect of elevated temperature on ripening of 'Bartlett' pear, *Pyrus communis* L. J. Amer. Soc. Hort. Sci. 99: 344-349.

Mayberry, K. S. and T. K. Hartz. 1992. Extension of muskmelon storage life through the use of hot water treatment and polyethylene wraps. HortScience 27: 324-326.

Mayne, R. G. and H. Kende. 1986. Ethylene biosynthesis in isolated vacuoles of *Vicia faba* L.-requirement for membrane integrity. Planta 167: 159-165.

McDonald, B. 1990. Precooling, storage and transport of kiwifruit. ***In*** I. J. Warrington and G. C. Weston (eds). Kiwifruit science and management. Ray Richards and New Zealand Soc. Hort. Sci., Aukland, pp 429-459.

McDonald, B. and J. E. Harman. 1982. Controlled-atmosphere storage of kiwifruit. I. Effect on fruit firmness and storage life. Scientia Hort. 17:113-123.

McGarvey, D. J. and R. E. Christoffersen. 1992. Characterization and kinetic parameters of ethylene-forming enzyme from avocado fruit. J. Biol. Chem. 267: 5964-5967.

McGarvey, D. J., R. Sirevag and R. E. Christoffersen. 1992. Ripening-related gene from avocado fruit. Ethylene-inducible expression of the mRNA and polypeptide. Plant Physiol. 98: 554-559.

McGarvey, D. J., H. Yu and R. Christoffersen. 1990. Nucleotide sequence of a ripening-related cDNA from avocado fruit. Plant Mol. Biol. 15: 165-167.

McKeon, T. A. and S.F. Yang. 1982. A comparison of the conversion of 1-amino-2-ethylcyclopropane-1-carboxylic acid stereoisomers to 1-butene by pea epicotyls and by a cell free system. Planta 84-87.

McMurchie, E. J., W. B. McGlasson and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237: 235-236.

Mencarelli, F., W. J. Lipton and S. J. Peterson. 1983. Response of 'Zucchini' squash to storage in low-O_2 atmospheres at chilling and nonchilling temperatures. J. Am. Soc. Hort. Sci. 108: 884-890.

Metzidakis, J. and E. M. Sfakiotakis. 1989. The control of autocatalytic ethylene production and ripening in avocado fruit by temperature, high carbon dioxide and low oxygen. ***In*** . H. Clijsters et al. (eds.).Biochemical and Physiological Aspects of ethylene production in lower and higher plants. Kluwer Academic Publishers, The Netherlands, pp 201-210.

Metzidakis, J. and E. M. Sfakiotakis. 1993. Inhibition of ethylene biosynthesis and supression of cellulase and polygalacturonase in avocado fruit subjected to low oxygen storage. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 129-135.

Mitchel, F. G. 1986. Influence of cooling and temperature maintenance on stone fruit quality. Decid. Fruit Grower 36: 205-211.

Mitchel, F. G. 1994. Composition, maturity and quality. ***In*** (J. K. Hasey, R. S. Johnson, J. A. Grant and W. O. Reil eds.). Kiwifruit growing and handling. ANR Publications, University of California, USA, pp 94-98.

Mitchell, F. G., G. Mayer and W. Biasi. 1991. Effect of harvest maturity on storage performance of 'Hayward' kiwifruit. Acta Hort. 297: 627-635.

Mitchell, T., A. J. R. Porter and P. John. 1988. Authentic activity of the ethylene-forming enzyme observed in membranes obtained from kiwifruit (*Actinidia deliciosa*). New Phytol. 109: 313-319.

Nakagawa, N., N. Nakajima and H. Imaseki. 1988. Immunochemical difference of wound-induced of 1-aminocyclopropane-1-carboxylate synthase from the auxin-induced enzyme. Plant Cell Physiol. 29: 1255-1259.

Nakagawa, N., H. Mori, K. Yamazaki and H. Imaseki. 1991. Cloning of a complementary DNA for auxin-induced 1-aminocyclopropane-1-carboxylate synthase and differential expression of the gene by auxin and wounding. Plant. Cell. Physiol. 32: 1153-1163.

Nakajima, N. and H. Imaseki. 1986. Purification and properties of 1-aminocyclopropane-1-carboxylate synthase of mesocarp of *Cucurbita maxima* Duch. fruits. Plant Cell Physiol. 27: 969-980.

Nakajima, N., H. Mori, K. Yamazaki and H. Imaseki. 1990. Molecular cloning and sequence of a complementary DNA encoding 1-aminocyclopropane-1-carboxylate synthase induced by tissue wounding. Plant Cell Phisiol. 31(7): 1021-1029.

Nakajima, N., N. Nakagawa and H. Imaseki. 1988. Molecular size of wound-induced 1-aminocyclopropane-1-carboxylate synthase from *Cucurbita maxima* Duch. and change of translatable mRNA of the enzyme after wounding. Plant Cell Physiol. 29: 989-998.

Nanos, D. G., R. J. Romani and A. A. Kader. 1992. Metabolic and other responses of 'Barlet' pear fruit and suspension-cultured 'Passe crassane' pear fruit cells held in 0.25% O_2. J. Amer. Soc. Hort. Sci. 117: 932-940.

Niklis, N., E. Sfakiotakis and C.C. Thanassoupoulos. 1993. Ethylene biosynthesis in "Hayward" kiwifruit infected by *Botritys cinerea*. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 255-256.

Odawara, S., A. Watanabe and H. Imaseki. 1977. Involvement of cellular membrane in regulation of ethylene production. Plant cell Physiol.18: 569-575.

Olson, D. C., J. A. White, L. Edelman, R. H. Harkins and H. Kende. 1991. Differential expression of two genes for 1-aminocyclopropane-1-carboxylate synthase in tomato fruits. Proc. Natl. Acad. Sci. USA. 88: 5340-5344.

Park, K. Y., A. Drory and W. R. Woodson. 1992. Molecular cloning of an 1-aminocyclopropane-1-carboxylate synthase from senescing carnation flower petals. Plant Mol. Biol. 18: 377-386.

Parkin, K. L. And S. J. Kuo. 1989. Chilling-induced lipid degradation in cucumber (*Cucumis sativa* L. Cv. Hybrid C) fruit. Plant Physiol. 90:1049-1056.

Pech, J. C., C. Balague, A. Latche and M. Bouzayen. 1994. Postharvest physiology of climacteric fruits: recent developments in the biosynthesis and action of ethylene. Sciences des Aliments 14: 3-15.

Peck, S. C., D. C. Olson and H. Kende. 1993. A cDNA sequence encoding 1-aminocyclopropane-1-carboxylate oxidase from pea. Plant Physiol. 140: 681-686.

Picton, S., S. L. Barton, M. Bouzayen, A. J. Hamilton and D. Grierson. 1993. Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene forming enzyme transgene. Plant J. 3: 469-481.

Pirrung, M. C. 1986. Mechanism of a lipoxygenase model for ethylene biosynthesis. Biochemistry 25: 114-119.

Platt-Aloia, K. A. and W. W. Thomson. 1981. Ultrastructure of the mesocarp of mature avocado fruit and changes associated with ripening. Ann. Bot. 48: 451-465.

Porritt, S. W. and P. D. Lidster. 1978. The effect of pre-storage heating on ripening and senescence of apples during cold storage. J. Amer. Soc. Hort. Sci. 103: 584-587.

Porter, A. J. R., J. T. Borlakoglu and P. John. 1986. Activity of the ethylene-forming enzyme in relation to plant cell structure and organization. J. Plant Physiol. 125: 207-216.

Pratt, H. K. and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. Annu. Rev. Plant Physiol. 20: 541-584.

Pratt, H. K. and M. S. Reid, 1974. Chinese gooseberry: seasonal patterns in fruit growth and maturation, ripening, respiration and the role of ethylene. J. Sci. fd. Agric. 25: 747-757.

Rando, R. R. 1974. Chemistry and enzymology of K_{cat} inhibitors. Science 185: 320-324.

Rattanapanone, N., D. Grierson and M. Stein. 1977. Ribonucleic acid metabolism during the development and ripening of tomato fruits. Phytochemistry 16: 629-633.

Reid, M. S., D. A. Heatherbell and H. K. Pratt. 1982. Seasonal patterns in chemical composition of the fruit of *Actinidia chinensis*. J. Amer. Soc. Hort. Sci. 107: 316-319.

Reinbothe, S., C. Reinbothe, J. Lehmann and B. Parthier. 1992. Differential accumulation of methyl jasmonate-induced mRNAs in response to abcisic acid and desiccation in barley (*Hordeum vulgare*). Physiol. Plant. 86: 49-56.

Riov, J. and S. F. Yang. 1982a. Autoinhibition of ethylene production in citrus peel discs: suppression of 1-aminocyclopropane-1-carboxylic acid synthesis. Plant Physiol. 69: 687-690.

Riov, J. and S. F. Yang. 1982b. Effects of exogenous ethylene on ethylene production in citrus leaf tissue. Plant Physiol. 70: 136-141.

Rodrigues-Pousada, D. Van Der Straeten, A. Dedonder and M. Van Montagu. 1993. Cloning and expression an *Arabidopsis thaliana* 1-aminocyclopropane-1-carboxylate synthase gene: pattern of temporal and spatial expression. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 24-30.

Ross, G. S., M. L. Knighton and M. Lay-Yee. 1992. An ethylene-related cDNA from ripening apples. Plant Mol. Biol. 19: 231-238.

Rothan, C. and J. Nicolas. 1994. High CO_2 levels reduce ethylene production in kiwifruit. Physiol. Plant. 92: 1-8.

Rottmann, W. H., G.F. Peter, P.W. Oeller, J. A. Keller, N. F. Shen, B. P. Nagy, L. P. Taylor, A. D. Campbell and A. Theologis. 1991. 1-aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J. Mol. Biol. 222: 937-961.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Northern hybridisation. ***In*** Molecular cloning: a laboratory manual. Vol. 1 (chapter 7), Cold Spring Harbour Laboratory, Cold Spring Harbour, New York, pp 39-53.

Sandmann, G. and P. Boger. 1980. Copper-mediated lipid peroxidation processes in photosynthetic membranes. Plant Physiol. 66: 797-800.

Sanz, L. C., J. C. Fernandez-Maculet, E. Gomes, B. Vioque and J. Olias. 1993. Effect of methyl jasmonate on ethylene biosynthesis and stomatal closure in olive leaves. Phytochemistry 33: 285-289.

Sato, T., P. W. Oeller, A. Theologis. 1991. The 1-aminocyclopropane-1-carboxylate synthase of Cucurbita. J. Biol. Chem. 266: 3752-3759.

Sato, T. and A. Theologis. 1989. Cloning the mRNA encoding 1-aminocyclopropane-1-carboxylate synthase, the key enzyme for ethylene biosynthesis in plants. Proc. Natl. Acad. Sci. USA. 86: 6621-6625.

Satoh, S. and Y. Esashi. 1986. Inactivation of 1-aminocyclopropane-1-carboxylic acid synthase of etiolated mung bean hypocotyl segments by its substrate, S-adenosil-L-methionine. Plant Cell Physiol. 27: 285-291.

Satoh, S. and S. F. Yang. 1988. S- adenosylmethionine-dependent inactivation and radiolabelling of 1-aminocyclopropane-1-carboxylate synthase isolated from tomato fruits. Plant Physiol. 88: 109-114.

Satoh, S. and S. F. Yang. 1989a. Inactivation of 1-aminocyclopropane-1-carboxylate synthase by L-vinylglycine as related to the mechanism-based inactivation of the enzyme by S-adenosyl-L-methionine. Plant Physiol. 91: 1036-1039.

Satoh, S. and S. F. Yang. 1989b. Specificity of S-adenosyl-L-methionine in the inactivation and the labelling of 1-aminocyclopropane-1-carboxylate synthase isolated from tomato fruits. Arch. Biochem. Biophys. 271: 107-112.

Schaller, G. E. and A. B. Bleecker. 1995. Ethylene-binding sites generated in yeast expressing the *Arabidopsis* ETR1 gene. Science 270: 1809-1811.

Schirra, M. And P. Sass. 1994. Fatty acid profile changes in Marsh Seedless grapefruit in response to storage conditions. Acta Hort. 368: 429-434.

Senaratna, T., C. E. Mackay, B. D. McKersie and R. A. Fletcher. 1988. Uniconazole-induced chilling tolerance in tomato and its relationship to antioxidant content. J. Plant Physiol. 133: 56-61.

Sfakiotakis, E. M. and D. R. Dilley. 1973. Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. J. Amer. Soc. Hort. Sci. 98: 504-508.

Sfakiotakis, E. M. and D. R. Dilley. 1974. Induction of ethylene production in Bosc pears by postharvest cold stress. HortScience 9: 336-338.

Sfakiotakis, E. M., P. Ververidis and G. Stavroulakis, 1989. The control of autocatalytic ethylene production and ripening in kiwifruit by temperature and controlled atmosphere storage. Acta Hort. 258: 115-123.

Sisler, E. C. and C. Wood. 1988. Interaction of ethylene and CO_2. Physiol. Plant. 73: 440-444.

Sitrit, Y., J. Riov and A. Blumenfeld. 1986. Regulation of ethylene biosynthesis in avocado fruit during ripening. Plant Physiol. 81: 130-135.

Slater, A., M. J. Maunders, K. Edwards, W. Schuch and D. Grierson. 1985. Isolation and characterization of cDNA clones for tomato polygalacturonase and other ripening-related proteins. Plant Mol. Biol. 5: 137-147.

Smith, J. J., P. Ververidis and P. John. 1992. Characterization of the ethylene-forming enzyme partially purified from melon. Phytochem. 31: 1485-1494.

Smock, R. M. 1979. Controlled atmosphere storage of fruits. Hort. Rev. 1: 301-336.

Spanu, P. and T. Boller. 1989. Ethylene biosynthesis in tomato plants infected by *Phytophthora infestans*. J. Plant Physiol. 134: 533-537.

Spanu, P., D. Reinhart and T. Boller. 1991. Analysis and cloning of the ethylene-forming enzyme from tomato by functional expression of its mRNA in *Xenopus laevis* oocytes. EMBO J. 10: 2007-2013.

Spychalla, J. P. and S. L. Desborough. 1990. Fatty acids, membrane permeability, and sugars of stored potato tubers. Plant Physiol. 94: 1207-1213.

Stavroulakis, G. and E. M. Sfakiotakis. 1993. Regulation by temperature of the propylene induced ethylene biosynthesis and ripening in 'Hayward' kiwifruit. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 142-143.

Stavroulakis, G. and E. M. Sfakiotakis. 1995. Time course study of thermoregulation in ethylene biosynthesis and ripening of 'Hayward' kiwifruit induced by propylene. Acta Hort. 379: 429-436.

Stavroulakis, G. and E. M. Sfakiotakis. 1997. Regulation of the propylene induced ripening and ethylene biosynthesis by oxygen concentration in 'Hayward' kiwifruit. Acta Hort. 444: 529-534.

Suttle, J. C. and H. Kende. 1980. Methionine metabolism and ethylene biosynthesis in senescing petals of *Trandescantia*. Phytochemistry 19: 1075-1079.

Tang, X., A. M. T. R. Gomes, A. Bhatia and W. R. Woodson. 1994. Pistil-specific and ethylene-regulated expression of 1-aminocyclopropane-1-carboxylate oxidase genes in petunia flowers. Plant Cell 6: 1227-1239.

Theologis, A. 1992. One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. Cell 70: 181-184.

Theologis, A. 1995. Ethylene perceptors: how perceptive!. Science 270: 1774.

Thomai, T. and E. M. Sfakiotakis. 1997. Effect of low oxygen atmosphere storage on quality changes, acetaldehyde and ethanol accumulation in early and late harvested 'Hayward' kiwifruit. Acta Hort. 444: 593-598.

Tsai, D. S., R. N. Arteca, J. M. Bachman and A. T. Phillips. 1988. Purification and characterization of 1-aminocyclopropane-1-carboxylate synthase from etiolated mung bean hypocotyls. Arch. Biochem. Biophys. 264: 632-640.

Tucker, G. A. 1993. Introduction. ***In*** G. B. Seymor, J.E. Taylor and G. A. Tucker (eds). Biochemistry of fruit ripening. Chapman & Hall, London, pp 3-43.

Tucker, G. A. And D. Grierson.1982. Synthesis of polygalacturonase during tomato fruit ripening. Planta 155: 64-67.

Tucker, G. A. And D. Grierson. 1987. Fruit ripening. ***In*** D. D. Davies (ed.). The biochemistry of plants. Vol.12. Academic Press, San Diego, USA, pp 265-318.

Tucker, M. L. and G. G. Laties. 1985. The dual role of oxygenin avocado fruit respiration: kinetic analysis and computer modelling of diffusion-affected respiratory oxygen isotherms. Plant, Cell and Environment 8: 117-127.

Tucker, G., N. Robertson and D. Grierson. 1980. Changes in polygalacturonase isoenzymes during ripening of normal and mutant tomato fruit. Eur. J. Biochem. 112: 119-124.

Van Der Straeten, D., R. A. Rodrigues-Pousada, R. Villarroel, S. Hanley, H. Goodman and M. Van Montagu. 1992. Cloning, genetic mapping and expression analysis of an *Arabidopsis thaliana* gene that encodes 1-aminocyclopropane-1-carboxylate synthase. Proc. Natl. Acad. Sci. USA. 89: 9969-9973.

Van Der Straeten, D., L. Van Wiemeersch, H. M. Goodman. 1990. Cloning and sequence of two different cDNAs encoding 1-aminocyclopropane-1-carboxylate synthase in tomato. Proc. Natl. Acad. Sci. USA. 87: 4859-4863.

Van Der Straeten, D., L. Van Wiemeersch, H. M. Goodman and M. Van Montagu. 1989. Purification and partial characterization of 1-aminocyclopropane-1-carboxylate synthase from tomato pericarp. Eur. J. Biochem. 182: 639-647.

Ververidis, P. 1991. Characterisation and partial purification of the enzyme responsible for ethylene synthesis from 1-aminocyclopropane-1-carboxylic acid in plant tissues. PhD Thesis. Department of Agricultural Botany, School of Plant Sciences, Faculty of Agriculture and Food, University of Reading, Reading, U.K.

Ververidis, P. and P. John. 1991. Complete recovery *in vitro* of ethylene-forming enzyme activity. Phytochemistry 30: 725-727.

Wang, C. Y. 1989. Chilling injury of fruits and vegetables. Food reviews international. 5: 209-236.

Wang, C. Y. and D. O. Adams. 1980. Ethylene production by chilled cucumbers (*Cucumis sativus* L.) Plant Physiol. 66: 841-843.

Wang, C. Y. and D. O. Adams. 1982. Chilling-induced ethylene production in cucumbers (*Cucumis sativus* L.). Plant Physiol. 69: 424-427.

Wang, C. Y., C. E. Sams and K. C. Gross. 1985. Ethylene, ACC, soluble polyuronide, and cell wall noncellulosic neutral sugar content in 'Eldorado' pears during cold storage and ripening. J. Am. Soc. Hort. Sci. 110: 687-691.

Wang, H. and W. R. Woodson. 1991. A flower senescence-related mRNA from carnation shares sequence similarity with fruit ripening related mRNA involved in ehtylene biosynthesis. Plant Physiol. 96: 1000-1001.

Wang, T. T and S. F. Yang. 1987. The physiological role of lipoxygenase in ethylene formation from 1-aminocyclopropane-1-1-carboxylic acid in oat leaves. Planta. 170: 190-196.

Whitaker, B. D. 1994. Lipid changes in mature-green tomatoes during ripening, during chilling, and after rewarming subsequent to chilling. J. Amer. Soc. Hort. Sci. 119: 994-999.

Whittaker, D. J., G. S. Smith and R. C. Gardner. 1997. Expression of ethylene biosynthetic genes in *Actinidia chinensis* fruit. Plant Mol. Biol. 34: 45-55.

Wilson, J. M. and R. M. M. Crawford. 1974a. Leaf fatty-acid content in relation to hardening and chilling injury. J. Exp. Bot. 25: 121-127.

Wilson, J. M. and R. M. M. Crawford. 1974b. The acclimatization of plants to chilling temperatures in relation to the fatty-acid composition of polar lipids. New Phytol. 73: 805-820.

Wise, R. R. and A. W. Naylor.1988. Stress ethylene does not originate directly from lipid peroxidation during chilling-enhanced photooxidation. J. Plant Physiol. 133: 62-66.

Woodson, W. R., K. Y. Park, A. Drory, P. B. Larsen and H. Wang. 1992. Expression of ethylene biosynthesis pathway transcrips in senescing carnation flowers. Plant Physiol. 99: 526-532.

Yakir, D., A. Sadovski, H. D. Rabiniwitch and J. Rudich. 1984. Effect of high temperature on quality of processing tomatoes of various genotypes ripened off the vine. Scientia Hort. 23: 323-330.

Yang, S. F. 1980. Regulation of ethylene biosynthesis. HortScience 15: 238-243.

Yang, S. F., J. G. Dong, J. C. Fernandez-Maculet and D. C. Olson. 1993. Apple ACC oxidase: purification and characterization of the enzyme and cloning of its cDNA. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 59-64.

Yang, S. F. and N. E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35: 155-189.

Yang, S. F., Y. Liu and O. L. Lau. 1986. Regulation of ethylene biosynthesis in ripening apple fruits. Acta Hort. 179: 711-720.

Yano, M. and Y. Hasegawa. 1993a. Ethylene production in harvested kiwifruit with special reference to ripe rot. J. Jap. Soc. Hort. Sci. 62: 443-449.

Yano, M. and Y. Hasegawa. 1993b. Some features of autocatalytic ethylene production in kiwifruit. J. Jap. Soc. Hort. Sci. 62: 625-632.

Yip, W. K. 1993. Characterization of the 1-aminocyclopropane-1-carboxylate (ACC) synthase izozymes (genes) in tomato. ***In*** J. C. Pech and C. Balague (eds.). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, The Netherlands, pp 13-18.

Yip, W. K., J. G. Dong, J. W. Kenny, G. A. Thompson and S. F. Yang. 1990. Characterization and sequencing of the active site of 1-aminocyclopropane-1-carboxylate synthase. Proc. Natl. Acad. Sci. USA. 87: 7930-7934.

Yip, W. K., J. G. Dong and S. F. Yang. 1991. Purification and caracterization of 1-aminocyclopropane-1-carboxylate synthase from apple fruits. Plant Physiol. 95: 251-257.

Yip, W. K., T. Moore and S. F. Yang. 1992. Differential accumulation of transcripts for four tomato 1-aminocyclopropane-1-carboxylate synthase homologs under various conditions. Proc. Natl. Acad. Sci. 89: 2475-2479.

Yoshii, H. and H. Imaseki. 1981. Biosynthesis of auxin-induced ethylene. Effects on indole-3-acetic acid, benzyladenine and abscissic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. Plant Cell Physiol. 22: 369-379.

Yoshii, H. and H. Imaseki. 1982. Regulation of auxin-induced ethylene biosynthesis. Repression of inductive formation of 1-aminocyclopropane-1-carboxylate synthase by ethylene. Plant Cell Physiol. 23: 639-649.

Yoshii, H., A. Watanabe and H. Imaseki. 1980. Biosynthasis of auxin-induced ethylene in mung bean hypocotyls. Plant Cell Physiol. 21: 279-291.

Yu, Y. B., D. O. Adams and S. F. Yang. 1979. 1-aminocyclopropane-1-carboxylic acid synthase, a key enzyme in ethylene biosynthesis. Arch. Biochem. Biophys. 198: 280-286.

Yu, Y. B., D. O. Adams and S. F. Yang. 1980. Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. Plant Physiol. 66: 286-290.

Yu, Y. B. and S. F. Yang. 1980. Biosynthesis of wound ethylene. Plant Physiol. 66: 281-285.

Zagory, D. and A. A. Kader. 1989. Quality maintenance in fresh fruits and vegetables by controlled atmospheres. ***In***: Quality factors of fruits and vegetables: chemistry and technology. J. J. Jen (ed.). American Chemical Society, symposium series, 405, pp 174-188.