PHYSIOLOGICAL RESPONSES OF PEARS TO EXOGENOUS ETHYLENE AND PRE-HARVEST FACTORS INFLUENCING POST-HARVEST QUALITY WITH REFERENCE TO 'FORELLE' AND 'PACKHAM'S TRIUMPH' PEARS.

BY

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Thesis presented in partial fulfillment of the requirements for the degree Master of Science in Agriculture in the Department of Horticultural Science, University of Stellenbosch, Stellenbosch.

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

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SUMMARY

The major sites for fruit ripening and softening are found in the middle lamella where enzymatic and non-enzymatic mechanisms seem to degrade pectin polymers to a more soluble state and in the primary cell wall bring about the release of neutral sugars. Calcium plays a very important role in sustaining cell wall structure and cell membrane integrity. Calcium regulates various cellular functions through calmodulin, a calcium binding protein. Cell wall hydrolytic activity is a consequence of, rather than a prelude to ethylene synthesis. Fruit firmness decreased linearly in reaction to exogenously applied ethylene and a threshold level exists to which immature fruit will react on ethylene treatment. The onset of autocatalytic ethylene production rather than the respiratory climacteric seems to be critical in the ripening sequence.

'Forelle' pears are held in cold storage for 12 weeks, otherwise they will not reach a proper climacteric and will ripen unevenly. The result is that this cultivar, which already has a very late harvesting date, reaches the marketplace even later. In 1998, 'Forelle' pears harvested at commercial maturity were treated with ethylene at 0, 10 or 100 $\mu L \cdot L^{-1}$. Both 10 and 100 $\mu L \cdot L^{-1}$ ethylene treatments significantly hastened ripening, but differences between these concentrations were negligible and it was concluded that the storage period for 'Forelle' can be shortened to 4 weeks by exogenous ethylene.

In 1999, 'Forelle' pears were harvested at three maturity levels and treated with ethylene at a concentration of 50 $\mu L \cdot L^{-1}$ for periods of 12, 24 or 36 hours to evaluate the effect of harvest maturity and treatment length on ethylene treatment. All harvesting times required a minimum of 24 hours of ethylene to attain a physiological response. It was concluded that the storage period for 'Forelle' could be shortened to four weeks with exogenous ethylene gassing irrespective of harvest maturity. The time of attainment of a yellow ground colour and a loss of firmness differed significantly between harvesting maturities. Ethylene treated fruit in 1998 and 1999 held their firmness in cold storage very well for the whole 12 week period and fruit held at 20°C for seven days ripened normally. It seems that there is no risk of deterioration in quality for fruit held in cold storage after treatment with ethylene.

'Packham's Triumph' pears are characteristically yellow, juicy and soft when ripe, but highly susceptible to bruising. Firmer fruit are less prone to bruising injury during picking, grading, packing and transport. In 1998, physiologically young and physiologically old 'Packham's Triumph' and 'Forelle' orchards were treated with foliar applied calcium nitrate (0.7%), calcium chloride (0.7%) and urea (750 g.100L^{-1} water) one week before harvest and harvested according to bearing position. Fruit was stored under controlled atmosphere (CA) for a period of 9 months. The four treatments had no significant effect on the storage potential of 'Packham's Triumph' and 'Forelle' pears.

FISIOLOGIESE REAKSIE VAN PERE OP EKSOGENE ETILEEN EN VOOR-OES FAKTORE WAT NA-OES KWALITEIT BEÏNVLOED MET VERWYSING NA 'FORELLE' EN 'PACKHAM'S TRIUMPH' PERE

OPSOMMING

Die belangrikste fokuspunte vir vrugsagwording en rypwording is in die middel lamella en in die primêre selwand waar ensiematiese en nie-ensiematiese maganismes onderskeidelik pektien polimere afbreek na 'n meer oplosbare vorm en waar neutrale suikers vrygestel word. Kalsium speel 'n belangrike rol in die onderhoud van selwand struktuur en integriteit. Kalsium dien as 'n reguleerder van 'n verskeidenheid sellulêre funksies deur calmodulin, 'n kalsium bindende protein. Selwand hidrolitiese aktiwiteit is meer as gevolg van, as 'n voorvereiste tot, etileen sintese. Vrugstewigheid daal liniêr in reaksie op eksogene etileen en daar bestaan 'n drempelwaarde waarby onvolwasse vrugte sal reageer op etileen. Die begin van autokatalitiese etileen produksie, nie die respiratoriese klimakterium, blyk krities te wees in die rywordings proses.

'Forelle' pere word vir 12 weke in koelopberging gehou anders sal hulle nie 'n voldoende klimakterium bereik nie en sal oneweredig ryp word. Die implikasie is dat die kultivar, wat reeds 'n laat oesdatum het, die markomgewing nog later sal bereik. In 1998 is 'Forelle' pere, wat om kommersieële volwassenheid geoes is, met onderskeidelik 0, 10 of 100 $\mu L \cdot L^{-1}$ etileen behandel. Beide die 10 en 100 $\mu L \cdot L^{-1}$ etileen behandelings het rypwording betekenisvol vervroeg, maar die verskil tussen die konsentrasies was weglaatbaar klein. Die gevolgtrekking is dat die koelopberging van 'Forelle' pere verkort kan word tot 4 weke met die behandeling met etileen.

Om die effek van vrug volwassenheid en behandeling lengte op etileen behandeling te evalueer is 'Forelle' pere tydens drie stadiums gedurende volwassewording geoes en behandel vir onderskeidelik 12, 24 en 36 ure met 50 $\mu L \cdot L^{-1}$ etileen. Alle stadiums van oesvolwassenheid het 'n minimum etileen behandeling van 24 uur benodig om 'n fisiologiese responsie te bewerkstellig. Die gevolgtrekking is dat die koelopberging van

'Forelle' pere verkort kan word tot vier weke met die behandeling met etileen ongeag oesvolwassenheid. Oesvolwassenheid het wel betekenisvol verskil in die tyd benodig tot die verkryging van 'n geel agtergrond kleur en 'n daling in vrugstewigheid. Etileen behandelde vrugte in 1998 en 1999 het hul vrugstewigheid uitstekend gehou gedurende die 12 weke koelopberging en het normaal sag en ryp geword wanneer pere gehou is teen 20°C vir sewe dae. Daar blyk geen gevaar te wees van 'n daling in kwaliteit in koelopberging wanneer vrugte behandel is met etileen nie.

'Packham's Triumph' pere is kenmerklik geel, sappig en sag wanneer ryp, maar baie kwesbaar vir beserings. Stewiger vrugte is minder kwesbaar vir besering gedurende oes, gradering, pak en vervoer. In 1998 is fisiologiese jong en fisiologiese ou 'Packham's Triumph' en 'Forelle' boorde een week voor oes met onderskeidelik kalsium nitraat (0.7%), kalsium chloried (0.7%) en ureum (750 g.100L^{-1} water) as 'n blaarvoeding behandel en geoes volgens draposisie. Vrugte was daarna vir 9 maande onder beheerde atmosferiese toestande (BA) opgeberg. Die vier behandelings het geen betekenisvolle effek op die opbergingspotensiaal van 'Packham's Triumph' en 'Forelle' pere gehad nie.

ACKNOWLEDGEMENTS

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CHAPTER 1: LITERATURE REVIEW: FRUIT SOFTENING WITH REFERENCE TO APPLE AND PEAR FRUIT

1.1 INTRODUCTION

Texture is recognized as one of the most important postharvest qualities of fruit. It not only influences palatability, but affects shelf-life, transportability and disease resistance. The degree to which fruit soften reflects, among other things, changes in the structure and cohesion of their cell walls. In order to manipulate textural properties of fruit we need to understand the biochemical basis of the ultrastructural changes in fruit. Current strategies rely on the optimization of storage conditions including the removal of ethylene gas, modification of atmospheric conditions and strict temperature control, but still a large proportion of fleshy fruits soften prematurely or too rapidly before consumption.

The biochemical basis of fruit softening is still poorly understood and this is mainly due to the complexity and interaction of enzymatic and non-enzymatic factors. Until recently the complexity of cell wall modifications was not fully appreciated, with attention focusing on just a few of the cell wall degrading enzymes, e.g. polygalacturonase. Texture is likely to involve a number of factors, including cell wall integrity, turgor pressure and anatomical characteristics. Traditional breeding methods offer one route to enhance the texture of fruits, but a more targeted approach would be through the use of genetic manipulation and recombinant DNA technology. This would depend upon the success with which the correct genes are identified and thus manipulated accordingly. The aim of this review however, is not to ponder the future possibilities in manipulating fruit softening, but to review the existing literature.

This review has four goals; firstly, it aims to describe the structural model considered to be the basis for pome fruit cell walls, secondly to discuss the cell wall components and the changes associated with ripening and softening. The third goal is to assess the enzymatic mechanisms involved, and fourthly to discuss the role that non-enzymatic factors play in softening of apple and pear fruit.

1.2 CELL WALL STRUCTURE AND COMPONENTS

1.2.1 Primary cell wall

Essentially every cell of higher plants is encased in a cellulosic wall (McNeil *et al.*, 1984). The walls of growing cells are called primary cell walls and are composed of approximately 90% polysaccharide and 10 % protein. The polysaccharides consist of approximately 30% cellulose, 30% hemicellulose and 30-35% pectin. Meristematic and parenchymous tissues are particularly rich in pectic substances. Pectic substances are closely related to each other and highly hydrated.

Proteins are mostly glycoproteins and other substitutes such as methyl ethers and esters, and acetyl and feruloyl esters (McNeil *et al.*, 1984). The extensins play an important role in cell growth and lectins help in recognition of foreign molecules (Salisbury & Ross, 1992)

Unbranched cellulose molecules are bound together in long cylindrical fibers, 4.5 to 8.5 nm thick (Salisbury & Ross, 1992). These microfibrils are embedded within a matrix of other materials which are chemically much more complex (Fry, 1988). Principal among these are hemicellulose, which forms a branching molecular network filled with water. The matrix materials in which cellulose is embedded are not crystalline like cellulose and thus the primary cell wall is adapted to growth. When the cell is not growing the primary cell wall resists stretching, but during growth the cell wall stretches plastically, irreversibly like bubblegum, through the ability of the microfibrils to move apart longitudinally and to slide past each other (Salisbury & Ross, 1992). In an attempt to model the effective parts of the cell wall, Preston (1979) considered the existing evidence. A few models exist using one or a combination of bonds expected to be present in primary cell walls: (a) Covalent bonding. This is the feature of the model which Keegstra *et al.*, (1973) proposed. They proposed that all the matrix polysaccharides and the glycoproteins are covalently linked though connected to the microfibrils by xyloglucan, which is only hydrogen bonded to them. This model is unlikely because it proposes that a growing cell wall is a single macromolecule and is thus incompatible with the known swelling and shrinkage of primary walls and extension during growth. (b) Bonding between glycoprotein chains. It was claimed that on the basis of the lack of cohesion in a wall after treatment with dithiothreitol, that the protein chains might be linked by disulfide bonds. (c) A sequence of calcium bonds between polyuronides (Figure 1a). These bonds, numbering about 20, working in collusion, might be needed (Rees, 1977). (d) Two or more polysaccharide chains are held together by numerous

closely spaced hydrogen bonds. This is the case with ribbon-like molecules (Figure 1c). (e) Simulated bonding induced by an entanglement between chains which tend to twist as figured for carrageenan (Rees, 1977) and possibly for all (1-3) linked polysaccharides and the protein which will lie on the α-helix (Figure 1b).
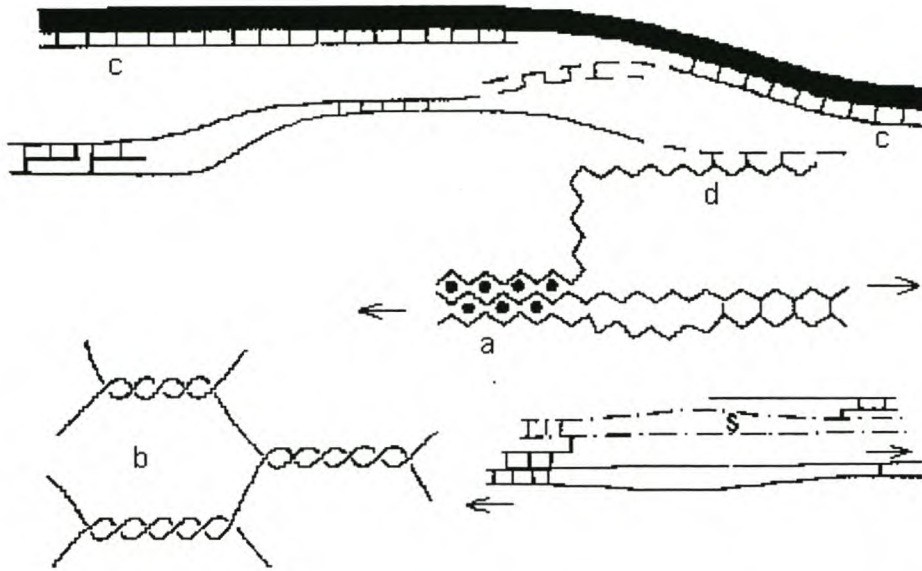

Figure 1: Diagrammatic presentations of some possible bonding schemes in primary walls. Solid, straight or smoothly curved lines. (c) = ribbonlike polysaccharides. Broken lines = twisted ribbon-like polysaccharides, e.g. xylan with a threefold screw axis. Zigzag lines (a) = rhamnogalacturonan (pectic acid) Broken and dotted lines = Protein; Helices = helical chain molecules such as 1-3 linked xylan. Full circles (a) = Calcium ions in the "egg-box". Short thinner lines represent hydrogen bonds and arrows show possible shear stresses allowing yield and creep. S = disulfide bond. (Preston, 1979)

According to Preston (1979) the cellulosic crystallite must be clothed with flat, ribbon like molecules, strongly hydrogen bonded because they are fully compatible with cellulose (Figure 1c). Such molecules are β-(1-4)-linked glucans, xylans or mannans. These in turn will preferentially hydrogen bond to lengths of a chain compatible with cellulose, such as xylan, of which neighboring lengths might have a threefold axis not so compatible. These lengths would in turn form suitable links to a polyuronide aggregate (Figure 1d) linked together by calcium bridges with hydrogen bonds in series. The kinking induced in a polygalacturonan chain by the presence of L-rhamnose might be a means of cross-linking many polysaccharide chains (Preston, 1979).

1.2.2 Middle lamella

The middle lamella consists of pectic substances that cement adjacent cells together and are ideally suited because they exist as gels (Salisbury & Ross, 1992). The fact that cells of non-woody plant tissues can be dissociated using chelating agents (Knee & Bartley, 1981) suggests that cohesion of the middle lamella depends on ionic rather than covalent bonds. Divalent cations, especially calcium are the obvious candidates. Single calcium ions would only form weak bonds between isolated carboxyl groups and thus more stable bonding occurs by cooperative effects when sequences of uronic acid lie parallel and each pair of residues encloses a calcium ion. This is called the "egg-box" model with junction zones (Grant *et al.*, 1973). Ultrastructural studies of ripening pome fruit showed that pectin in the middle lamella are being broken down by certain enzymes and this plays the predominant role in cell separation and fruit softening (Ben-Arie *et al.*, 1979a). When referring to the cell wall in this review it includes the primary cell wall and the middle lamella.

1.2.3 Secondary cell wall

In certain cells, e.g. xylem, which when mature provide support for the plant or conduct fluids under negative pressure, the protoplast secretes a secondary wall after the cell has stopped enlarging (Salisbury & Ross, 1992). These secondary walls consist of about 45% cellulose, 30% hemicellulose and 22-28% lignin, which is very rigid and resists changes in form.

1.3 CELL WALL COMPONENTS AND RIPENING-ASSOCIATED CHANGES

There are two major compositional changes which occur in the cortical cell walls during ripening and softening of apples, namely the loss of galactose residues and the solubilization of polygalacturonide (Bartley, 1974). It is thought that the loss of galactose residues arises mainly from the hydrolysis of the galactan of the primary cell wall and that the polygalacturonide is derived from the middle lamella (Knee, 1975).

1.3.1 Cellulose

Cellulose is a linear β-(1-4) glucan (Figure 2) that provides the mechanical strength of the plant cell wall (Fisher & Bennett, 1991). Cellulose self-associates by intermolecular hydrogen bonding to form microfibrils of at least 36 glucan chains and as already noted, becomes strongly associated with hemicellulose in the cell wall. Because of this association the physical properties

of the microfibril are determined by the intermolecular hydrogen bond. Faust & Shear (1972) claimed that high cellulose content of apples may play a role in the rigidity of cell walls.
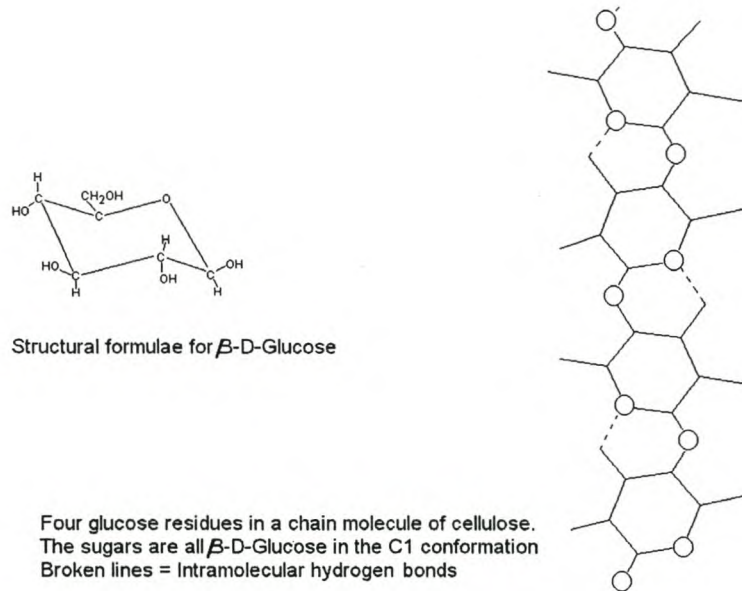
Structural formulae for β-D-Glucose

Four glucose residues in a chain molecule of cellulose. The sugars are all β-D-Glucose in the C1 conformation Broken lines = Intramolecular hydrogen bonds

Figure 2: Structure of a cellulose chain (linear β-(1-4) glucan) (Preston, 1979)

When fruit ripen it might be anticipated that it is associated with a change in cellulose structure. This is apparently not the case as could be deducted by Ahmed & Labavitch (1980a) with chemical analyses of cellulosic glucan levels. It appears that cellulose levels remain constant during the ripening of pears (Figure 3). Alternatively it could be possible that the ultrastructural changes associated with ripening may result from the degradation of a component of the noncellulosic matrix. Consequently a loss of microfibrillar organization may be found (Fisher & Bennett, 1991).

1.3.2 Hemicellulose
All cell wall models agree that cellulose is associated with a monolayer of hemicellulose bound to the microfibril surface by hydrogen bonds (Keegstra et al., 1973; Varner & Lin, 1989; Fry, 1989a). In dicotyledonous cell walls the principal hemicellulose is xyloglucan. It has a linear β-(1-4)-glucosyl backbone, identical to that of cellulose. Xylose and more complex side chains containing xylose, galactose, and fructose are attached to carbon 6 of glucosyl residues of the glucan backbone at regular intervals. In some cases xylose side chains are attached in a highly

regular fashion at three consecutive glucose residues followed by an unsubstituted glucose (Fry, 1989a; Hayashi, 1989). The small quantities of monomers characteristic of hemicellulose, xylose, glucose and mannose do not decline in the ripening of apples (Bartley, 1976) and pears (Knee & Bartley, 1981).

1.3.3 Pectin

Pectin is a class of complex polysaccharides defined by their extractability in hot water, chelators or dilute acid (Fisher & Bennett, 1991). Pectin is often described as consisting of "smooth" and "hairy" blocks, which may reside as components of a single pectin polymer. "Smooth" blocks are characterized by a linear conformation of α–(1–4)–linked galacturonic acid and its methyl ester. Within these smooth homogalacturonan polymers are α-(1-2)-linked rhamnosyl residues spaced irregularly. It has been suggested by Fry (1988) that the rhamnosyl residues delineate homogalacturonan domains for methyl esterification or de-esterification, thus enabling Ca^{2+} cross-linking at regular intervals.

The so-called "hairy" blocks include rhamnogalacturonans I and II and are complex heteropolymers comprised of 12 different sugars. The backbone, rich in galacturonic acid and rhamnose, bears numerous side chains rich in arabinose and galactose but also containing fucose, methylfucose, methylxylose, apiose, glucuronic acid, aceric acid, keto-deoxyoctulosonic acid and glucose (Fry, 1988). The "hairy" blocks are, in general, not susceptible to degradation by pectinase. The rhamnogalacturonan polymers are distributed throughout the primary cell wall, with the middle lamella constituting the richest source (Albersheim & Killias, 1963; Albersheim et al., 1960). The carboxyl groups may be esterified, and branches (usually branched arabinans and/or linear galactans (Knee, 1975)) are frequently found. The diversity in the D-galacturonans is thereby largely a function of their molecular weight (i.e. degree of polymerisation), the presence of acetyl groups, the proportion and location of neutral sugars, and the degree of esterification.

Studies on the conformation of the D-galacturonans (Rees, 1977) showed that these polymers may interact in two basic fashions depending largely on the degree of esterification. The polymers are composed of regions of periodic sequences of uninterrupted galacturonan, which can engage in some degree of ordered, intermolecular packing. In this manner, chains form a

network by joining through these regions of association termed "junction zones" (Figure 3). The nature of the ordered associations has been interpreted in terms of an "egg-box" model (Grant *et al.*, 1973) involving co-operative binding of cations, particularly Ca^{2+}, between the associated D-galacturonan chains (Figure 2). Galacturonans are thus able to form a gel.
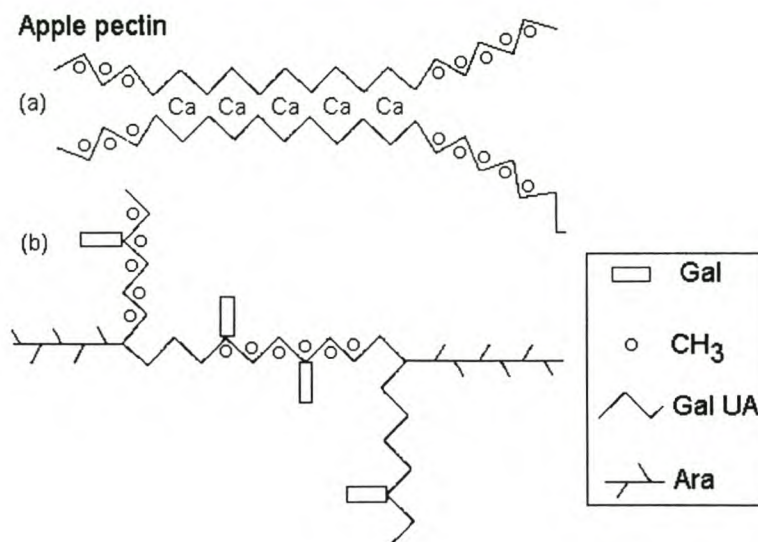

Figure 3: Proposed structures for polygalacturonate fractions in apple fruit. (a) sections of two "homogalacturonan" chains with linkage region. (b) Portion of branched rhamnogalacturonan. The kinks in the backbone represent rhamnose residues, which are possibly the site of attachment of arabinan branches (Knee & Bartley, 1981).

The extent of packing is apparently limited by the presence of L-rhamnose units, which serve to kink/bend the polymers and/or by the presence of branches and the degree of esterification (Rees, 1977; Preston, 1979). These interruptions result in a deviation from conformational regularity and cause the polysaccharides to leave ordered associations and engage with additional partners. Because of the extended, ribbon-like conformation of the D-galacturonans (Rees, 1977), more highly esterified polymers may also engage in some degree of interchain packing. High-methoxy pectins show little affinity for cations, probably explaining the ease with which these polymers may be extracted from fruit tissues (Pilnik & Voragen, 1970).

That D-galacturonans are modified during ripening is apparent, primarily from the increased quantities of soluble polymers found in homogenates of ripening fruit. An overall loss in pectins has sometimes been noted, but an increase in solubility is more frequently observed (Knee 1978a, 1978b; Yamaki *et al.*, 1979; Ahmed & Labavitch, 1980a).

During ripening in apple and pear there is a dramatic increase in water- and chelator-soluble pectins (Ben-Arie *et al.,* 1979b). This observation suggests that pectin polymers are cleaved from covalent cross linkages in the cell wall (Huber, 1983). The process is matched by a decline in the insoluble pectin fraction (Knee & Bartley, 1981) and it is natural to conclude that a change in part of the insoluble fraction converted it to a soluble form. The soluble pectin in ripe apples has a low proportion of neutral monomers (Knee, 1973b) and this is in keeping with its supposed origin in the middle lamella, which is believed to be rich in this type of pectin.

Apple is exceptional among other fruit because it seems to contain no endo-polygalacturonase (endo-PG). In other fruit, like pear (Pressey & Avants, 1976), this enzyme seems to be the means to achieve solubilization of pectin. The viscosity of the wall-bound pectin in apples declines during ripening although one should expect the neutral glycan branches on the polymer to block exo-polygalacturonase attack (Knee & Bartley, 1981). It was noted by Bartley (1977) that the hydrolysis of the galactan is closely related to the observed loss of firmness and this points to the importance of the polymer in the maintenance of the three-dimensional structure of the cell wall.

1.3.4 Cell wall protein

The best characterized, and perhaps the most abundant, structural proteins of dicot cell walls are the extensins, a family of hydroxyproline rich glycoproteins (Varner & Lin, 1989). Glycoprotein is in some way attached to the polysaccharides in the wall and is of structural significance (Preston, 1979). There is, as yet, no agreement as to the sugar covalently bound in the glycoprotein, to the polysaccharide to which the glycoprotein is bound, or indeed whether there is a single protein or several (Preston, 1979). The protein, containing hydroxyprolyl residues, is glycosylated with arabinose and is believed to be somehow linked to cellulose microfibrils (Knee, 1975). This means that it plays a structural role.

Knee (1975) found little change in the distribution or total amount of hydroxyproline residues during the ripening of apples. An increase in solubility of protein in ripe fruit was noted but was considered as a contamination with cytoplasmic protein and does not mean that proteins are released during ripening.

The possible linkages between polyuronide and glycoproteins containing hydroxyproline,

arabinose and galactose are, according to Knee (1973b), difficult to establish because of aggregation between these products after extraction. Other cell wall glycoproteins containing xylose and glucose residues are thought to have a possible role in stabilizing hemicellulose structure.

1.3.5 Neutral sugars

Other neutral cell wall polymers undergo extensive changes related to ripening. The loss of neutral sugars, especially galactose and arabinose, is quantitatively the largest ripening associated change in cell wall composition in many fruit (Fisher & Bennett, 1991). As mentioned earlier, the pectic substances display remarkable structural heterogeneity. The frequency and distribution of side chains, the degree of esterification, and other modifications may confer a variety of biological and structural functions. It is not surprising that the presence of nongalacturonan structural variables has long been considered as possibly constituting other means by which the rheology of the cell wall is influenced (Huber, 1983). According to Pressey (1977) the only galacturonosyl-galacturonic acid linkage identified in pectin is the α-(1-4) linkage. On this basis, it is unlikely that branches of galacturonan, if they exist, are attached directly to the galacturonan backbone. It is possible, however that such branches are attached through neutral sugars on the chain. Neutral sugars other than rhamnose may be present in fruit pectins. Purified pectin (Pressey, 1977) from apples contained 1.2% rhamnose, 9.3% arabinose, 1.4% galactose, 0.8% xylose, traces of fucose, 2-0-methylxylose and 2-0-methylfucose. It is not clear if the neutral sugars occur in the galacturonan chain or as branches on the chain.

There is considerable evidence that wall neutral sugars are subjected to altered metabolism during fruit softening. This evidence has been largely derived from studies of the sugar composition of fruit cell walls. Thus, information is limited to those polymers that are metabolised to an extent that they are no longer associated with the isolated cell wall. In many fruit types the quantities of wall arabinose and galactose, sugars commonly found associated with pectic polymers, were observed to decrease (Figure 4) during ripening (Bartley, 1974; Bartley, 1976; Yamaki *et al.*, 1979; Ahmed & Labavitch, 1980a).

In ripening pear (Ahmed & Labavitch, 1980a), the arabinose lost during ripening (50% of total) was recovered as a component of an 80% ethanol-insoluble polymer. The arabinose was identified as a component of pectin arabinan. Ahmed & Labavitch (1980a) identified cell wall arabinose, solubilized during the ripening of pear, as a component of pectin arabinan and thus

indicated that arabinose loss occurred as a consequence of pectin degradation. In contradiction to these findings other studies have shown that neutral sugar loss occurs independently of pectin degradation (Knee, 1973a; Bartley, 1976). The authors concluded that there was no evidence that an enzyme other than D-galacturonanase was required for neutral sugar loss in pear. Knee (1973a) and Bartley (1976) reported for apple tissue that a water-soluble pectin released during softening was low in neutral sugar, yet arabinose and primarily galactose residues were lost from the wall. Two stages of wall breakdown were identified during the softening of apple, one resulting from galactan metabolism and the other from polyuronide solubilization. These events were observed occurring together (in air at 12°C) and under certain circumstances (e.g., low O_2) in sequence, with galactose loss preceding polyuronide solubilization (Bartley, 1977).
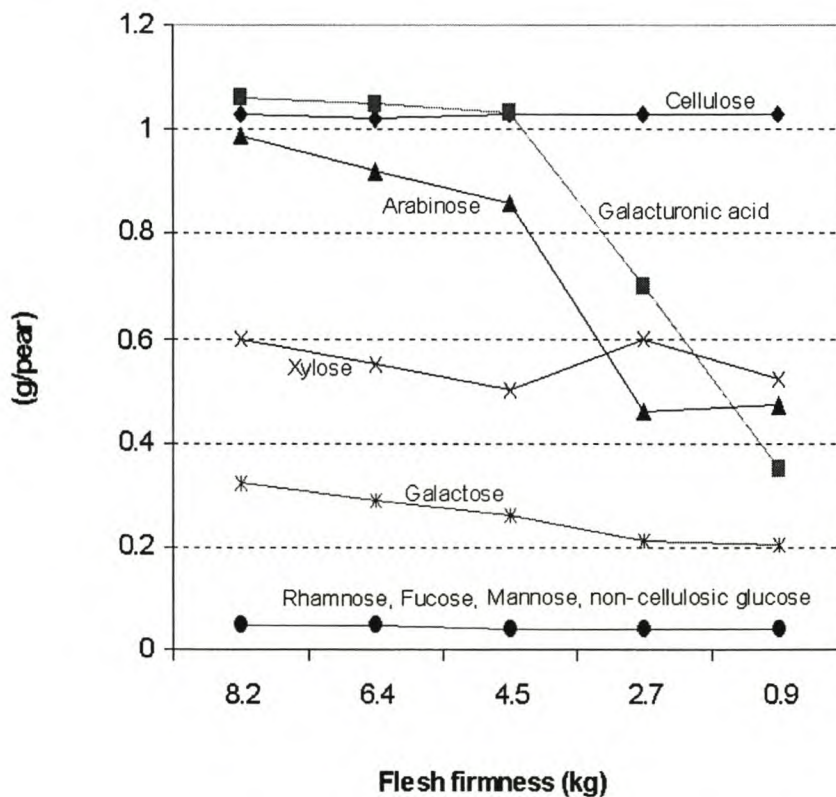

Figure 4: Changes in cell wall composition during the ripening of 'Bartlett' pears (Ahmed & Labavitch, 1980a).

Information regarding the metabolism of wall neutral sugars in ripening fruit has been obtained largely from studies of the composition of isolated wall and of the soluble polymers recovered from homogenates of fruit tissues. Unfortunately, neither method would estimate the occurrence of more subtle wall changes - those that might alter wall polymers without resulting in quantitative changes in levels of the component sugars (Huber, 1983).

1.4 ENZYMATIC MECHANISMS

Textural changes such as fruit softening are believed to occur as a result of enzymatic and/or nonenzymatic mechanisms that somehow influence the architecture of the cell wall. Enzymes reported and believed to be involved with the softening of fruit are the D-galacturonanases, also known as the polygalacturonases (PG), pectinmethylesterases (PME), cellulases and transgalacturonases (Huber, 1983).

1.4.1 D-galacturonanases/Polygalacturonases (PG)

Due to their ubiquitous distribution and temporal association with ripening, the D-galacturonanases are most often implicated in cell wall metabolism responsible for fruit softening (Huber, 1983). The preferred substrates for these enzymes are the D-galacturonans and thus they are referred to as D-galacturonanases, with the prefix *endo* and *exo* employed to designate a random or terminal hydrolytic pattern, respectively. These enzymes catalyze the hydrolytic cleavage of α-(1-4) galacturonan linkages.

According to Bartley *et al.* (1982), cell wall degradation and softening of cortical tissue is more extensive in pears than in apples and this may be explained by the differences in the polygalacturonase compliment of the fruits. PG activity (Ben-Arie *et al.*, 1979b) becomes pronounced after softening commences and continues to increase for as long as the fruit continue to soften.

The hydrolysis of intrapolymer bonds by the D-galacturonanases provides but one of a number of mechanisms by which the functional properties of pectins might be altered. The removal of galacturonan side chains, usually arabinans and galactans, and Ca^{2+}-metabolism, for example, may also directly influence fruit firmness (Ben-Arie *et al.*, 1979b).

1.4.1.1) Exo-polygalacturonase

Exo-polygalacturonase (exo-PG) hydrolyses galacturonans one monomer unit at a time, from the non-reducing end (Pressey & Avants, 1976). According to Pressey & Avants (1976) both endo- and exo-polygalacturonase can be found in pear. In the case of apple, only exo-PG is present (Bartley, 1978), which accounts for the loss of about 10% of total uronic acid residues during ripening. The enzyme attacks preferentially non-esterified polygalacturonate but it is capable of degrading apple cell walls to release monomers and some polymeric material.

Degradation of pectin by exo-PG, the only polygalacturonase in apples, is more gradual than the cleavage catalyzed by endo-polygalacturonase, and is expected to be limited by esterification and branching of the polysaccharide (Bartley *et al.*, 1982). The role of exo-PG is not obvious and has been questioned earlier by Pressey & Avants (1976). It is possible that exo-PG simply completes the hydrolysis of pectate initiated by endo-polygalacturonases.

1.4.1.2) Endo-polygalacturonase

Endo-polygalacturonase (endo-PG) cleaves the pectate chain randomly at any site where twelve or more free carboxyl groups are found (Pressey & Avants, 1976). It is clear that endo-PG will have a much greater effect on pectin degradation.

Endo-PG activity has been correlated with increases in soluble pectins and softening of a number of fruit (Brady, 1987; Huber, 1983), and thus it has often been suggested that polygalacturonase is primarily responsible for ripening associated pectin degradation. The evidence supporting this conclusion includes: (1) *in vitro* degradation of cell walls by polygalacturonase mimics the pectin degradation that occurs *in vivo,* (2) the correlation between polygalacturonase levels and fruit softening and (3) the absence of polygalacturonase in ripening impaired tomato mutants that fail to soften (Fisher & Bennett, 1991; Huber, 1983).

A role for an endo-PG in the softening of pear is reasonable, according to Pressey & Avants (1976), because this enzyme has a pronounced effect on the size of pectate molecules and hence their contribution to cell wall rigidity. If pectin are responsible for the integrity of cell walls, their degradation by an endo-PG, following deesterification by pectinesterase, would lead to tissue softening. According to Bartley *et al.* (1982) pear endo-PG is not active during storage at −1°C but only appears after two days at 18°C (Figure 4). This is concurrent with the initiation of softening. In 'Bartlett' pears (Ahmed & Labavitch, 1980b) polygalacturonase activity increased and decreased in parallel with the respiratory climacteric. Bartley *et al.* (1982) concludes that the activity of endo-PG in ripening pears accounts for the loss of viscosity of endogenous pectin.

However, work done on ripening *rin* (ripening inhibitor) tomato fruit with the incorporation of a PG gene failed to cause normal softening (Giovannoni *et al.,* 1989) despite a sixty-fold increase

in extractable PG, and extensive polyuronide solubilization and degradation (Giovannoni *et al.*, 1990). In accordance with these findings, PG gene expression was suppressed in wild-type fruit with the use of antisense PG RNA (Smith *et al.*, 1988). They found a 90% reduction in PG mRNA, PG protein and PG activity in heterozygous fruits, and a 99% reduction in homozygous fruits (Schuch *et al.*, 1991; Fisher & Bennett, 1991). The antisense gene greatly suppressed the depolymerisation of polyuronides but seemingly its solubilization was not affected (Smith *et al.*, 1990). Suprizingly there did not appear to be a marked effect on fruit softening (Smith *et al.*, 1990; Schuch *et al.*, 1991), although storage life and the ability to withstand transport without damage improved. Tomato juice made made from PG antisense fruit had significantly higher consistency (Schuch *et al.*, 1991). On the other hand, studies in semi-commercial trials have shown significant reductions in softening in transgenic fruits (Kramer *et al.*, 1992).

1.4.2 Pectinmethylesterase (PME)

Pectinmethylesterase (PME) is an enzyme which cleaves methyl groups from the backbone of pectin. Pressey & Avants (1976) noted that pectin molecules have to be deesterified by a pectinesterase (PME) before the glycosidic links of the pectic chain can be cleaved by an endo-PG. Wiley (1977) pointed out that the mode of action of PME resembles that of a zipper. Zipper action may account for the major softening during the first 60-90 days of cold storage. This is done by separating the pectin and hemicellulose complex from the cellulose matrix or by causing internal separation of the "dynamic" constituents such as the galacturonans, pentosans and hexosans.

Cold storage work done by Lee (1969) on 'Golden Delicious' and 'York Imperial' apple varieties showed that PME activity increased in storage up to 60 days and then levelled off at a high plateau. Results indicated that as PME activity increased the fruit became softer. He also showed that PME activity was approximately twice as high in the soft variety 'Golden Delicious' than in the firmer 'York Imperial'.

Bartley *et al.* (1982) also found an increase in PME activity during ripening and an increase in protein content (Figure 5). The decline in the degree of esterification of the total pectin during ripening (Knee, 1982a) shows that PME is active in pears; this enzyme is present in apples but the constancy of its level of esterification (Knee, 1978a) suggests that it is inoperative.
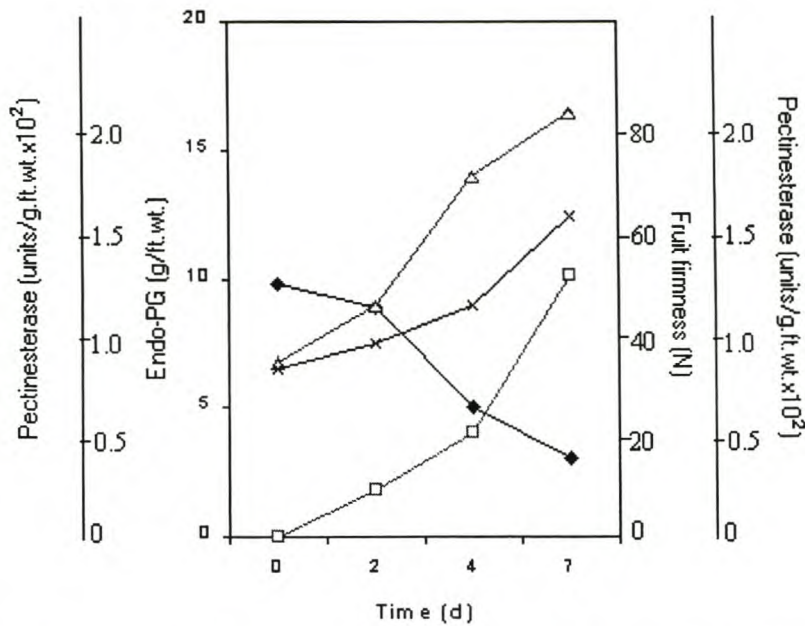
Figure 5: Softening, endo-PG and pectinesterase activities and protein content of 'Conference' pears ripening at 18°C after storage at 1°C for 40 weeks. ◆—◆ Fruit firmness; —□— endo-PG; —△— pectinesterase; —×— protein (Bartley *et al.*, 1982).

Findings made by Ben-Arie *et al.* (1979b) showed that PME activity in ripening pears declined throughout the harvest and storage season and they postulated that PME action is a prerequisite for PG activity. Although PME activity is important to fruit softening, its activity remains constant throughout the majority of the softening period until it drops off once the respiratory climacteric maximum has been attained (Ahmed & Labavitch, 1980b). This suggests that whilst PME plays an important role in softening of fruit, its availability is not a controlling factor.

1.4.3 Cellulases

The activity of cellulases, like endo-PG, is often low or undetectable in unripe fruit, but increases dramatically during ripening and softening (Yamaki & Kakiuchi, 1979; Ben-Arie *et al.*, 1979b). Cellulase generally reaches a peak and thereafter declines as the fruit continues softening (Ben-Arie *et al.*, 1979b). Reports of a loss of wall cellulose by Yamaki *et al.* (1979) have been made. However, reports of unchanged cellulosic levels in ripening "Bartlett" pears (Ahmed & Labavitch, 1980a) and apples (Bartley, 1976; Knee, 1973a), indicated no cellulase activity.

A number of arguments for the role of cellulases in fruit softening have been based on ultrastructural studies of the cell wall of ripening fruits. In studies on ripening avocado fruit, Pesis *et al.* (1978) observed dissolution of the middle lamella, presumably due to the action of D-

galacturonanases. Additionally, the cell wall fibrillar network (presumably representing cellulose) became less discernible and more diagonally oriented fibers were observed. Similar observations were reported for ripening pear and apple (Ben-Arie, *et al.* 1979a). In both fruit types, disintegration of the middle lamella was an early event. Modification of the closely packed fibrillar material, evident in pear fruit, was not apparent in ripening apple cortical tissue. Incubation of tissue discs from mature apple and pear fruit in solutions containing D-galacturonanase (apple) or a combination of D-galacturonanase and cellulase (pear) resulted in ultrastructural changes similar to those that occurred in naturally ripening fruit.

Apple has been reported to have no cellulase activity (Bartley, 1976) so presumably this enzyme was not required. In contradiction to this, Abeles & Takeda (1990) found that cellulase levels declined in apples during the respiratory climacteric and partial loss of fruit firmness. They concluded that the loss of flesh firmness could be caused by the continued action of cellulase or, alternatively, the action of polygalacturonase.

The *in vitro* studies performed with pear fruit discs should be interpreted with some caution because the cellulase employed was of fungal origin and may not possess the characteristics of the fruit cellulases. Both endo- and exocellulases (Cx type) have been reported in Japanese pear (Yamaki & Kakiuchi, 1979), although Ahmed & Labavitch (1980b) were unable to detect the activity of either in 'Bartlett' pear.

1.4.4 Other cell wall hydrolases

The complexity of the cell wall structural polysaccharides indicates that there is a large number of enzymes that are ultimately involved with ripening and softening of fruit (Fisher & Bennett, 1991). Enzymes reported to be present in ripening apple and pear include glycosidases (Ahmed & Labavitch, 1980b), xylanases (Yamaki & Kakiuchi, 1979), α- and β- galactosidases (Ahmed & Labavitch, 1980b) and endo-β-(1-3) glucanases (Hinton & Pressey, 1980).

The activity of these enzymes generally remains constant during ripening, with slight increases (20%) observed for β-galactosidase (Huber, 1983). Seven glycosidases were found in cell walls from 'Bartlett' pears, with α-galactosidase and α-mannosidase increasing twofold and fivefold, respectively, during softening (Ahmed & Labavitch, 1980b). Yamaki & Kakiuchi (1979) also

reported a variety of glycosidases and glycanases in the Japanese pear and their activity during development and ripening. Activities were generally high during cell division, decreasing thereafter and again increasing with ripening. D-galacturonase (PG) along with mannase were thought to initiate softening, whereas xylanase, β-galactosidase and β-xylosidase were thought to be associated with over-ripening. In spite of the presence of xylanase and β-xylosidase activities, cell wall xylose quantities remained relatively constant during softening of pear (Yamaki *et al.*, 1979; Ahmed & Labavitch, 1980a). Bartley (1974) reported that a β–galactosidase from apple fruit also degraded polymeric galactan.

Perhaps the most convincing evidence for a role of glycosidases in fruit softening has emerged from detailed studies with apples. A two-stage mechanism has been proposed for the breakdown of apple cell wall (Knee, 1975; Bartley, 1976). The initial phase involved the loss of wall galactosyl residues with little change in firmness, whereas the second involved polyuronide solubilization with a major decrease in firmness. A β-galactosidase was implicated in the removal of wall galactose, and such an enzyme with the capacity to degrade a pectin galactan has been reported for apple fruit (Bartley, 1974; Bartley, 1977).

Wall-associated β-galactosidase has been assayed *in vitro* in discs of apple cortical tissue (Bartley, 1977) and in acetone-extracted cell wall, which when incubated in aqueous media released neutral sugars, among them galactose and arabinose, in a reaction apparently mediated by wall-bound enzymes (Knee, 1973a). The β-galactosidase in apple tissue was presumed to function in debranching pectin galactan (Knee, 1978a), possibly facilitating the hydrolysis of pectins by exo-D-galacturonanase (Bartley, 1978).

The importance of β-galactosidase in fruit softening has been lessened by many investigators (Yamaki & Kakiuchi, 1979) and furthermore there exists little evidence that β-galactosidase and their presumed substrates, the noncellulosic glucans, are involved in fruit softening. A loss of wall glucan during apple ripening (Knee, 1973a) was later attributed to the metabolism of starch (Bartley, 1976). Wallner (1978) demonstrated for a number of apple cultivars that enzyme preparations (derived from apple tissue) containing β-galactosidase activity were unable to degrade apple fruit cell wall. He also found that β-galactosidase activity correlated poorly with the rate of softening. In observing that β-galactosidase activity was high prior to galactose loss, Bartley (1977) suggested that declining rates of synthesis might be responsible, in part, for the

reduced level of wall galactose in ripening apple (Figure 6). Knee & Bartley (1981) suggested that the reduction might reflect a declining rate of synthesis of pectin galactan.
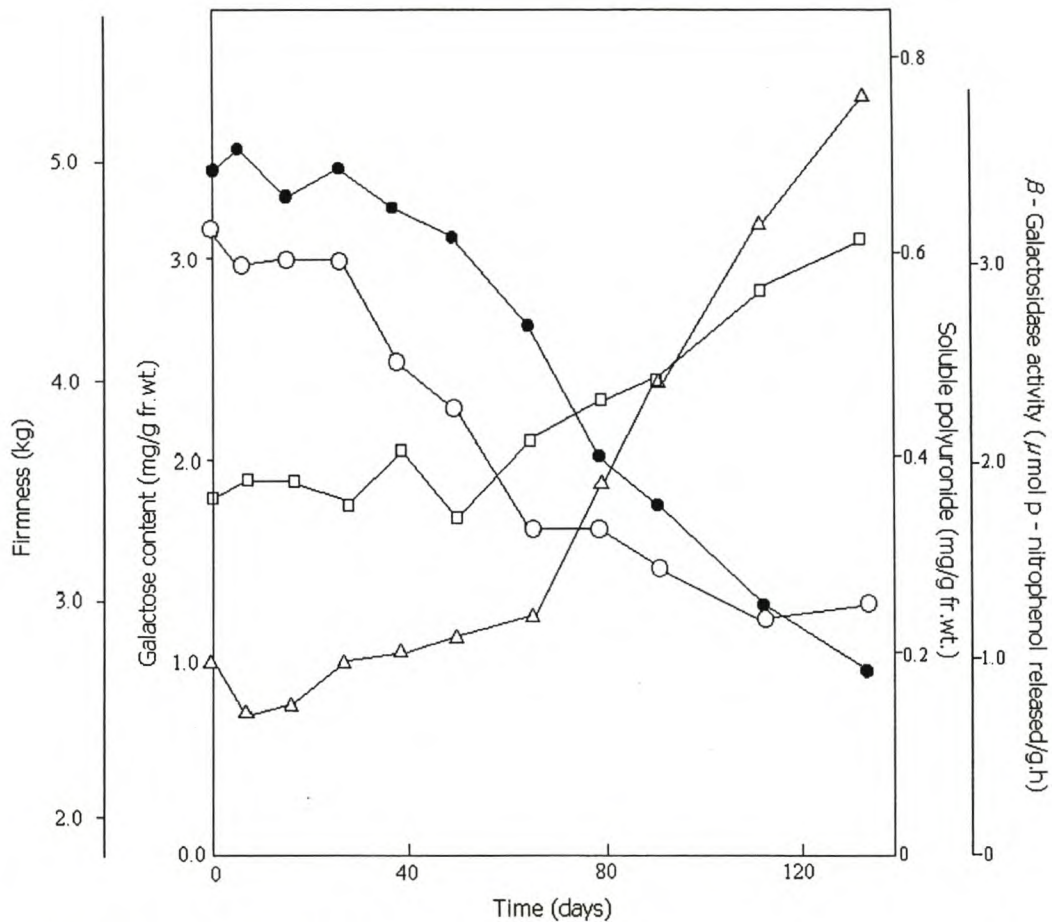

Figure 6: Changes in fruit firmness, galactose content of cortex cell walls and β-galactosidase activity in 'Cox Orange Pippen' apples stored in 2% O_2 at 3°C. —●— Fruit firmness; —▲— Soluble polyuronide —□— Galactose content; —○— β-galactosidase activity (Bartley, 1977).

Rhamnogalacturonase A (RGase A) activity was recently detected in apples (Gross *et al.*, 1995). RGase A is a pectic hydrolase that was first purified and characterised from the fungus *Aspergillus aculeatus* (Seymour & Gross, 1996). It is known that two RGase activities exist: RGase A (RG-hydrolase) cleaves α-1-2 linkages between galacturonosyl and rhamnosyl residues on the non-reducing side of the rhamnosyl residue and RGase B (RG-lyase) cleaves α-(1-4) linkages on the reducing side of the rhamnosyl residue (Mutter *et al.*, 1996). Since RGases are capable of degrading the pectin backbone they could potentially play an important role in fruit softening and/or fruit fungal decay (Seymour & Gross, 1996). The lack of convincing evidence that many of the wall-associated enzymes are involved in fruit softening is perhaps an indication that these enzymes may perform other functions.

1.4.5 Transgalacturonases

Xyloglucan, the principal hemicellulose in dicotyledonous cell walls, is composed of a backbone of β-(1-4)-D-galactopyranose residues (Fry *et al.,* 1992). Xyloglucan can form hydrogen bonds with cellulose microfibrils (Hayashi *et al.* 1987) and may cross-link them (Fry, 1989b), restraining cell expansion.

The enzymatic cleavage of xyloglucan is thought to loosen the wall, enabling cell growth (Fry *et al.,* 1992). The enzyme responsible was assumed to be cellulase (Fry, 1989b). However, hydrolysis is irreversible whereas bond breakage during growth is reversible (Taiz, 1984), which argues against the involvement of cellulase in xyloglucan degradation.

A possible explanation for this discrepancy arises from the finding that xyloglucan is subjected to *in vivo* endotransglycosylation, i.e. a xyloglucan chain can be cleaved and then transferred to a different acceptor (Fry, 1995). Substrate specificity exists in so far as to fucose which is not required in the donor substrate, and the acceptor which requires only glucose and xylose. Galactose increase the affinity of the enzyme (decreases the K_m) and the requirement of α-xylose is absolute.

Endotransglycosylation loosens the wall, enabling growth, but is followed by a repair step (Fry *et al.,* 1992). The newly cited enzyme responsible for this endotransglycosylation is called xyloglucan endotransglycosylase (XET).

XET may be universal in land plants (Fry, 1995). The highest activity of XET is found in growing stems, but it is also found in roots, leaves, fruits and in cell cultures. XET is a glycoprotein with a M_r of 33,000 and is a true transgalacturonase (lacks hydrolase activity). XET cleaves the xyloglucan chain, weakening the cell wall and allowing incremental expansion (Fry, 1989b), then rejoins the cut portion to the nonreducing terminus of a different xyloglucan chain restoring, but altering, the wall fabric (Smith & Fry, 1991).

Fry *et al.* (1992) proposed that XET contributes to growth promotion in two ways. Firstly, in the absence of oligosaccharides XET causes a polysaccharide-to-polysaccharide transglycosylation,

reversibly loosening the cell wall and permitting molecular creep. Secondly, in the presence of suitable oligosaccharides it causes polysaccharide-to-oligosaccharide transglycosylation, effectively cleaving the polymer resulting in a more permanent wall-loosening. XET could play a role in the softening and ripening of fruit due to the alteration of the cell wall structure through transglycosylation. Enzymes that catalyse only exo-transglycosylation have not been found in plant cell walls. This enzyme catalyses the transfer of single glucose residues from the nonreducing terminus of the donor to the [^{3}H]glucose (Fry, 1995).

Although it has been reported that very little XET activity is bound to the cell wall (Fry *et al.*, 1992), indications of cell wall bound activity of XET in kiwifruit, tomato and apple fruit have been found (Percy *et al.,* 1996). The activity of XET in apples (Figure 7) peaked twelve days after anthesis and declined towards the end of fruit expansion at about 20 weeks after anthesis. Most of the activity was in the soluble fraction during fruit expansion but between 8 and 16 weeks the XET became proportionally more associated with the cell wall. A second peak was found between 16 and 26 weeks, thereafter XET activity decreased as fruit became senescent (Percy *et al.,* 1996).

The largest increase in both soluble and insoluble XET activity detected throughout the growth of apple fruit was found during the stage of declining growth (Percy *et al.,* 1996). This is in contrast to kiwifruit where the XET activity remained unchanged. Apple furthermore contrasts with kiwifruit and tomato fruit during the period of ripening and softening. A decrease in XET activity and a little increase in pectin solubilization during ripening and softening was found in 'Braeburn' apples, whereas kiwifruit and tomato both showed an increase in XET activity and pectin solubilization. Percy *et al.* (1996) concluded that the correlation between XET activity and pectin solubilization is a loose one and that determination of XET activities in a wider range of fruit, some which behave as apples and some like kiwifruit and tomatoes, needs to be done to corroborate this relationship.
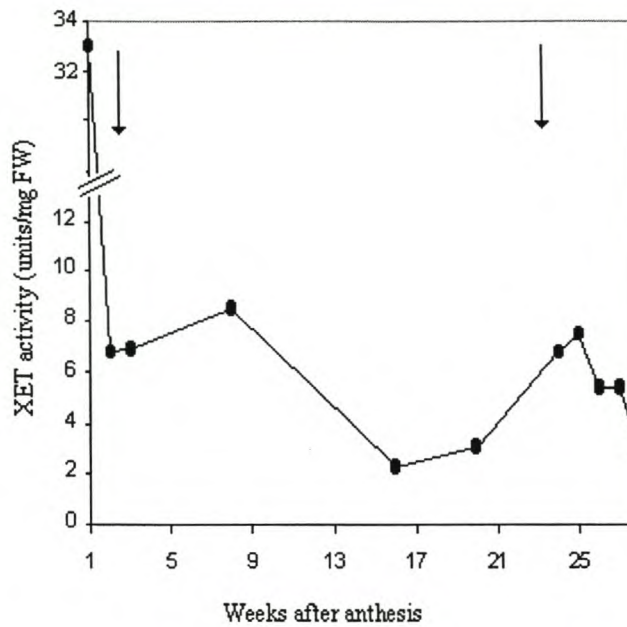
Figure 7: Total XET during development and ripening of apple fruit. Arrows indicate the end of rapid cell division phase and time of harvest (Percy *et al.,* 1996) .

The fact that XET shows such marked differences between apple and kiwifruit indicates that the function of the enzyme is too broadly based (Percy *et al.,* 1996). A few factors should be taken into account: (1) Not all xyloglucan is hydrogen bonded to cellulose in plant cells. (2) If XET is a multifunctional enzyme, then its assay in crude plant extracts in an attempt to correlate activity with changing growth rates will give ambiguous results. (3) An additional danger of crude extracts could be that a non-specific 1,4-β-glucanase could modify the xyloglucan substrate and XET activity.

1.5 NON-ENZYMATIC FACTORS

The lack of endo-PG in apple and the short term inhibitory effects of anoxia on pear tissue softening (Knee, 1982b) suggests that a process other than enzymatic pectin degradation is also involved in fruit softening. It is believed that softening is in some way dependent upon the operation of the respiratory enzyme, cytochrome oxidase. The simplest explanation is that an energy requiring process (other than enzyme synthesis) is involved. The hypothesis that this process is the demethylation of carboxyl groups on pectin has been rejected (Knee, 1982a) but it could involve an active ion pump regulating the ionic status of the cell wall (Knee, 1982b). The main evidence for this came from the reversal of pear softening with pH buffers and calcium ions (Knee, 1982b).

Furthermore, depending on the conditions chosen for the ripening of apples the loss of galactose residues from the cortical cell walls can occur concurrently with an increase in β-galactosidase activity (in air at 12°C) (Bartley, 1974) or precede the increase in enzyme activity, as occurs in 2% O_2 (Figure 5). These results suggest that an increase in enzyme activity is not required to initiate the loss of galactose residues from the cell wall. Thus the coincidence of change in enzyme activity and cell wall composition does not necessarily imply a causal relationship between the two. Knee (1982b) demonstrated that fruit softening and pectic degradation was halted by an atmosphere of nitrogen. Softening was also halted in an atmosphere of low oxygen (2% O_2 and 98% N_2) although pectin degradation still continued. Brennan & Frenkel (1977) postulated that the effect of oxygen, in the formation of hydrogen peroxide may be involved in oxidative processes required in the initiating and promotion of ripening.

1.5.1 Calcium

At least 60% of the total calcium in the plant is associated with the cell wall fraction (Fallahi *et al.*, 1997). Calcium affects fruit softening because it is an essential part of the cell wall structure, and it also influences cell membrane integrity. As noted earlier, calcium serves as an intermolecular binding agent that stabilises pectin-protein complexes of the middle lamella. Calcium also plays an important role in the cell membrane by inducing rigidification at the membrane surface of apple fruit tissue. Fruit senescence and quality are affected by calcium by altering intracellular and extracellular processes (Fallahi *et al.*, 1997). According to Faust and Shear (1972), calcium preserves the cellular organization not only by preserving the cell membranes, but also by maintaining the nucleic acid and protein synthesis.

Calcium also plays an important role in ethylene synthesis, because an intact membrane is important for the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. This reaction resides in the plasma membrane (Lieberman & Wang, 1982). Interesting to note is that Gerasopoulos & Richardson (1996) found a slower increase in internal ethylene in fruit with a high calcium content. The deduction to be made is that calcium plays not only an important role in membrane integrity, and thus the maintenance of ethylene biosynthesis, but also plays an antagonistic role in relation to ethylene biosynthesis.

As calcium ions have been shown to bind to pectin molecules it has been suggested that these ions form bridges between pectin molecules in the middle lamella and are responsible for cell cohesion (Knee & Bartley, 1981). Thus softening could be a result of a loss of calcium from the middle lamella and/or a loss of calcium binding sites in the pectin molecules (Knee, 1982b).

The rate of fruit softening appears to be correlated with fruit calcium status (Lidster & Porritt, 1978; Mason et al., 1975; Poovaiah, 1988). Poovaiah (1988) found a linear relationship between fruit flesh calcium status of 'Golden Delicious' apples after four months of cold storage and fruit firmness (Figure 8).
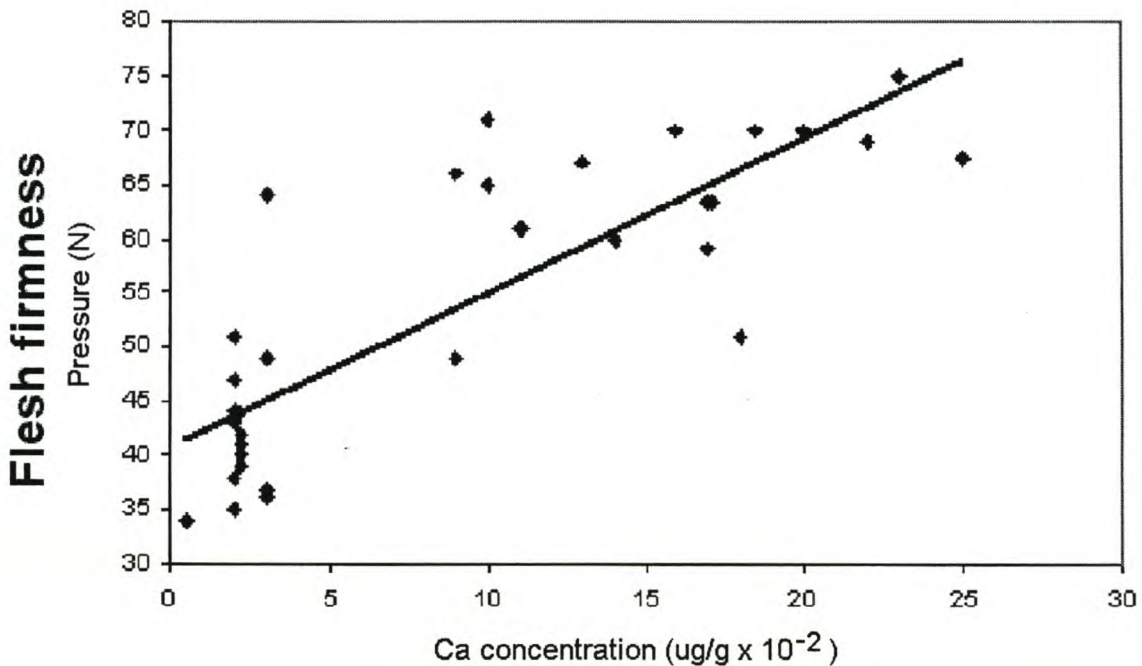

Figure 8: Correlation of calcium concentration of apple cylinders with flesh firmness in 'Golden Delicious' apples after 4 months of cold storage (Poovaiah, 1988).

Historically, non-enzymatic mechanisms of fruit softening have been explained on the basis of the central role of Ca^{2+} in pectin chemistry (Huber, 1983). In essence the model visualises that the removal of Ca^{2+} destabilises the pectic matrix (the eggbox model, Grant et al., 1973) resulting in loss of cell cohesion. Knee (1978a) also emphasised the importance of Ca^{2+} in regulating apple fruit cell cohesion and suggested that the increase in soluble pectins might be explained, in part, by the synthesis of more highly methylated polygalacturonate (Knee, 1978b). This displacement of Ca^{2+} by protons is thought of as an important facet of apple fruit softening.

Saks *et al.* (1990) found a decrease in calcium solubility preceding senescent breakdown, indicating that a loss of calcium mobility within the fruit could be the cause of a disorder or rapid cell wall degradation. Application of Ca^{2+} to ripening fruit suppressed both ripening and the associated respiratory climacteric (Faust & Shear, 1972; Gerasopoulos & Richardson, 1996).
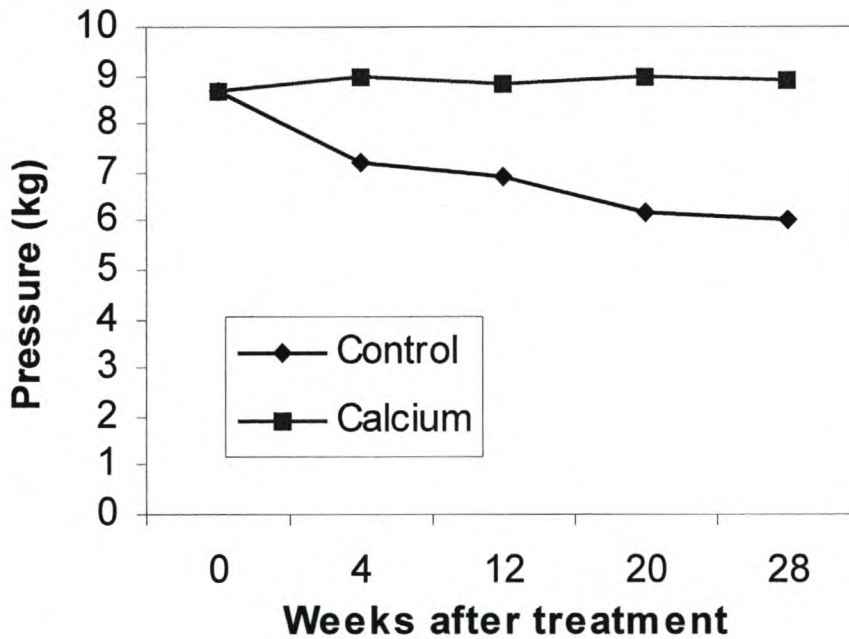

Figure 9: The effect of calcium infiltration (4%) soon after harvest on softening in 'Golden Delicious' apples (Poovaiah, 1988).

According to Knee (1982b) the softening of pear tissue can be reversed by the infiltration with pH 8.0 buffer or calcium chloride ($CaCl_2$) solution up to three days at 18°C. The most effective treatment was a mixture of buffer at pH 8.0 and $CaCl_2$. A similar reversal of softening could be achieved after storage for 28 weeks. Stow (1993) agreed with these findings regarding the reversal of softening with infiltration of calcium, and concluded that the loss of calcium from the middle lamella is a factor, but unlikely to be the sole factor in softening of apples. Poovaiah (1988) illustrated the retarding effect of infiltrated calcium on fruit softening in 'Golden Delicious' apples (Figure 9).

1.5.2 Calmodulin

Calmodulin, a ubiquitous calcium-binding protein, is known to be involved in many of the calcium dependent changes in plants. Calmodulin mediates calcium signals (Cheung, 1980; Klee *et al.*, 1980). About 90% of calmodulin in plants is found in the cytoplasm. The remaining

portion is localized in the mitochondria (5 to 6%), chloroplast (1 to 2%), and in the remaining microsomal fraction. Calmodulin is a small acidic protein and is very resistant to denaturation from heat or acid treatments. The tertiary structure of calmodulin contains four similar domains, which are able to bind to calcium (Marmé, 1986). Calcium binding induces a large conformational change in calmodulin, which enables it to function as an activator of a number of enzymes, eventually leading to slow and rapid physiological responses. Such responses may occur when the calcium-calmodulin complex acts directly on an effector system or indirectly on a regulatory system. This is usually found where a protein kinase activates or inactivates other enzymes via phosphorylation. Enzymes that are known to be regulated by calcium and calmodulin in plants (Poovaiah et al., 1988) include NAD kinase (Anderson et al., 1980), calcium ATPase, H^+ ATPase, quinate: NAD^+ oxidoreductase and protein kinases (Poovaiah, 1985).

Many reversible covalent modifications are known to take place in proteins following translation. One important regulatory modification of proteins that controls various cellular metabolic activities is phosphorylation (Trewavas, 1976). Phosphorylation and dephosphorylation reactions induce small changes in the conformation of many important enzymes thereby altering their activity. The altered activity of various key enzymes ultimately results in a physiological response. Calcium regulates a number of biochemical processes through activation of calcium- and calmodulin-dependent protein kinases. The marked effect of calcium on fruit softening and senescence may involve calcium-calmodulin-dependant processes (Poovaiah, 1988) or calcium-induced changes in the microenvironment of membrane proteins, both of which could influence their conformation and function (Paliyath and Poovaiah, 1985a).

The changes in the pattern of membrane protein and their phosphorylation have been studied in control (stored at 2°C) and senesced apples (stored at 20°C) by Paliyath and Poovaiah (1985a). A 30% decrease in membrane protein content was observed in senesced fruit as compared to control fruit. A further marked reduction in calcium- and calmodulin-promoted protein phosphorylation was observed in membrane preparations from the senesced apples. No detectable increase was found in protein phosphorylation in senesced apple membrane proteins when calcium and calmodulin were included in the assay mixture. This suggests that apple fruit may lose the capability to respond to calcium during advanced stages of senescence.

Paliyath and Poovaiah (1985b) detected a heat-stable, dialyzable, low-molecular weight component that inhibited calmodulin activity in senesced apple extracts and they succeeded in isolating calmodulin inhibitors from apple tissue. These inhibitors were identified as flavonoids, one of which was catechin. Of the various phenolic compounds tested only caffeic acid proved to be a potent inhibitor of calmodulin-promoted phosphodiesterase (PDE) activity. Caffeic acid and catechin are effective in inhibiting calcium-calmodulin-dependent phosphorylation in apple preparations (Paliyath and Poovaiah, 1985b). The occurrence of calmodulin inhibitors in apple (Paliyath and Poovaiah, 1985a) suggests a possible role in fruit ripening and softening.

1.5.3 Ethylene

Huber (1983) is of the opinion that the importance of the cell wall hydrolases in the early stages of ripening is based almost entirely on circumstantial evidence. Other studies have shown that wall hydrolytic activity is a consequence of, rather than a prelude to, ethylene synthesis.

Rhizobitoxine (Knee & Bartley, 1981) suppressed the production of ethylene and the increase in soluble pectins in ripening apple, yet its effect on exo-D-galacturonanase activity (Bartley, 1978) was not determined. Similarly, fruit stored (Bangerth, 1984) under hypobaric conditions (66 mbar) never produced autocatalytic ethylene or developed a respiratory climacteric. Only a slight decrease in fruit firmness was observed. When exogenous ethylene was continuously supplied a considerable decrease in firmness was noted.

Pectic components released from the cell wall during ripening may participate in the control of ripening associated ethylene production (Tong et al., 1986). According to Brady (1987) enough evidence exists to believe that ethylene is involved in ripening of fruits. This is why it is of great concern to the commercial post-harvest horticultural industry to limit the exposure of harvested fruit to ethylene. This is because ethylene reduces the time before ripening occurs. Results obtained by Gerasopoulos & Richardson (1996) suggest that the enzymes responsible for cell wall breakdown are quite sensitive to very small amounts of ethylene (in the range of 0.05 to 0.1µl/l) and can also respond directly to propylene as an ethylene homologue at low concentrations. Further evidence suggests that the ability of fruit tissue to respond to ethylene with elevated EFE (ethylene-forming enzyme) activity increased exponentially during maturation on the tree (Bufler, 1986).

Two systems (Figure 10) of ethylene production during fruit maturation and ripening have been distinguished (Yang, 1985; McGlasson, 1985): System 1 is the low level of ethylene present in fruit before the onset of ripening, while System 2 is the autocatalytic burst of ethylene production accompanying the ripening process. In preclimacteric fruit the resistance to ripening or resistance to ethylene action (ripening inhibitor) is so high that the ripening process is not initiated.
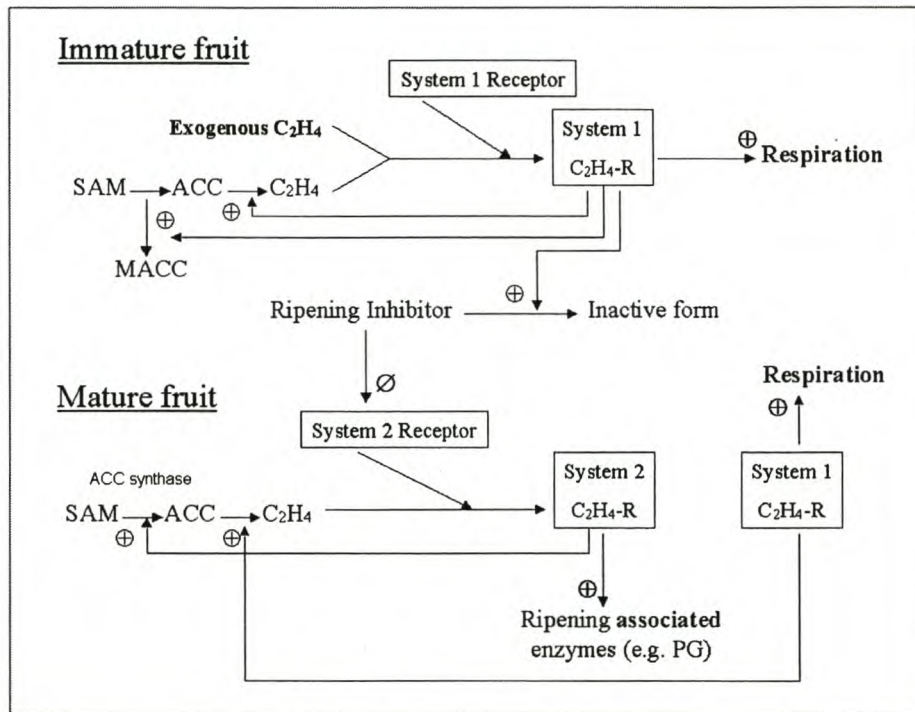

Figure 10: A model showing the sequence of ethylene action on the regulation of ethylene biosynthesis in fruit maturation and ripening.$\oplus$ and $\varnothing$ indicate the metabolic processes that are positively or negatively regulated by the specific ethylene-receptor complex or the ripening inhibitor (adapted from Yang, 1985; McGlasson, 1985).

As fruit undergo maturation there is a progressive decrease in resistance (shift to inactive form) to ethylene action (or an increase in sensitivity) and this process is thought to be controlled by endogenous ethylene. The decrease in resistance to ethylene action is accelerated by the external application of ethylene and is retarded by low O_2/high CO_2 atmosphere and by removal of ethylene. When the resistance to ethylene action decreases to a point at which the fruit becomes responsive to their endogenous ethylene levels, the ripening process is initiated, resulting in the autocatalytic burst of ethylene production (System 2). Hence the important factor that triggers the onset of ripening is the decrease in the resistance (or increase in ethylene sensitivity) to ethylene action.

The molecular mechanisms by which plants convey signals in response to environmental and hormonal stimuli, like exogenous ethylene, which lead to cellular responses are largely unknown. Extensive work done with animal cells (Berridge & Irvine, 1989) has shown the presence of a signal transduction mechanism involving hydrolysis of inositolphospholipids to produce two secondary messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Figure 11). There is growing evidence that this IP3 cycle may function in plants (Martinoia *et al.*, 1993, Morse *et al.*, 1989). Verhey & Lomax (1993) suggested that plant growth hormones might utilize the phosphatidylinositol cycle for the transduction of signals to plant cells.

In plant and animal cells, cytosolic free calcium plays a central role in signal transduction (Berridge & Irvine, 1989). Intracellular Ca^{2+} storage pools are responsible for the modulation of cytosolic free Ca^{2+} levels during signaling. In animal cells the primary site of Ca^{2+}-release is at the ER, whereas in plants, Ca transport activity is detected at the plasma membrane, the ER and the tonoplast (Figure 11). Several studies have shown that IP3 causes a specific and saturable transient release of calcium from intact vacuoles or tonoplast vesicles (Schumaker & Sze, 1987). Increased cytosolic free Ca^{2+}, either alone or with the Ca^{2+}-binding protein such as calmodulin, affects numerous cellular reactions.
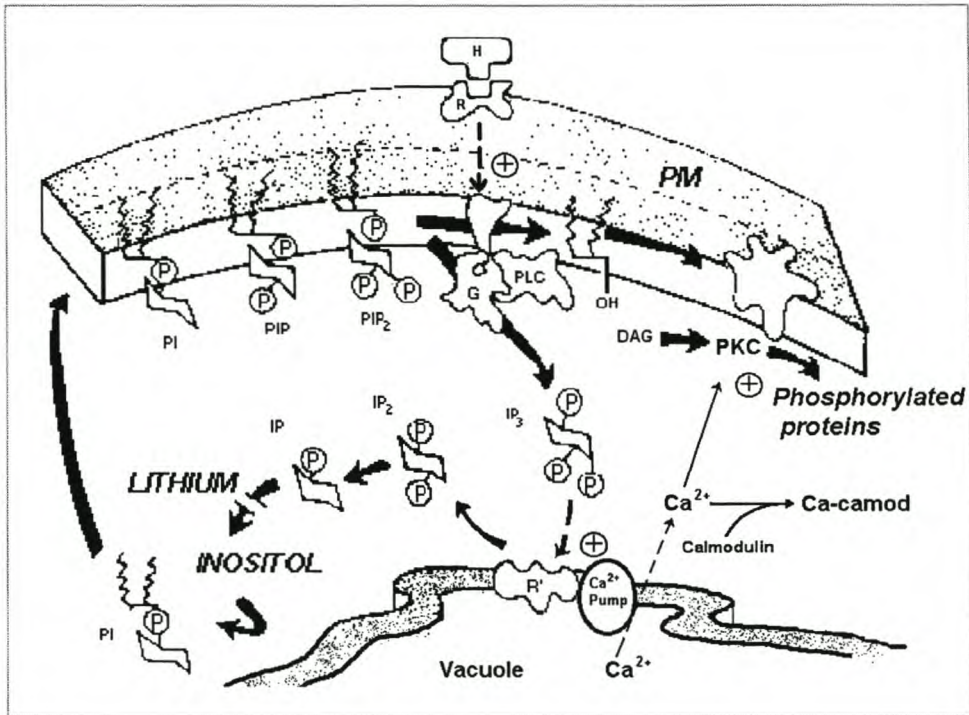
Figure 11: Schematic representation of phosphatidylinositol turnover during signal transduction in plant tissues. Binding of hormone (H) to its receptor causes the activation (+) of nearby phosphoinositidase (PLC). Activated PLC hydrolyses nearby phosphatidylinositol 4,5-bisphosphate (PIP2) to yield diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). IP3 binds to a receptor on the tonoplast (R') and stimulates Ca2+ efflux. DAG remains in the plasma membrane, where it activates protein kinase C (PKC). PKC is further activated by Ca2+ released from the vacuole so various enzymes become phosphorylated by PKC. Ca2+ also activates other protein kinases and other enzymes, when free or bound with calmodulin. IP3 loses phosphates by hydrolyses to form IP2 and IP and finally to inositol (modified from Morse *et al.*, 1989).

Wang *et al.* (1972) found that a rapid linear decrease in fruit firmness followed with the application of exogenous ethylene. In fully mature pears softening preceded the climacteric rise in both fruit treated with ethylene and the untreated fruit. These data led Wang *et al.* (1972) to believe that softening occurs prior to and is not dependent on the development of the climacteric rise in respiration. They also noted that there exists a threshold level of ethylene in immature fruit where softening is initiated and concluded that the onset of autocatalytic ethylene production rather than the respiratory climacteric is the most critical in the ripening sequence. 'Anjou' pears treated with exogenous ethylene by Wang & Hansen (1970) attained a decrease in flesh firmness and an increase in protein, nitrogen and soluble pectin even though the fruit remained in the preclimacteric condition. The positive effect of exogenously applied ethylene on decreasing fruit firmness found by Wang & Hansen (1970) is shown in Figure 12.
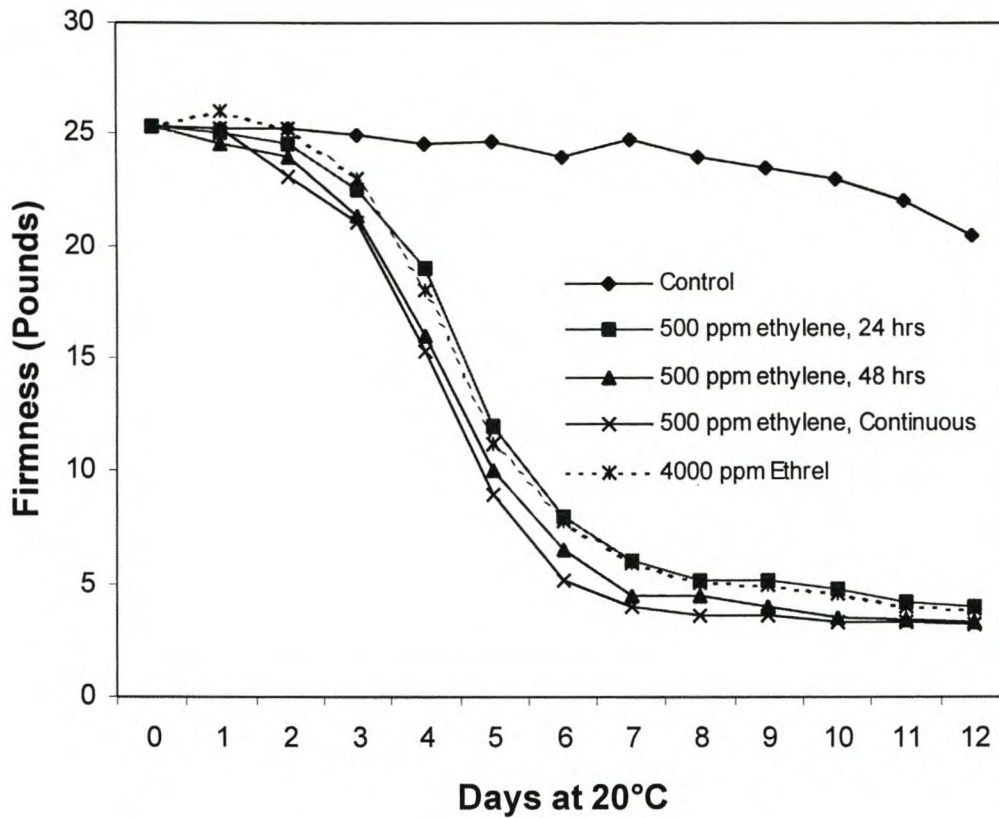
Figure 12: Effect of ethrel and different lengths of ethylene treatment on firmness of immature 'Anjou' pears (Wang & Hansen, 1970).

1.5.4 Polyamines

Among the free polyamines there are three which have significance to this review. They are putrescine, spermidine and spermine. Putrescine may be directly synthesised from ornithine by ornithine decarboxylase (ODC) or indirectly through a series of intermediates from arginine by arginine decorboxylase (ADC). Spermidine and spermine are synthesized from putrescine by addition of aminopropyl groups (Smith, 1985).

Polyamines are involved in the promotion of growth, stabilizing membranes, minimizing stress and shown to delay senescence of detached leaves. Polyamines usually increase where rapid cell division and protein synthesis occur. In apples, high levels of polyamine are found at fruit set and it was found that fruit set of apples (Costa *et al.*, 1986) and pears (Crisosto *et al.*, 1986) could be increased with exogenously applied putresine. Polyamines, typically putrescine, accumulate in response to certain stresses (nutrient deficiency, cold, drought or salinity) (Smith, 1985).

Controlled atmosphere storage (CA) involving low oxygen and high carbon dioxide is widely used to prolong the storage life of apples. Low oxygen (1% O_2 at 1°C) storage induced higher levels of all three polyamines and significantly inhibited the softening of apples compared to apples stored in air. Polyamines also inhibited polygalacturonase activity *in vitro* indicating that they may act *in vivo* in protecting cell walls from degradation (Kramer *et al.*, 1989).

Polyamine biosynthesis in connection with fruit ripening and senescence is of great interest because ethylene and polyamines are antagonistic and share a common intermediate, S-adenosylmethionine (AdoMet) (Faust and Wang, 1992). Kramer *et al.* (1991) and Kramer and Wang (1990) found that the application of polyamines to mature apples retarded softening and reduced chilling injury wihout any effect on ethylene biosynthesis. They concluded that polyamines may affect fruit softening through the protection of cell walls. Infiltration studies done with polyamines and calcium on apples (Wang *et al.*, 1993) indicated that calcium and polyamines may be competing for the same binding sites in the cell wall. The improvement of fruit quality during storage by these cations could involve strengthening of the cell wall.

Exogenously applied polyamines can reduce ethylene production in apple slices (Ben-Arie *et al.*, 1982). The resulting decrease in ethylene production prevented senescence. Polyamine levels in pears are relatively high when fruit are young and the concentration slowly decreases as the fruits enlarge and mature (Toumandje and Richardson, 1988).

1.5.5 Auxin

The primary effect of auxin involves wall loosening, and presumably enhanced synthesis of pectin destined for incorporation into the cell wall as a secondary effect (Knee, 1982a). Auxin treatment of cereal coleoptiles stimulated the incorporation from [$^{14}CH_3$]methionine into methyl ester groups on pectin (Knee, 1982a). Pectin synthesis may be a normal concomitant of wall loosening whenever it occurs in plant tissues. The extent of synthesis in intact ripening fruit is uncertain. It must be less than is required to replace the losses through enzymatic degradation so that structural cohesion is lost, or alternatively it could be a synthesis of a structurally altered polymer which, by way of its bonding, influences and initiates softening.

Fry (1989b) argued that the wall loosening that is found in reaction to auxin may be a result of an

increase in activity of extracellular cellulase – a group of enzymes that cleave hemicellulose chains in the walls of both monocotyledons and dicotyledons. Mousdale & Knee (1981) found that indole-3-acetic acid (IAA) concentration showed a three to four fold increase prior to the rapid rise in ethylene concentration but fell to about its original level as the rise occurred.

1.5.6 Cell wall synthesis

The possible role that cell wall synthesis may play in the softening of fruit has rarely been considered (Knee & Bartley, 1981). According to Huysamer (1992) it is not surprising since there would be no incentive for fruit to start synthesizing new tissue when in a period of overall degradation.

The synthesis could be homeostatic as proposed for tomato by Mitcham *et al.* (1989) and by Romani (1987) to explain the climacteric response. An alternative explanation (Huysamer, 1992) is that the newly synthesized material plays a direct role in the loss of cell wall integrity leading to softening. The incorporation of the newly synthesized material, which may differ from the existing material, may alter the cell wall rheology (possible role of XET).

Examples of this could be 1) a more-highly esterified pectin – but less capable of ionic binding, 2) a less branched pectin which would be more susceptible to PG hydrolysis, and 3) an alteration in the types and numbers of neutral sugars and their glycosidic linkages in the hemicellulose, which could lead to the alteration of cellulose binding characteristics (Huysamer, 1992).

1.6 SUMMARY

Although the components of the cell wall have already been elucidated, the exact basis of intermolecular interaction and bonding is still under speculation. Covalent bonding has been rejected but the possibility of a combination of disulfide, hydrogen, simulated and ionic calcium bonds seems to be accepted. L-rhamnose in cross linkage of polysaccharide chains serves to kink the polygalacturonan chain, thus restricting packing. Calcium plays an important role in stabilizing interchain bonding and is thought to act in an "egg-box" model where calcium ions fit into interchain spaces to form "junction zones".

The major sites for fruit softening are in the middle lamella where enzymatic and non-enzymatic

mechanisms seem to degrade pectin polymers to a more soluble state and in the primary cell wall bring about the release of neutral sugars from galactan. In general no changes are noted in the cellulose or hemicellulose fraction during ripening and softening of apples and pears, although a rise and subsequent decline is found in cellulase activity during maturation. A possible role of cellulase is thought to be in the disintegration of the middle lamella where cellulosic microfibrils become less discernible.

Evidence supports the role of enzymatic mechanisms in fruit softening, especially the D-galacturonanases. This is mainly due to their ubiquitous distribution and temporal association with ripening. It is thought that PME plays a role in deesterifying the pectin molecule before the glycosidic links can be cleaved by a PG. Endo-PG is thought to play a much greater role in softening than exo-PG because of its ability to cleave the pectate chain at random sites where twelve or more free carboxyl groups are present, whereas exo-PG can only cleave one monomer at a time from the non-reducing end. Exo-PG completes the hydrolysis of pectate. Interesting to note is that apple does not contain endo-PG, while pear does. This may explain the higher softening rate of pears in relation to apples. The long held notion that PG is the enzyme responsible for softening came under heavy criticism due to the results obtained by antisense PG tomatoes. It seems that although PG activity could be greatly reduced, a comparable decline in fruit softening and polyuronide solubilization could not be attained. When a chimeric PG gene was incorporated into *rin* tomatoes the fruit failed to soften in spite of a sixty-fold increase in PG.

As a result of the complexity of the biochemical processes involved in fruit softening, the role of other cell wall hydrolases, like the glycosidases, xylanases, α- and β-galactosidases and endo-β-(1-3) glucanases, should not be ignored. β-galactosidases are cited to play a predominant role in the release of neutral sugars and the debranching of pectin galactan in apple cortical tissue. However, the importance of β-galactosidases has been questioned by many investigators. A declining rate of pectin galactan synthesis may be the cause of reduced level of galactose. RGases may possibly play a role in fruit softening due to their ability to degrade the pectin backbone.

A possible role of a transgalacturonase has recently been proposed in fruit softening. XET is thought to cleave the xyloglucan chain, weakening the cell wall and allowing incremental expansion, then rejoin the cut portion to the non-reducing terminus of a different xyloglucan

chain restoring, but altering, the wall fabric. This alteration of wall structure as a result of wall synthesis may be an alternate route to fuit softening. Apple tissue contrasts with other fleshy fruit, like kiwifruit and tomato, in XET activity throughout the growing season. XET activity in apple seems to decline during ripening and softening, whereas the solubilization of pectin does not show marked increases.

The lack of endo-PG in apple and the short term inhibitory effects of anoxia on pear tissue softening suggests that a process other than enzymatic pectin degradation is also involved in fruit softening. The operation of the respiratory enzyme cytochrome oxidase seems to be important to fruit softening if the negative effect of low oxygen is taken into account. An active ion pump regulating the ionic status of the cell wall could be involved as evidenced by the reversal of softening of pear with pH buffers and calcium ions.

Calcium plays a very important role in cell wall structure and cell membrane integrity. Calcium preserves the cellular organisation not only by preserving cell membranes, but also by maintaining nucleic acid and protein synthesis. Displacement of calcium ions by protons in the methylation of polygalacturonate which destabilizes the pectic matrix ("egg-box" model) is an important facet in fruit softening. Rapid cell wall degradation and disorder are correlated with a loss of calcium mobility. Although the loss of calcium from the middle lamella is considered an important event in fruit softening it is not the sole factor responsible. Infiltration studies of pear tissue with $CaCl_2$ determined that high calcium status has a negative effect on ethylene synthesis and the softening of fruit flesh. Investigators during the last few years have revealed that calcium regulates various cellular functions through calmodulin, a calcium binding protein. A calcium-calmodulin complex has been shown to activate several enzymes including protein kinases.

Cell wall hydrolytic activity is a consequence of, rather than a prelude to ethylene synthesis. Fruit firmness decreased linearly in reaction to exogenously applied ethylene and a threshold level was showen to exist to which immature fruit will react on ethylene treatment. The onset of autocatalytic ethylene production rather than the respiratory climacteric seems to be critical in the ripening sequence. Furthermore it seems that enzymes responsible for cell wall breakdown are quite sensitive to very small amounts of ethylene.

Polyamines are able to delay senescence of fruit due to a double action. They protect membranes and maintain macromolecular synthesis, both of which are essential in preventing senescence.

The role of auxin in fruit softening involves the loosening and enhanced synthesis of pectin, destined for incorporation in the cell wall. A rapid increase in IAA has been noted prior to the rise in ethylene but it was found to subside as the rise occurred. For softening to occur the rate of synthesis of pectin should be less than required to replace the losses due to enzymatic degradation. The alteration of the cell wall rheology due to cell wall synthesis could possibly play a role in fruit softening when a more-highly esterified pectin, a less branched pectin or a pectin with an alteration in the types and numbers of neutral sugars and their glycosidic linkages is incorporated in the cell wall.

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Chapter 2: Paper 1

Exogenous ethylene shortens the prerequisite cold storage period for normal ripening of 'Forelle' pears.

Abstract

Commercial handling of 'Forelle' pears *(Pyrus communis* L.) entails a minimum period of 12 weeks of storage at -0.5°C after harvest to ensure uniform ripening. Results presented in this paper indicated a potential to reduce this 12 week cold storage period, thereby allowing earlier marketing when demand and prices are high. 'Forelle' pears harvested at commercial maturity were treated with ethylene at 0, 10 or 100 $\mu L \cdot L^{-1}$ for 24 hours at 20°C. Thereafter, pears were stored at -0.5°C for 12 weeks, with periodic sampling to evaluate ripening. Both 10 and 100 $\mu L \cdot L^{-1}$ ethylene treatments significantly hastened ripening, as judged by changes in peel colour from green to yellow, fruit softening and increases in soluble solids. Differences between these concentrations were negligible, and it was concluded that the storage period for 'Forelle' can be shortened to 4 weeks by exogenous ethylene.

Keywords: 'Forelle' pears *(Pyrus communis* L.); ethylene concentration; cold storage

INTRODUCTION

In contrast to summer pear varieties that do not have a chilling requirement to ripen properly, winter pears are harvested at a maturity stage well in advance of the capability for ethylene biosynthesis that develops during cold storage. Chilling after harvest during the storage period induces an accelerated ripening process in fruit when taken out of storage, leading to uniform ripening, a decrease in flesh firmness, and development of flavour and juiciness. This holds true for cultivars like 'Bon Chretien' (Looney, 1972), 'Beurre Bosc' (Sfakiotakis & Dilley, 1974), 'Conference' (Knee *et al.*, 1983), 'Eldorado' (Wang *et al.*, 1985), 'd'Anjou' (Blankenship & Richardson, 1985) and 'Comice' (Richardson & Gerasopoulos, 1994).

'Forelle', one of the most important pear cultivars in South Africa due to its high export value, requires a minimum of 12 weeks of cold storage before being considered marketable (De Vries & Hurndall, 1993). 'Forelle' is therefore held in cold storage (-0.5°C) for eight weeks in South

Africa and for a further four weeks during shipping and distribution otherwise they will not reach a proper climacteric and will ripen unevenly. The result is that this cultivar, which already has a very late harvesting date, reaches the marketplace even later. Efforts to shorten the prerequisite storage period using heat treatments (De Vries & Hurndall, 1993) or a combination of controlled atmosphere (CA) and normal atmosphere storage (De Vries & Hurndall, 1994; De Vries & Moelich, 1995) were unsuccessful.

Cold storage stimulates ripening in 'Forelle' pears by its influence on ethylene biosynthesis and action. The enzyme converting ACC to ethylene in apple is sensitive to temperature and is believed to be cell membrane bound (Mattoo *et al.*, 1977). Temperatures below 12°C are shown to inhibit the conversion of ACC to ethylene (Apelbaum *et al.*, 1981). Furthermore, distinct increases in endogenous ACC content in 'Eldorado' pears were observed by Wang *et al.* (1985) after 4 weeks at 0°C. It could be postulated then, that cold storage of pears causes a build-up of ACC, until a threshold is reached and temperatures rise, leading to accelerated ripening and attainment of a proper climacteric.

Ethylene is commonly used as a fruit-ripening hormone in avocados, bananas, citrus fruits, cantaloupe and tomatoes (Reid, 1992). It is thought that apples will remain in an unresponsive state to either endogenous or exogenous ethylene until a hypothetical ripening inhibitor has decreased or some effector has accumulated in the tissue (Bufler, 1986). Exogenous or endogenous ethylene, however, is effective in shortening the time to the eventual onset of autocatalytic ethylene production, *e.g.* by destroying the ripening inhibitor. Evidence of this was found as early as 1972 when ethylene gas applied to 'd'Anjou' pears held at 20°C induced ripening and softening, while untreated fruit did not ripen (Wang *et al.*, 1972). Recently ethylene gassing of 'Anjou' pears led to the induction of a normal climacteric rise and the capability of fruit tissue to convert ACC to ethylene without the required chilling (Chen *et al.*, 1997). Preliminary trials on the treatment of 'Forelle' pears with ethylene before cold storage (Conradie & Huysamer, unpublished data) indicated that it is possible to reduce the prerequisite cold storage period. Treatment with ethylene after a four week cold storage period did not have any effect. In the present study 'Forelle' pears were treated with ethylene gas in a attempt to substitute or reduce the minimum 12 week cold storage requirement.

MATERIALS AND METHODS

'Forelle' *(Pyrus communis* L.) pears were harvested at commercial maturity from a commercial orchard in the Ceres area, Western Cape Province, South Africa. Mean fruit weight was 147.8 g. Fruit was harvested when flesh firmness reached ± 7 kg. Fruit were subjected to two ethylene treatments within 6 h of harvest, at the Department of Horticulture, University of Stellenbosch. Bearing in mind the optimal ripening conditions proposed by Reid (1992, ethylene was premixed in air by Afrox (Ltd). The trial consisted of a total randomised design consisting of 3 treatments each with 13 repetitions of 30 fruit. A constant flow of ethylene gas was obtained by two two-stage ethylene regulators into Conviron chambers kept at a constant temperature of 20°C while ensuring adequate air circulation and ventilation. Ethylene gas at a concentration of 10 and 100 $\mu L \cdot L^{-1}$ was applied for 24 h. Control fruit were kept in a well ventilated room at 20°C for 24 h. Thereafter all fruit was stored at –0.5°C for 12 weeks at normal atmospheric conditions (90% RH).

Every two weeks over a period of 12 weeks, two samples of each treatment were removed from cold storage. Maturity tests were immediately conducted on one sample, to determine the maturity of fruit kept in cold storage, while the other sample was held for a further seven days at 20°C to simulate the marketing period, and then tested for maturity. Replicates were stored in open plastic bags in a commercial cold storage facility. Peel ground colour was evaluated with a Unifruco colour chart (0.5 = dark green through to 5 = deep yellow). A critical value of 3.5 on the Unifruco colour chart is accepted as marketable for 'Forelle' pears. Colour measurements were taken from the non-blushed side with a Nippon Denshoku colorimeter (Handy colorimeter, HR-3000). Colour is reported as a set of L*, C* and H° values. The L* value refers to the lightness of the colour on a scale of 0 to 100 where 0 = black through to 100 = white. The C* value refers to the chroma or intensity of colour, with a higher value referring to a colour of greater intensity. The H° value refers to the hue angle on a scale of 0° = red/purple, 90° = yellow, 180° = blue/green and 270° = blue. (McGuire, 1992). Flesh firmness (kg) was tested with a penetrometer (Southtrade fruit pressure tester, mod. FT 327) with an 8 mm tip. Two measurements were taken from each fruit, at opposite sides, after a piece of skin (roughly 2 cm in diameter) was removed. Total soluble solids (TSS; %) were measured by an digital refractrometer (Brix % Palette PR-100 (0-32%) Refractometer). Titrable acidity (TA; %) was calculated as a % Malic acid, by titrating juice from liquidised segments, taken from groups of 6 made from the 30 fruit in each batch, with

0.1 NaOH to a pH of 8.2. Data was analysed using the SAS System (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Ground colour

Significance levels indicate definite interactions between treatments and cold storage periods (weeks) for fruit in cold storage (Figure 1) and after seven days at 20°C (Figure 2). 'Forelle' pears treated with exogenous ethylene attained a yellow ground colour significantly earlier than untreated fruit. Fruit that has attained the critical value of 3.5, and higher, reached the proper maturity to be marketed and consumed (De Vries & Moelich, 1995). A significant difference at the 5% level was found between the control and the ethylene treatments for fruit immediately out of cold storage (Figure 1) and for fruit held at 20°C for seven days (Figure 2), starting at week four. Fruit subjected to both treatments, 10 $\mu L \cdot L^{-1}$ and 100 $\mu L \cdot L^{-1}$ ethylene, could be considered marketable at this stage and did not differ significantly. The ethylene treatments were significantly more yellow than the control for fruit in cold storage, and for fruit held for seven days at 20°C, until week 8 (Figure 1). Thereafter the control and ethylene treatments did not differ significantly and all fruit yellowed equally. Control fruit when held at 20°C for seven days attained the 3.5 critical value only after eight weeks of cold storage (Figure 2) whilst both ethylene treatments had already attained the critical value after four weeks.

These results obtained from the evaluation of ground colour indicate that the treatment of 'Forelle' pears with ethylene gas has the potential of reducing its prerequisite cold storage of 12 weeks to 4 weeks. It also indicates that 'Forelle' pears may be sensitive to a lower concentration of ethylene gas than used in the present study because no significant differences could be found between ethylene treatments.
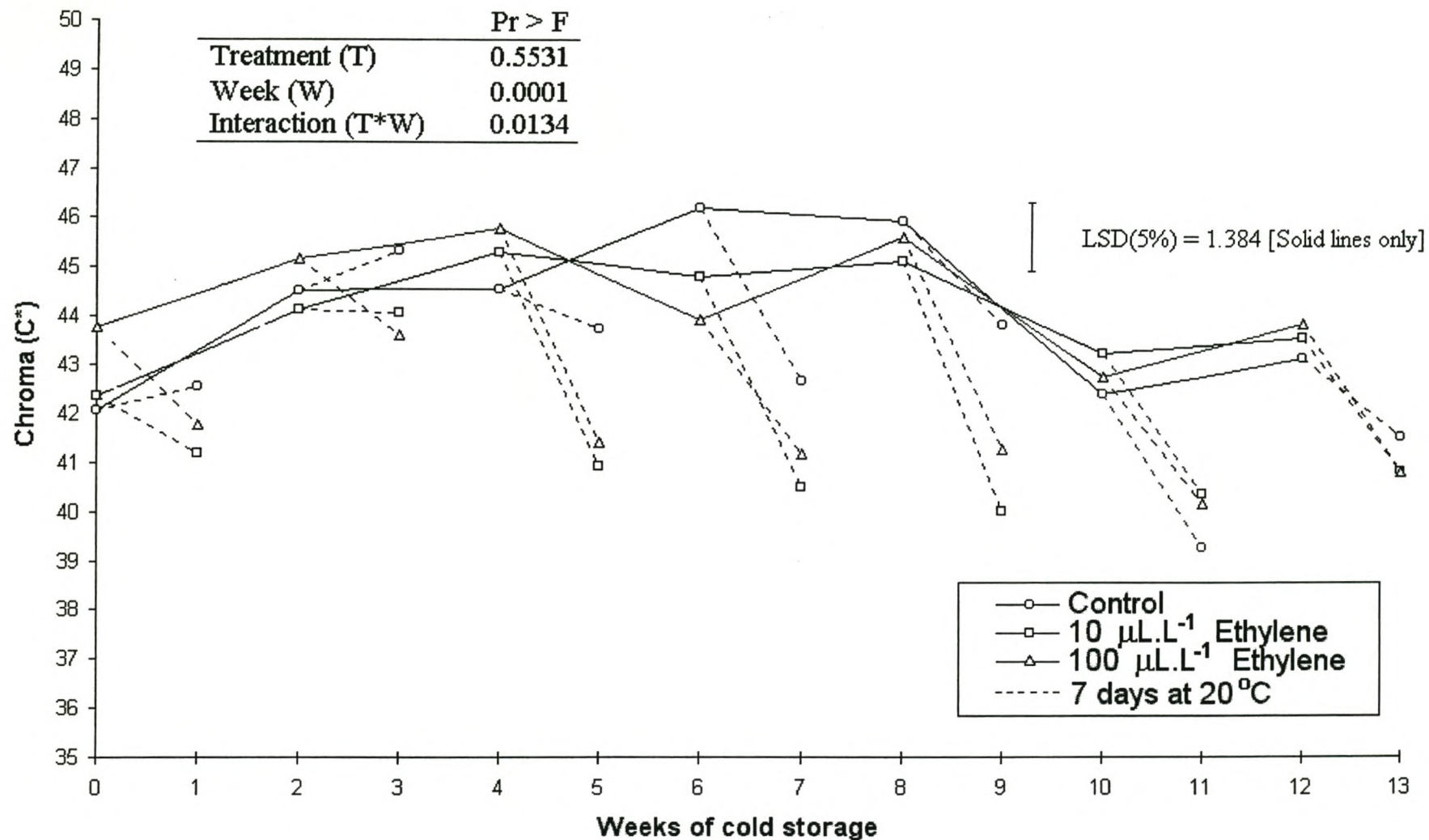
Figure 1: Peel ground colour in 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines repr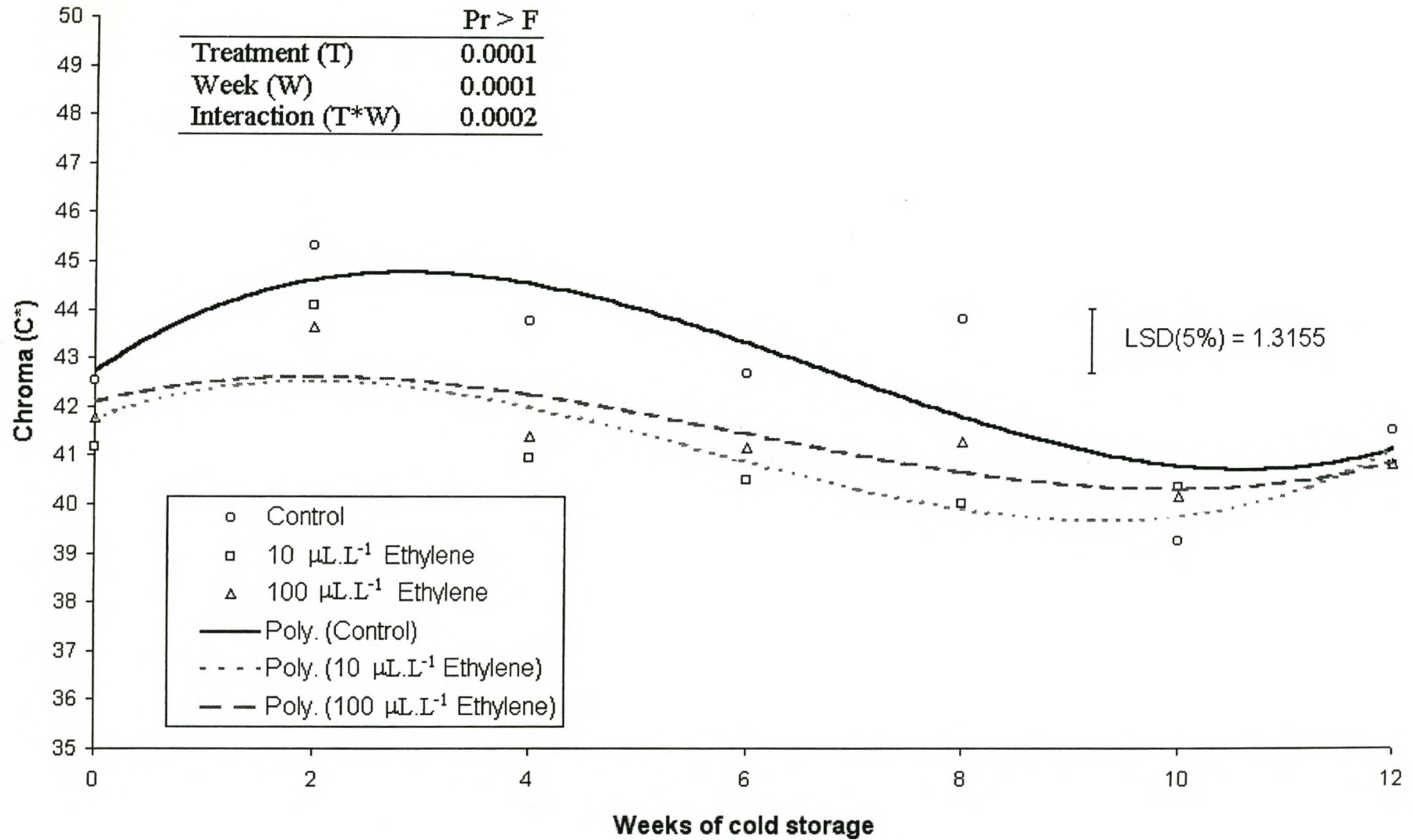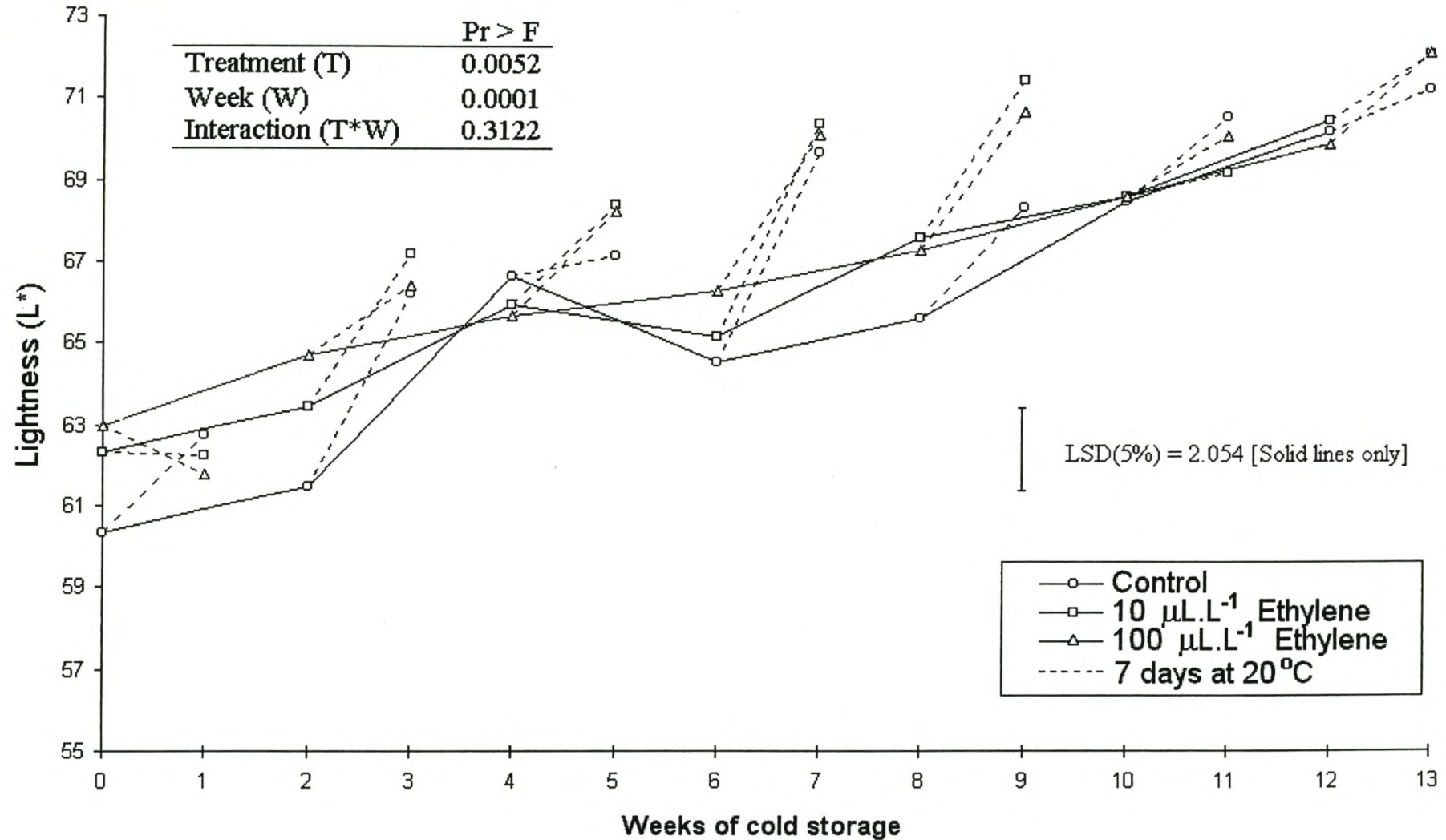esent fruit held at 20°C for seven days. The line at 3.5 represents the critical minimum value for marketing and/or consumption.

	Pr > F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

LSD(5%) = 0.251

- o Control
- □ 10 µL.L^{-1} Ethylene
- △ 100 µL.L^{-1} Ethylene
- ▬ ▬ Crit.Value
- ── Poly. (Control)
- - - - Poly. (10 µL.L^{-1} Ethylene)
- ─ ─ Poly. (100 µL.L^{-1} Ethylene)

Ground colour

Weeks of cold storage

Figure 2: Peel ground colour in 'Forelle' pears held at 20°C for seven days after cold storage for different periods. The line at 3.5 represents the critical minimum value for marketing and/or consumption. R²(Control) = 0.9939; R²(10 µL·L^{-1}) = 0.9858; R²(100 µL·L^{-1}) = 0.9794.

Fruit colour

Fruit colour taken objectively by the colorimeter support the results obtained using the colour chart. Significance levels indicate definite interactions in C* between treatments and cold storage periods (weeks) for fruit in cold storage (Figure 3) and after seven days at 20°C (Figure 4). As fruit ripened there was a decrease in chroma, indicating a shift from a more intense green colour to a less intense yellow colour (lower chroma). No significant differences were found between treatments for fruit held in cold storage (Figure 3), but when fruit were held for a further seven days at 20°C, the ethylene treated fruit had a significantly greater decrease ($P \leq 0.05$) in chroma (Figure 4). No significant differences were found in the decrease of chroma between ethylene concentrations.

Significance levels indicate no definite interactions in L* between treatments and cold storage periods (weeks) for fruit in cold storage (Figure 5), but when fruit were held at 20°C for seven days a strong interaction was found (Figure 6). Ethylene treated fruit in cold storage and those held for seven days at 20°C were lighter in colour than control fruit, although there was not always a significant difference during some weeks.

As the hue angle (H°) drops, fruit changes to a more yellow colour, indicating ripening. Fruit in cold storage (Figure 7) showed a poor interaction in hue angle between treatments and weeks. Control fruit in cold storage were greener than ethylene treated fruit, but the difference was not significant. Fruit held at 20°C for seven days (Figure 8) showed strong interactions between treatments and weeks. Ethylene treated fruit had significantly lower ($P \leq 0.05$) hue angles than control fruit after four weeks of cold storage and seven days at 20°C (Figure 8). The hue angles for ethylene treated fruit held for seven days at 20°C remained significantly lower than control fruit until 10 weeks of cold storage. No significant differences could be found between fruit treated with different concentrations of ethylene. These results verify those obtained with ground colour evaluation by colour chart.

Figure 3: Chroma (C*) of peel in 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
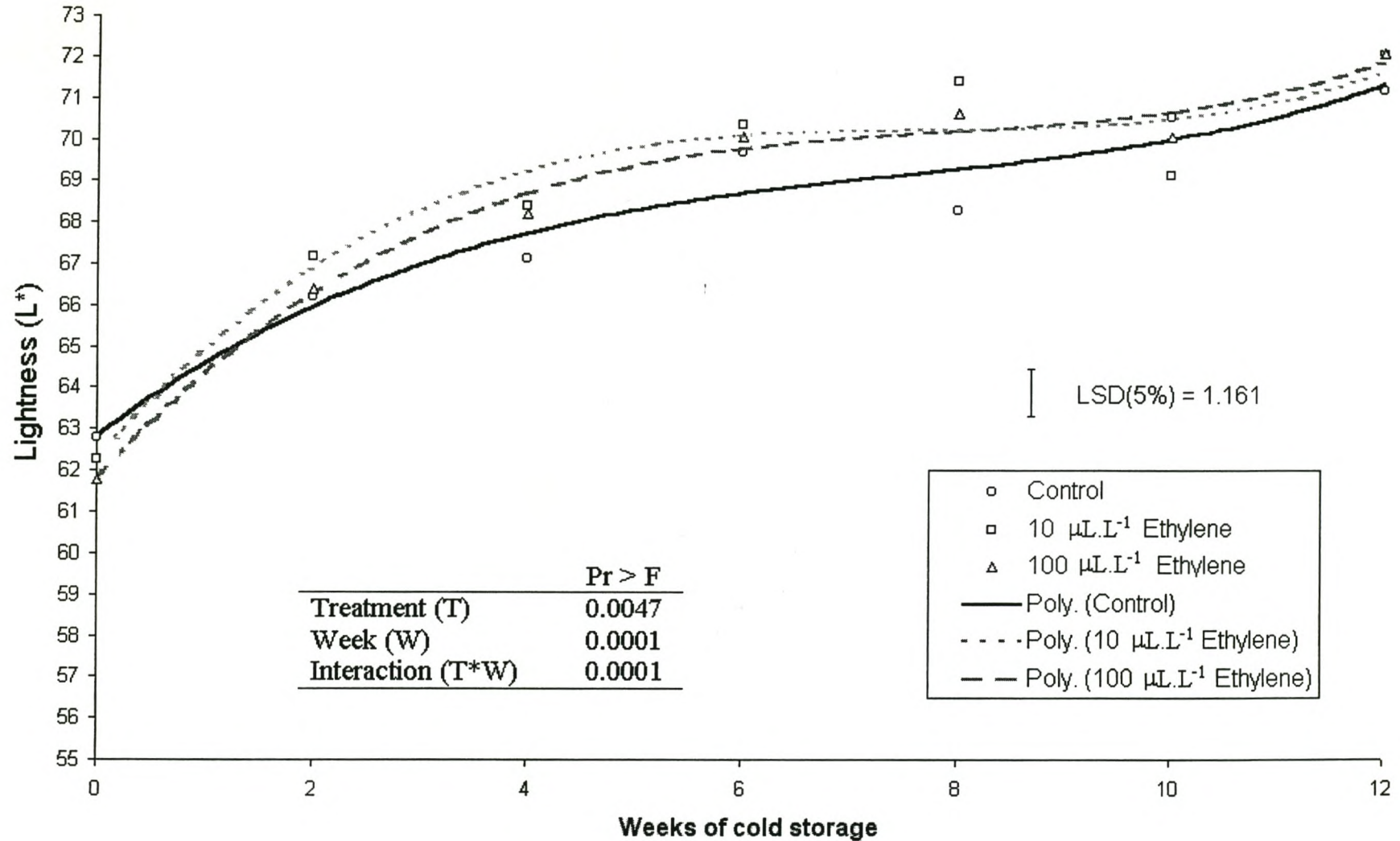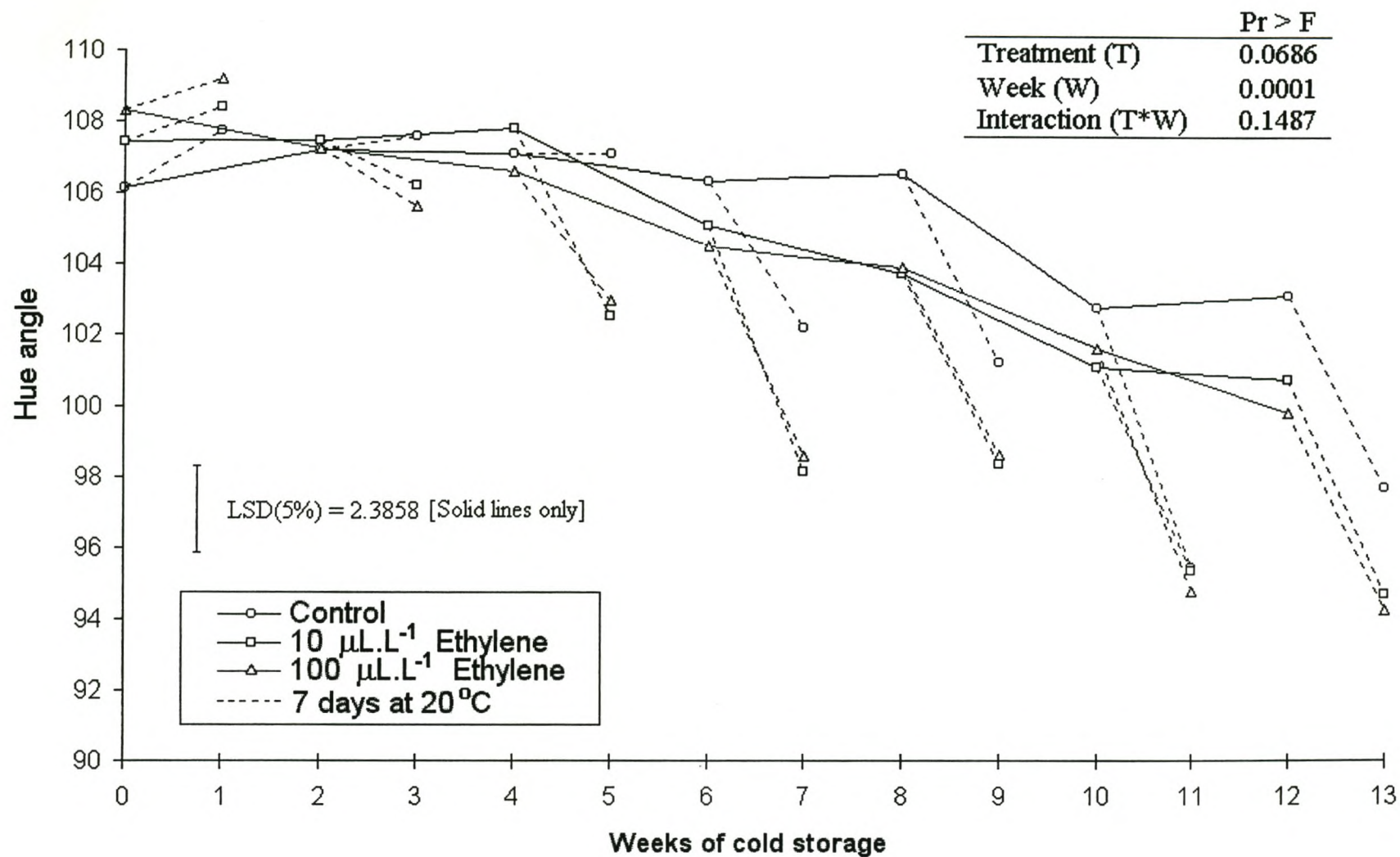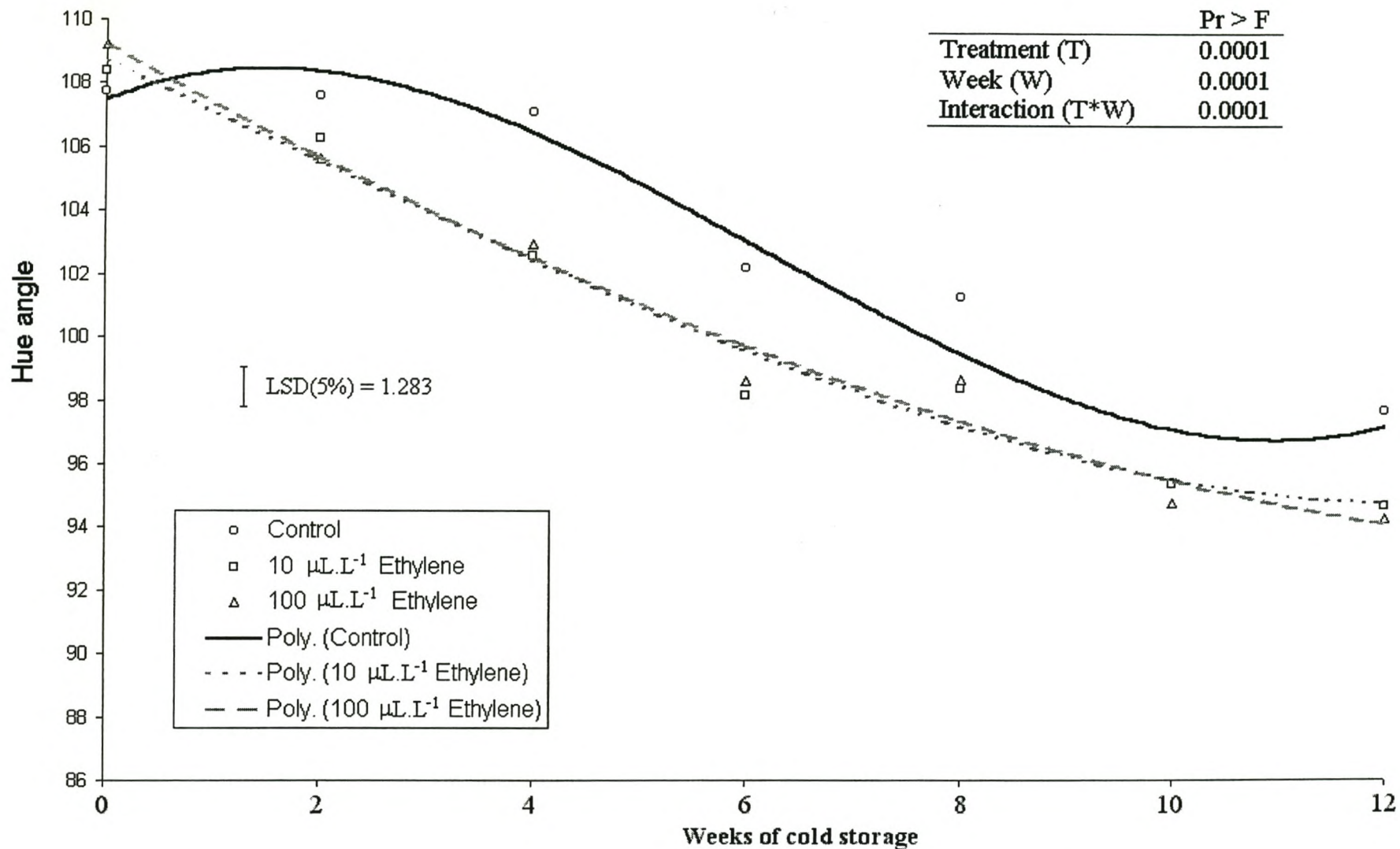
Figure 4: Chroma (C*) of peel in 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.6336; R^2(10 $\mu L \cdot L^{-1}$) = 0.5877; R^2(100 $\mu L \cdot L^{-1}$) = 0.6602.

Figure 5: Lightness (L*) of peel in 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

Figure 6: Lightness (L*) of peel in 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9488; R^2(10 μL·L^{-1}) = 0.9351; R^2(100 μL·L^{-1}) = 0.987.
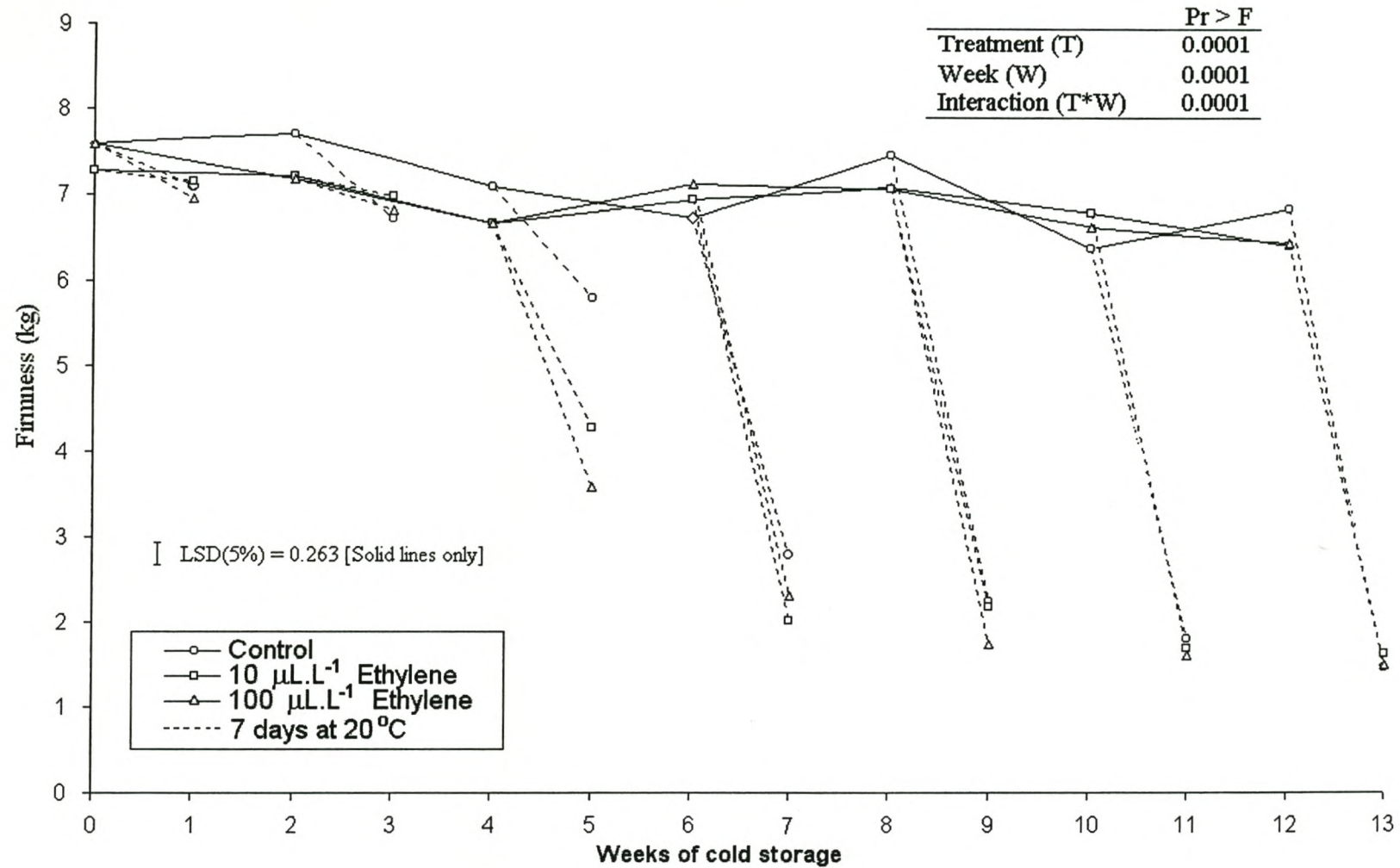
Figure 7: Hue angle (H°) of peel in 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
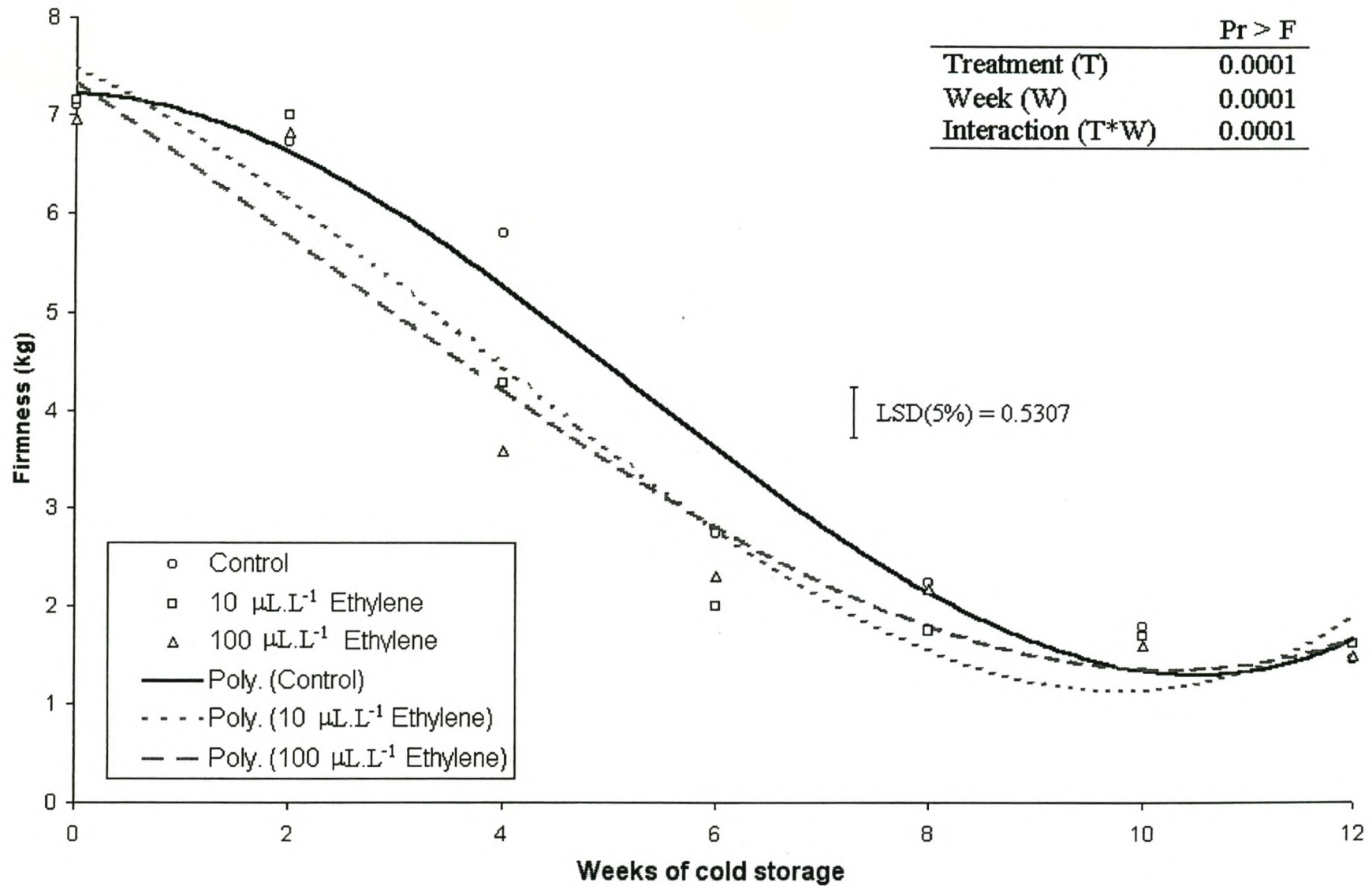
Figure 8: Hue angle (H°) of peel in 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9471; R^2(10 µL·L^{-1}) = 0.9764; R^2(100 µL·L^{-1}) = 0.9808.

Flesh firmness

Significance levels indicate a strong interaction between treatments and weeks for fruit in cold storage (Figure 9), and fruit held at 20°C for seven days (Figure 10). It has been reported that after ethylene treatment the uniformity in firmness of 'Bartlett' pears increased (Clayton *et al.*, 1999). This effect could be seen (Figure 9) where firmness of firmness of ethylene treated fruit in cold storage remained relatively constant and did not differ significantly from one another, while the control fruit were at some stages significantly softer ($P \leq 0.05$) and at other stages significantly firmer.

Ethylene treated fruit held their firmness in cold storage very well for the whole 12 week period. All treatments were still above the minimum export standard of 5.5 kg at the end of the trial. My personal observation was that minimum wooliness occurred and only in the latter stages of storage. The ethylene treated fruit did not show a greater tendency to wooliness. There appears to be no risk of deterioration in quality for fruit held in cold storage after treatment with ethylene.

'Forelle' pears treated with ethylene softened significantly earlier than control fruit when held for seven days at 20°C (Figure 10). The local standard of eating pressure for 'Forelle' pears are set at 4,5 kg while it is internationally set between 4,1 and 3,6 kg (Crouch, 2000). A significant drop in firmness was seen after ethylene treated fruit had four weeks of cold treatment opposed to the six weeks control fruit needed for a similar drop. A significant difference at the 5% level was found at week 4 between ethylene treatments, with 100 $\mu L \cdot L^{-1}$ ethylene being the softest.

	Pr > F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

I LSD(5%) = 0.263 [Solid lines only]

Legend:
- Control
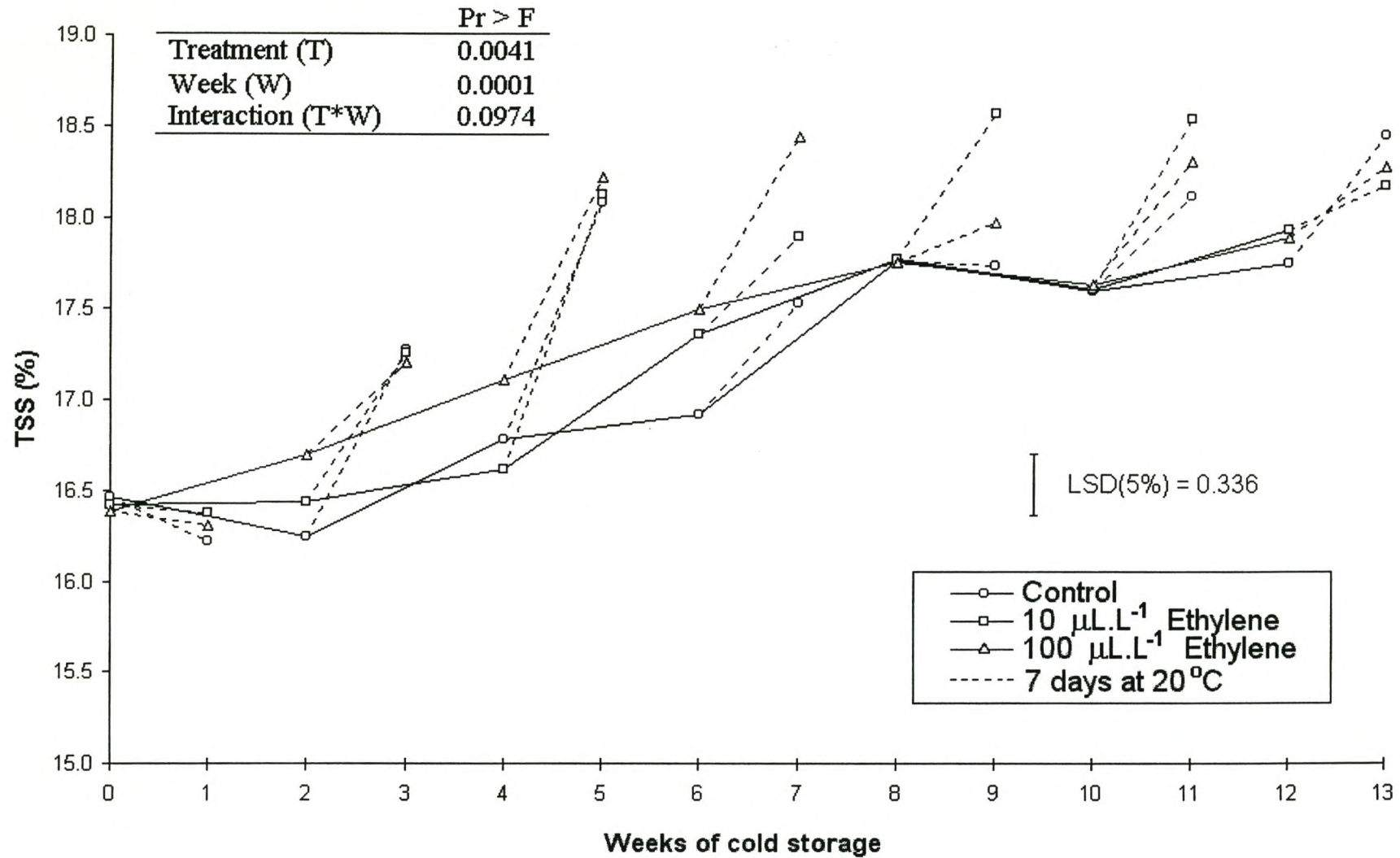- 10 µL.L⁻¹ Ethylene
- 100 µL.L⁻¹ Ethylene
- 7 days at 20 °C

Figure 9: Firmness (kg) of 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
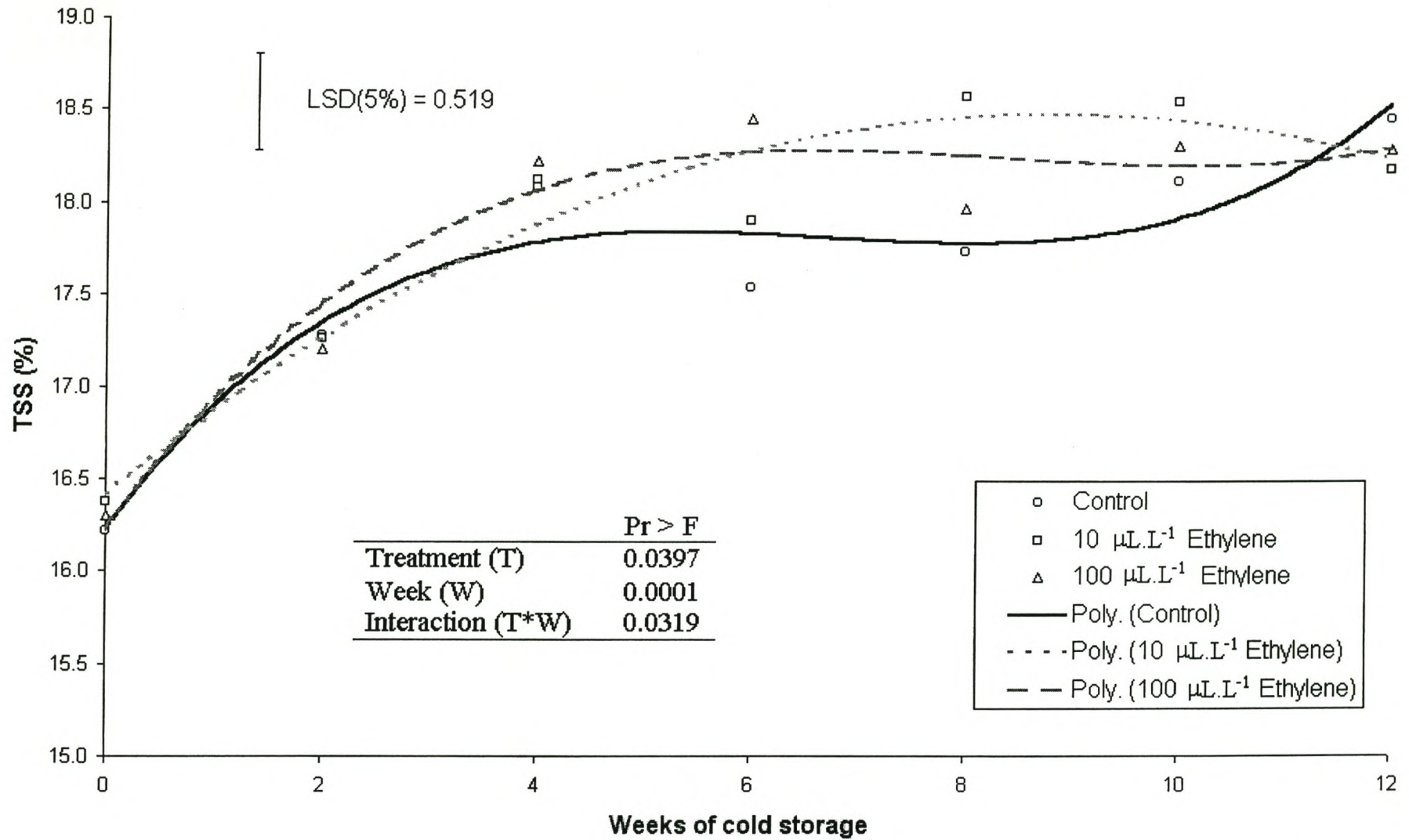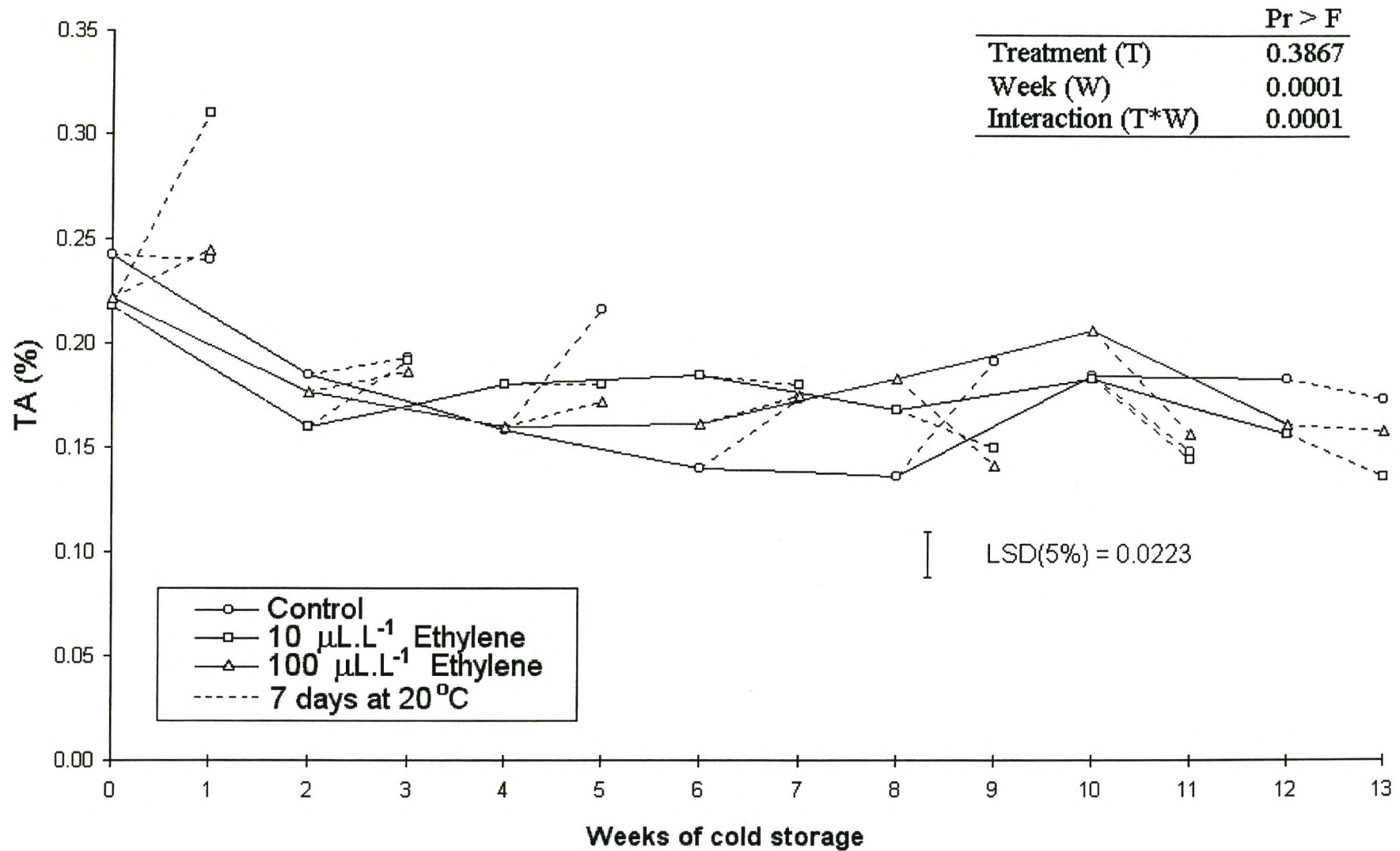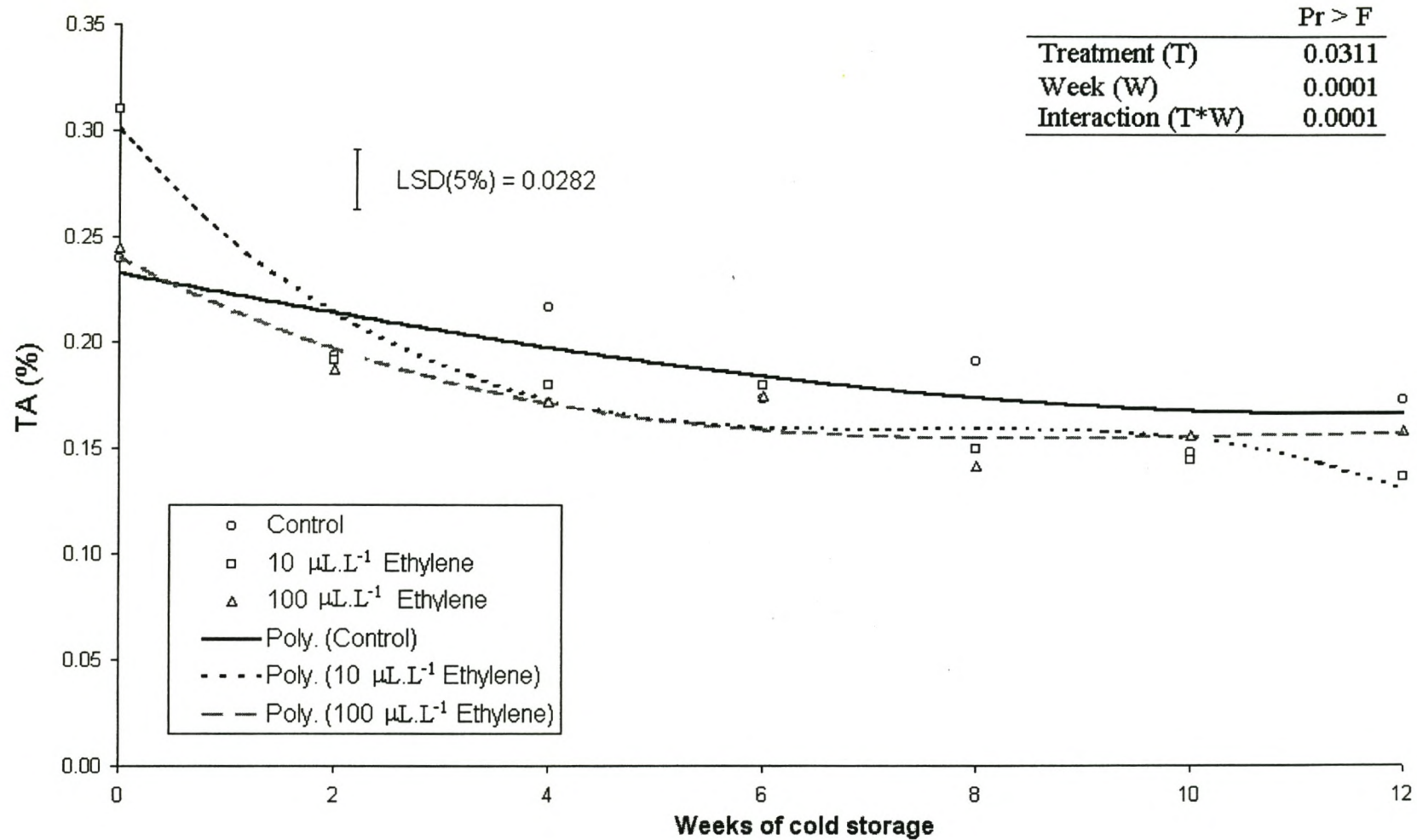
	Pr > F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

LSD(5%) = 0.5307

Legend:
- ○ Control
- □ 10 μL.L⁻¹ Ethylene
- △ 100 μL.L⁻¹ Ethylene
- —— Poly. (Control)
- ····· Poly. (10 μL.L⁻¹ Ethylene)
- – – Poly. (100 μL.L⁻¹ Ethylene)

Weeks of cold storage

Firmness (kg)

Figure 10: Firmness (kg) of 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9635; R^2(10 μL·L⁻¹) = 0.9497; R^2(100 μL·L⁻¹) = 0.9376.

Total soluble solids (TSS) and titrable acidity (TA)

Significance levels indicate a strong interaction in TSS for fruit held at 20°C for seven days after cold storage (Figure 12), but not for fruit held in cold storage at –0.5°C (Figure 11). Fruit treated with 100 µL·L^{-1} ethylene had a higher TSS content in the early stages of cold storage, but did not differ significantly from the other treatments (Figure 11). When fruit was held at 20°C for seven days (Figure 12) it was found that ethylene treated fruit attained higher TSS contents than control fruit after 6 weeks of cold storage.

Significance levels indicate definite interactions in TA between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 13) and after seven days at 20°C (Figure 14). No definite trends could be found in the change in TA of fruit in cold storage (Figure 13). Although control fruit had a higher TA when fruit was held for seven days at 20°C (Figure 14) it was not significantly so.

Figure 11: TSS (%) of 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

Figure 12: TSS (%) of 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.93; R^2(10 µL·L^{-1}) = 0.9395; R^2(100 µL·L^{-1}) = 0.9439.

Figure 13: TA (%) of 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

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Figure 14: TA (%) of 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.691; R^2(10 $\mu L \cdot L^{-1}$) = 0.9387; R^2(100 $\mu L \cdot L^{-1}$) = 0.9149.

DISCUSSION

'Forelle', one of the most important pear cultivars in South Africa due to its high export value, requires a minimum of twelve weeks cold storage at $-0.5°C$ before being considered marketable (De Vries & Hurndall, 1993). Results obtained, in this study, by ethylene treatment indicate a potential to reduce this 12 week cold storage period to $4 - 6$ weeks, thereby allowing earlier marketing when demand and prices are high.

The possible mechanism by which ethylene has the ability to induce a physiological response and a shortening of the prerequisite cold storage of 'Forelle' pears could be explained by Yang's model of System 1- and System 2- ethylene receptors (Yang, 1985; McGlasson, 1985). Two systems (Figure 15) of ethylene production during fruit maturation and ripening have been distinguished: System 1 represents the low level of ethylene present in fruit before the onset of ripening, while System 2 represents the autocatalytic burst of ethylene production accompanying the ripening process. In pre-climacteric fruit the resistance to ripening or resistance to ethylene action (ripening inhibitor) is so high that the ripening process is not initiated.

As fruit undergo maturation there is a progressive decrease in resistance (shift to inactive form) to ethylene action (or an increase in sensitivity) and this process is thought to be controlled by endogenous ethylene. The decrease in resistance to ethylene action is accelerated by the external application of ethylene and is retarded by low O_2/high CO_2 atmospheres and by removal of ethylene. When the resistance to ethylene action decreases to a point at which the fruit become responsive to their endogenous ethylene levels, the ripening process is initiated, resulting in the autocatalytic burst of ethylene production (System 2). Hence the important factor that triggers the onset of ripening is the decrease in the resistance, or increase in ethylene sensitivity, to ethylene action.
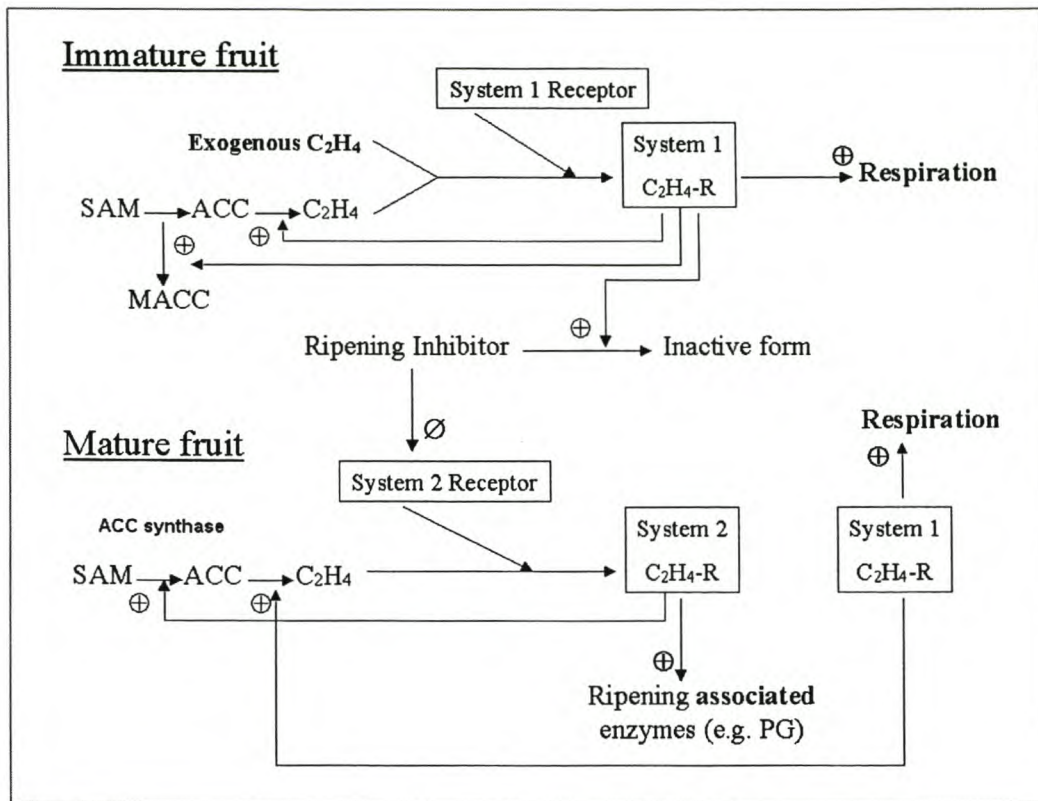
Figure 15: A model showing the sequence of ethylene action on the regulation of ethylene biosynthesis in fruit maturation and ripening.⊕ and ∅ indicate the metabolic processes that are positively or negatively regulated by the specific ethylene-receptor complex or the ripening inhibitor (adapted from Yang, 1985; McGlasson, 1985).

It can be concluded from the data presented that the treatment of 'Forelle' pears with ethylene gas reduces its cold requirement from 12 weeks to four weeks, as judged by changes in peel colour from green to yellow and fruit softening. A tenfold increase in concentration of ethylene did not have a significantly different effect than 10 $\mu L \cdot L^{-1}$ ethylene. This phenomena could be seen in all maturity indexes measured. It was concluded that the ethylene biosynthesis pathway of 'Forelle' pears to exogenous ethylene is highly sensitive to small amounts of ethylene. It is likely to be more sensitive to concentrations lower than that used in the present study.

Ethylene treated fruit held their firmness in cold storage very well for the whole 12 week period and fruit held at 20°C for seven days ripened normally. My personal observation was that minimum wooliness occurred and only in the latter stages of storage. The ethylene treated fruit did not show a significantly greater tendency to wooliness. There appears to be no risk of deterioration in quality for fruit held in cold storage after treatment with ethylene.

Although ground colour measurements indicated that ethylene treated fruit required only four weeks of cold storage to attain the minimum required value (Unifruco colour chart value of 3.5), a properly ripened fruit is accompanied by a yellow skin colour, soft texture and correct TSS/TA ratio. Therefore it is suggested that ethylene treated 'Forelle' pears be stored for a minimum of six weeks at $-0.5°C$ before being marketed. This would ensure a yellow fruit accompanied by a favourable palatability.

ACKNOWLEDGEMENTS

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CHAPTER 3: PAPER 2

EFFECT OF HARVEST MATURITY AND DURATION OF ETHYLENE APPLICATION ON THE PREREQUISITE COLD STORAGE PERIOD FOR 'FORELLE' PEARS.

Abstract

Commercial handling of 'Forelle' pears *(Pyrus communis* L.) entails a minimum period of 12 weeks of storage at -0.5°C after harvest to ensure uniform ripening. Results presented in this paper indicate a potential for reducing this 12 week cold storage period, thereby allowing earlier marketing when demand and prices are high. 'Forelle' pears were harvested at three maturity levels and treated with ethylene at a concentration of 50 $\mu L \cdot L^{-1}$ at 20°C for periods of 12, 24 or 36 hours. Thereafter, pears were stored at -0.5°C for 12 weeks, with periodic sampling to evaluate ripening. All harvest maturities required a minimum of 24 hours of ethylene to attain a physiological response in ground colour and firmness. It was concluded that the storage period for 'Forelle' could be shortened to 4 weeks with exogenous ethylene gassing irrespective of harvest maturity. The time of attainment of a yellow ground colour and a loss of firmness differed significantly between harvesting maturities. Quality of 'Forelle' pears held in cold storage was not adversely affected by ethylene, regardless of the harvesting date.

Keywords: 'Forelle' pears *(Pyrus communis* L)*; ethylene; treatment length; harvest maturity; fruit maturity; ripening

INTRODUCTION

Ripening of pears, due to their climacteric nature, is associated with a large increase in the rate of respiration and ethylene production. This is the stage where the potential for good taste and quality generally reaches a maximum, hence restricting the period of optimum harvest because climacteric fruit become highly perishable once ripening is underway. 'Forelle' pears differ somewhat in that they need a minimum of 12 weeks of cold storage before they have the potential to reach a proper climacteric (De Vries & Hurndall, 1993). 'Forelle' is therefore held in cold storage (-0.5°C) for eight weeks in South Africa and for a further four weeks during shipping and

distribution otherwise they will not reach a proper climacteric and will ripen unevenly. The result is that this cultivar, which already has a very late harvesting date, reaches the marketplace even later. Efforts to shorten the prerequisite storage period using heat treatments (De Vries & Hurndall, 1993) or a combination of controlled atmosphere (CA) and normal atmosphere storage (De Vries & Hurndall, 1994; De Vries & Moelich, 1995) were unsuccessful.

Temperatures below 12°C are shown to inhibit the conversion of ACC to ethylene (Apelbaum *et al.*, 1981) and distinct increases in endogenous ACC content in 'Eldorado' pears were observed by Wang *et al.* (1985) after 4 weeks cold storage at 0°C. It has been postulated that cold storage of pears causes a build-up of ACC until a threshold is reached and temperatures rise, leading to accelerated ripening and attainment of a proper climacteric. The increases in respiration and ethylene production can be induced prematurely by treatment with exogenous ethylene (McGlasson, 1985). The ripening process is irreversible once endogenous (autocatalytic) ethylene production increases to a certain threshold level. The biogenesis of ethylene in climacteric fruit is regulated with 2 systems (Yang, 1985; McGlasson, 1985): System 1, which is involved in the regulation of aging processes and is responsible for the low rate of ethylene production during growth, and System 2, which is responsible for the autocatalytic increase in ethylene production which accompanies ripening. Climacteric fruit will therefore not respond to exogenous ethylene after normal ripening has started (Wang & Hansen, 1970) due to autocatalytic ethylene production. Sensitivity to ethylene is not constant throughout the life of fruit (Burg & Burg, 1965) in that the threshold for ethylene action decreases with fruit age. This means that fruit harvested earlier than the optimal harvesting time will be less sensitive to ethylene. This effect was noted by Sfakiotakis & Dilley (1973) where the efficiency of propylene (ethylene analog) increased progressively during the maturation of 'Red Delicious' apples. More mature fruit required less time to initiate autocatalytic ethylene production. Although the respiration rate and ethylene production increased in fruit of lower maturity, flesh softening and an increase in soluble solid content was lacking. This indicated that the mechanisms for autocatalysis of ethylene production and respiration were functional quite early in maturation although the fruit had not attained the required potential for ripening. Softening of pears, on the other hand, occurs prior to, and is not dependant on, development of the climacteric rise in respiration (Hansen & Blanpied, 1968; Wang *et al.*, 1972).

It is generally recommended that climacteric fruit be exposed to a minimum of 12 h of ethylene to initiate full ripening (Reid, 1992). 'D'Anjou' pears treated longer than 24 h with exogenous ethylene decreased in firmness after 12 days at 20°C while untreated fruit remained firm (Wang & Hansen, 1970). Hansen & Blanpied (1968) found that the optimum length of treatment with ethylene for induction of ripening in 'Bosc' pears varied according to fruit maturity. Pears picked at a pre-mature stage required 48 h compared to 24 h for fully mature fruit. Ripening in the post-mature stage proceeded without exposure to ethylene but was stimulated by a 12 h treatment.

The present study examines the effect of ethylene as a method for treating 'Forelle' pears in order to reduce the minimum period of 12 weeks of cold storage. This investigation was undertaken to determine the importance of fruit maturity at harvest and the duration of exposure to exogenous ethylene on the prerequisite cold storage period for 'Forelle' pears.

MATERIALS AND METHODS

'Forelle' *(Pyrus communis* L.) pears were harvested from a commercial orchard in the Villiersdorp area, Western Cape Province, South Africa. Fruit was harvested when flesh firmness reached ± 7 kg. Fruit were harvested at three different maturities to evaluate the effect of maturity on receptiveness of 'Forelle' pears to exogenous ethylene before storage. Fruit was harvested two weeks prior to optimal maturity, at optimal maturity and two weeks after the optimal maturity date. Fruit were subjected to three ethylene treatments within 6 h of harvest, at the Department of Horticulture, University of Stellenbosch.

Bearing in mind the optimal ripening conditions proposed by Reid (1992), 'Forelle' pears were treated with 50 $\mu L \cdot L^{-1}$ ethylene (premixed in air by Afrox (Ltd)), at 20°C for periods of 12, 24 or 36 hours respectively. A two-stage ethylene regulator kept a constant flow of ethylene through tightly sealed plastic buckets that vented to the outdoors to prevent ethylene build up. Ethylene concentration was regularly checked by sampling from sealed buckets, and analysed with a gas chromatograph (Varian, GC 3300). Control fruit was held at 20°C for 24 hours in an ethylene free room under normal atmospheric conditions. Thereafter all fruit was stored at –0.5°C for 12 weeks at normal atmospheric conditions (90% RH).

Every two weeks over a period of 12 weeks, two samples of every treatment were removed from cold storage. Maturity tests were immediately conducted on one sample, to determine the

maturity of fruit kept in cold storage, while the other sample was held for a further seven days at 20°C to simulate the marketing period. Peel ground colour was evaluated with a Unifruco colour chart (0.5 = dark green through to 5 = deep yellow). A critical value of 3.5 on the Unifruco colour chart is accepted as marketable for 'Forelle' pears. In addition, colour measurements were taken from the non-blushed side with a Nippon Denshoku colorimeter (Handy colorimeter, HR-3000). Colour is reported as a set of L*, C* and H° values. The L* value refers to the lightness of the colour on a scale of 0 to 100 (0 = black through to 100 = white). The C* value refers to the chroma or intensity of colour, with a higher value referring to a colour of greater intensity. The H° value refers to the hue angle on a scale of 0° = red/purple, 90° = yellow, 180° = blue/green and 270° = blue. (McGuire, 1992). Flesh firmness (kg) was tested with a penetrometer (Southtrade fruit pressure tester, mod. FT 327) with an 8 mm tip. Two measurements were taken from each fruit, at opposite sides, after a piece of skin (roughly 2 cm in diameter) was removed. The local standard of eating pressure for 'Forelle' pears are set at 4,5 kg while it is internationally set between 4,1 and 3,6 kg (Crouch, 2000). Total soluble solids (TSS, %) were measured by a handheld refractrometer (Brix % Palette PR-100 (0-32%) Atago refractometer). Titratable acidity (TA; %) was calculated as % Malic acid, by titrating juice from liquidised segments, taken from groups of 5 made from the 20 fruit in each sample, with 0.1 NaOH to a pH of 8.2. Data was analysed using the SAS System (SAS Institute Inc., Cary, North Carolina, USA). The trial consisted of a factorial design: 3 harvesting maturities x 4 treatments each with 13 samples of 20 fruit.

RESULTS

PRE-OPTIMUM HARVEST

Ground colour

Significance levels indicate strong interaction in ground colour between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 1), and when held at 20°C for seven days (Figure 2). Pre-optimally harvested 'Forelle' pears treated with exogenous ethylene attained a yellow ground colour significantly earlier than untreated fruit. Fruit that attained the critical value of 3.5 and higher have reached the proper ground colour to be marketed and consumed (De Vries & Moelich, 1995). A significant difference, at the 5% level, was found between the control and ethylene treatments for fruit immediately out of cold storage and for fruit held for seven days at 20°C (Figure 1 and 2). The longer ethylene treatments (24 and 36 h) had a more pronounced

yellowing effect on ground colour during cold storage, and when fruit was held for seven days at 20°C, than only 12 h of ethylene. While the 24 h and 36 h treatments started to differ significantly from the control after 6 weeks of cold storage, the 12 h treatment only showed a significant difference (P ≤ 0.05) after 8 weeks. This was also evident for fruit taken out of cold storage and held for seven days at 20°C (Figure 2). Here the 24 h and 36 h ethylene treatments attained the critical value of 3.5 after 8 weeks of cold storage, while the 12 h treatment and control only reached the same value after 12 weeks. This indicates a shortening of the prerequisite cold storage period of 12 to 8 weeks when pre-optimally harvested 'Forelle' pears were treated with ethylene for periods longer than 24 h.

Figure 1: Peel ground colour of pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days. The line at 3.5 represents the critical minimum value for marketing and/or consumption.

Figure 2: Peel ground colour of pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. The line at 3.5 represents the critical minimum value for marketing and/or consumption. R^2(Control) = 0.998; R^2(12h) = 0.9755; R^2(24h) = 0.9851; R^2(36h) = 0.9672.

Fruit colour

Fruit colour measured objectively by the colorimeter supported the results obtained using the colour chart. Significance levels indicate strong interactions in L* values between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 3), and after seven days at 20°C (Figure 4). The change in L* values shows a distinct trend towards lighter fruit. Treatment of pre-optimally harvested 'Forelle' pears with ethylene, 24 hours and longer had a significant effect on the lightness of fruit (Figure 3). Surprisingly, fruit treated with 24 h ethylene and stored at –0.5°C for 6 weeks were significantly lighter in colour than fruit treated for 0, 12 or 36 h. Throughout the storage period control fruit had the lowest L* values, indicating a darker (green) colour. When fruit was held for seven days at 20°C (Figure 4) both 24 and 36 h ethylene treated fruit were significantly (P ≤ 0.05) lighter in colour than 12 h and control fruit after 6 weeks of cold storage. The 1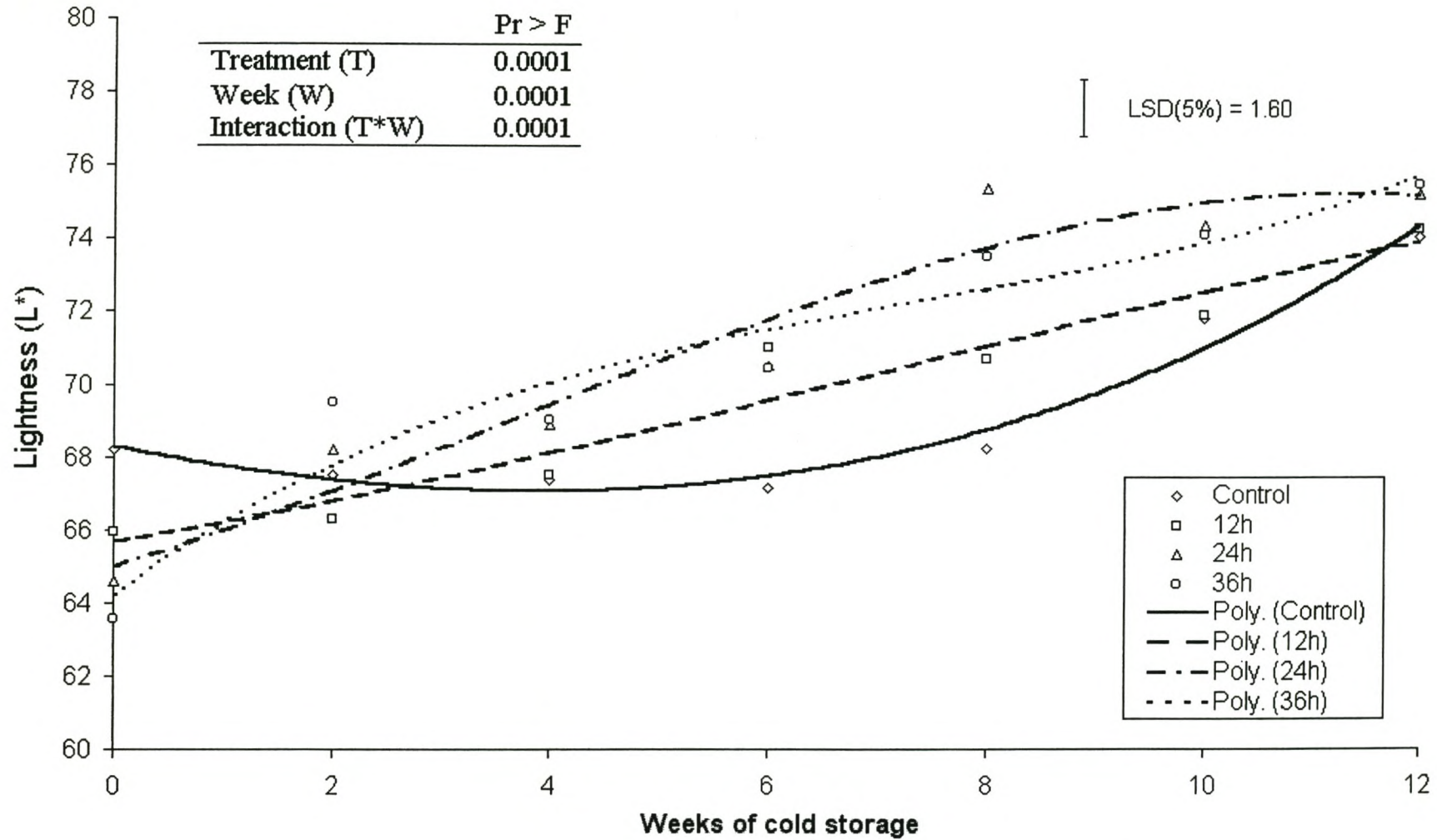2 h ethylene treatment did not have the same pronounced effect as the 24 and 36 h treatments. Control fruit after being held at 20°C for seven days still had the lowest L* value, indicating a darker (green) colour.

Although C* is an important component of colour, it is of lesser importance than L* or H° in establishing maturity. There were strong interactions in chroma between treatments and weeks for pre-optimally harvested pears in cold storage (Figure 5), and fruit held for seven days at 20°C (Figure 6). Although no clear trend can be seen in fruit in cold storage (Figure 5) it can be seen that 24 and 36 h ethylene treated fruit had significantly lower C* values than 12 h and control fruit after 6 weeks of cold storage and seven days of 20°C (Figure 6).

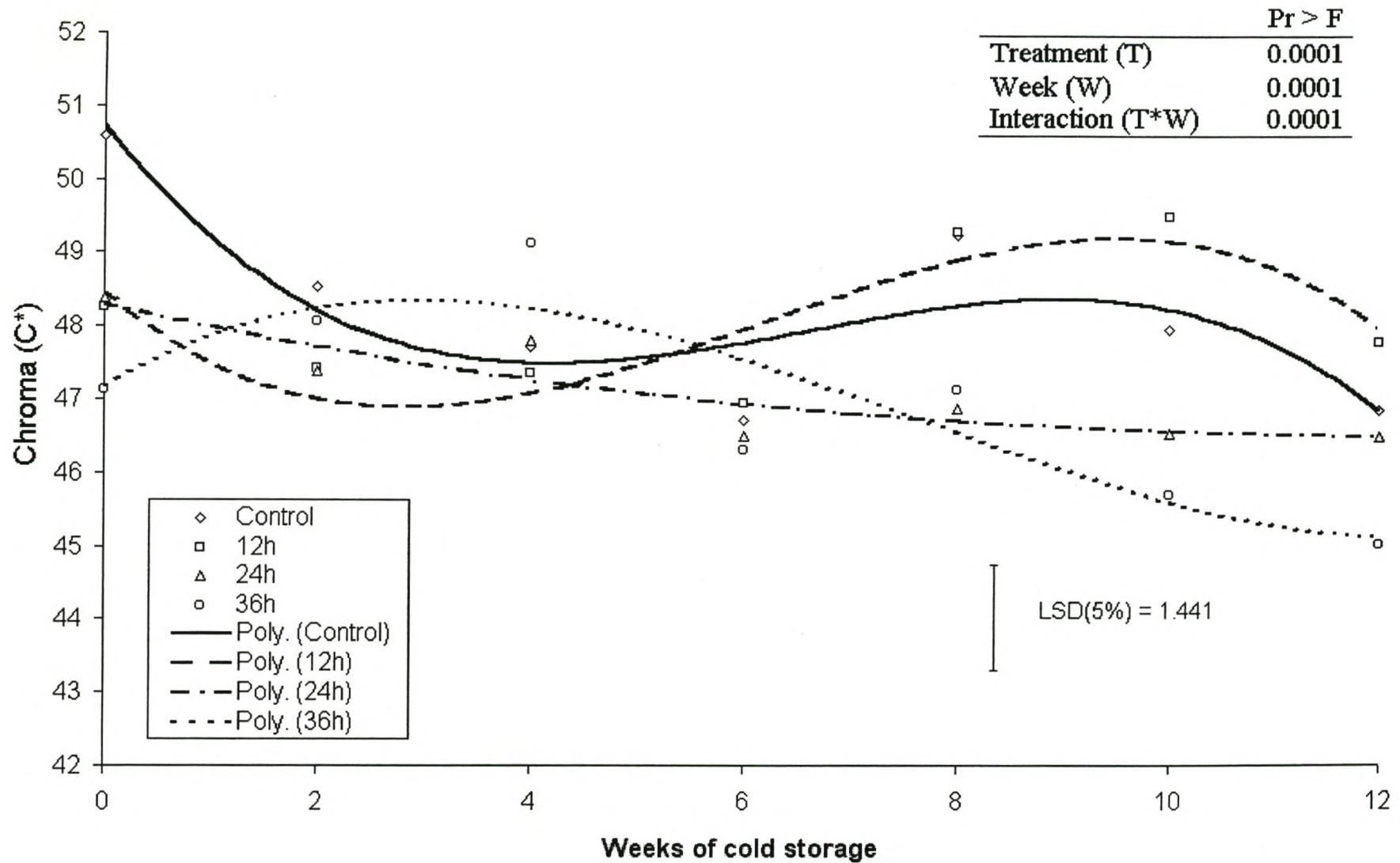As the hue angle drops fruit change to a more yellow colour, which indicates ripening. There were strong interactions in hue angle for pre-optimally harvested fruit between treatments and weeks for fruit in cold storage (Figure 7), and fruit held at 20°C for seven days (Figure 8). Treatment of 'Forelle' pears with ethylene had a significant yellowing effect (Figure 7 and 8) with 24 and 36 h ethylene treatments having a more pronounced effect than the 12 h treatment. It can be seen that the hue angle for the control remained higher than the ethylene treated fruit in cold storage, although not always significantly higher. It was interesting to note a 'greening' effect of taking pre-optimally harvested 'Forelle' pears from cold storage to 20°C. 'Forelle' pears held for seven days at 20°C had higher hue angles (greener) than fruit in cold storage. The reason for this is unclear and is possibly because fruit were picked before attaining maturity.

Although there was a 'greening' effect, fruit were still more yellow after seven days at 20°C than control fruit (Figure 8). Treatment with ethylene for 24 or 36 h had a more pronounced yellowing effect than 12 h. No significant difference could be seen between the 24 and 36 h treatments at any stage. The significantly early fall in hue angle of 24 and 36 h treatments held for seven days at 20°C began after 8 weeks of cold storage. After 12 weeks the effect of cold storage resulted in a drop in hue angle of control fruit.

Figure 3: Lightness (L*) of peel in pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
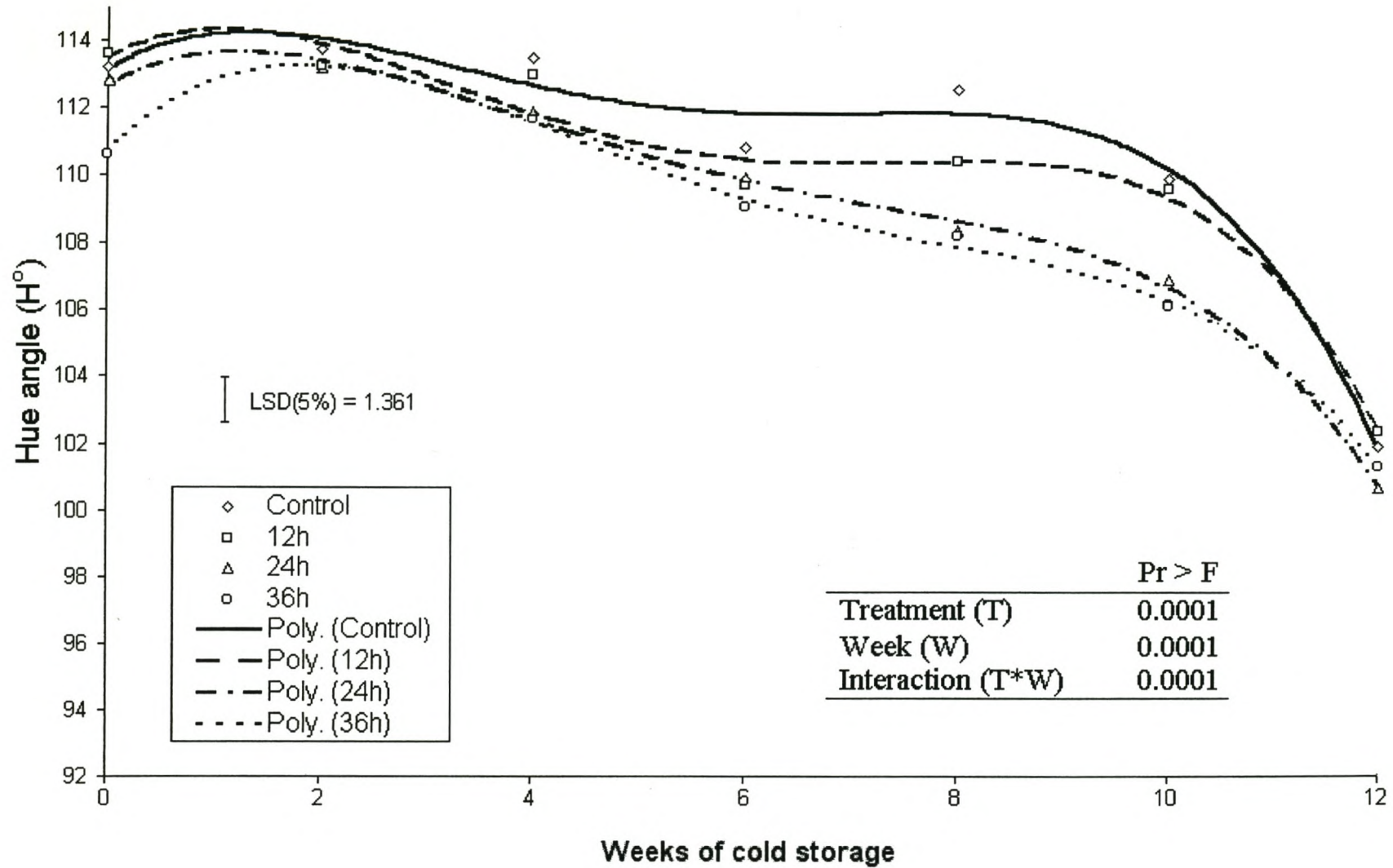
Figure 4: Lightness (L*) of peel in pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9706; R^2(12h) = 0.9427; R^2(24h) = 0.9372; R^2(36h) = 0.9336

Figure 5: Chroma (C*) of peel in pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

	Pr > F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

Legend:
- ◇ Control
- □ 12h
- △ 24h
- ○ 36h
- —— Poly. (Control)
- – – Poly. (12h)
- –·– Poly. (24h)
- ···· Poly. (36h)

LSD(5%) = 1.441

Figure 6: Chroma (C*) of peel in pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.7948; R^2(12h) = 0.7226; R^2(24h) = 0.8059; R^2(36h) = 0.7656.
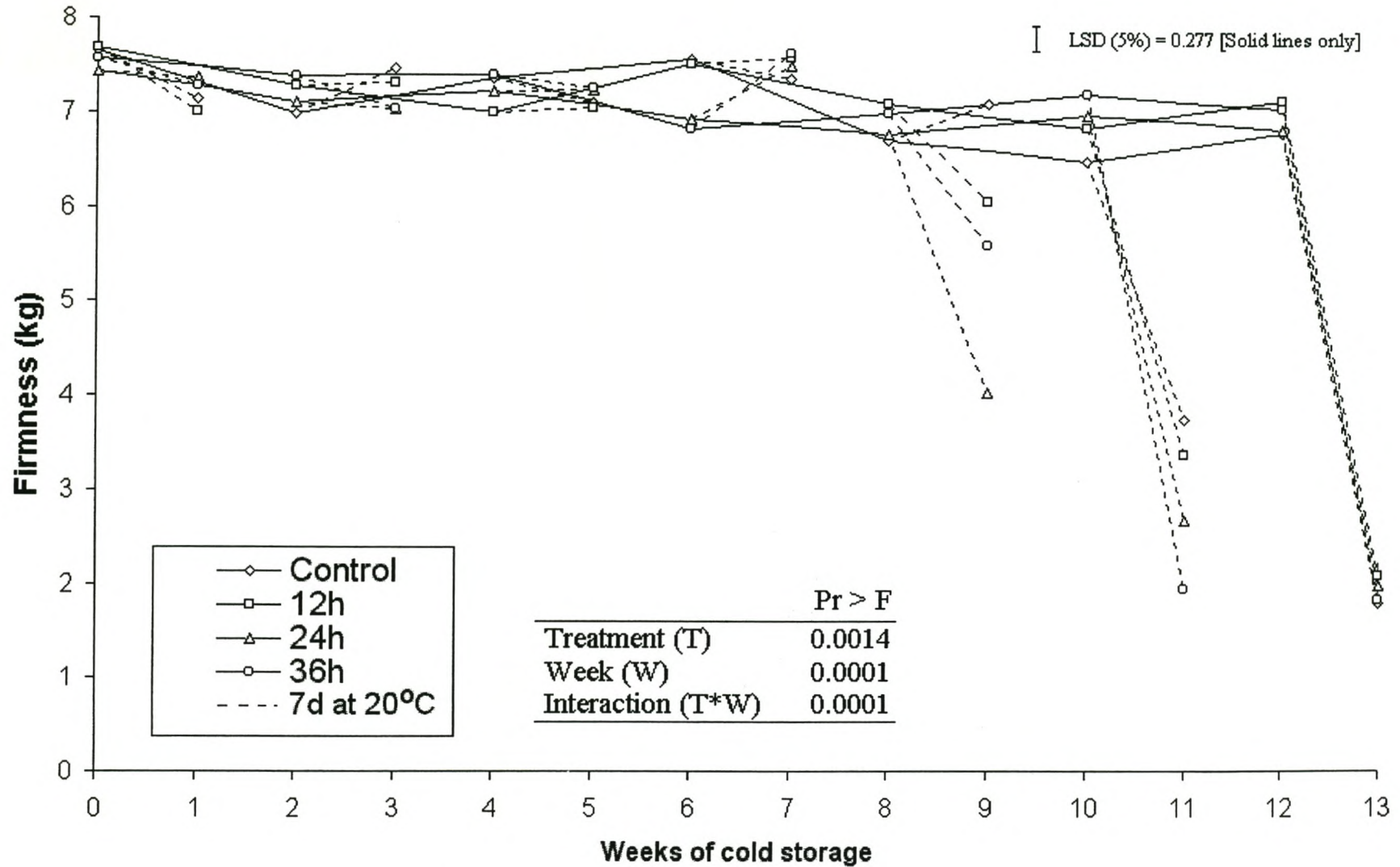
Figure 7: Hue angle (H°) of peel in pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
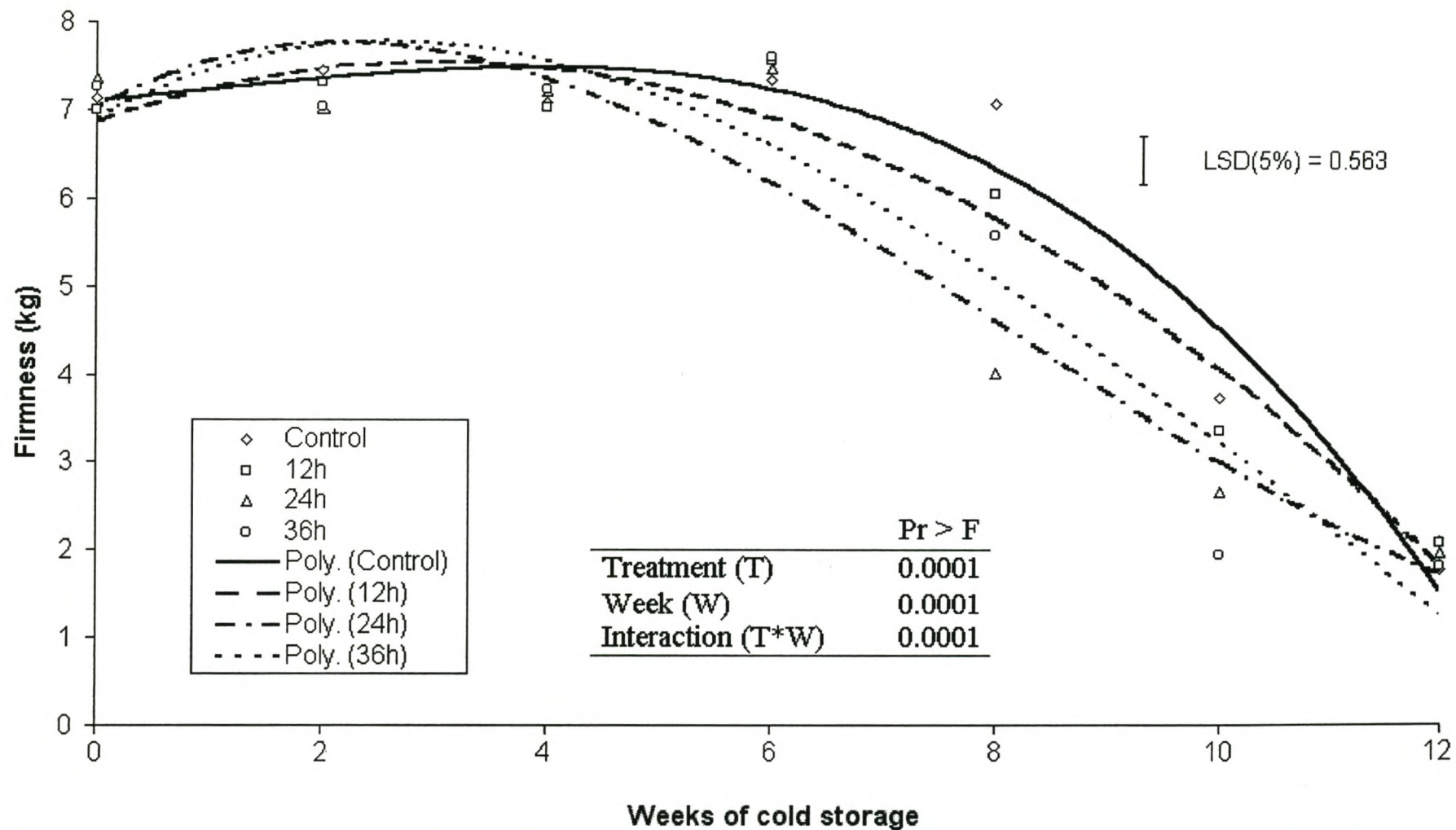
Figure 8: Hue angle (H°) of peel in pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9777; R^2(12h) = 0.9731; R^2(24h) = 0.9978; R^2(36h) = 0.9975.

Flesh firmness

Significance levels indicate strong interactions in firmness between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 9), and after seven days at 20°C (Figure 10). Pre-optimally harvested 'Forelle' pears held in cold storage were not adversely affected by ethylene. The ethylene treated fruit were, at some stages, even firmer than control fruit (Figure 9). Ethylene treated fruit can thus be stored at low temperatures equally as long as untreated fruit without danger of a loss in firmness. Ethylene treated fruit softened faster than control fruit when fruit was taken out of cold storage and held for seven days at 20°C (Figure 10). This was most evident after 8 weeks of cold storage, with the 24 h ethylene treatment having the significantly greatest drop. The decrease in firmness between the 12 h ethylene treatment and the control did not differ significantly. The 24 and 36 h treatments were significantly softer ($P \leq 0.05$) than the control. After 10 weeks the effect of cold storage resulted in a softening of control fruit but all treatments were still above the minimum export standard of 5.5 kg. My personal observation was that minimal wooliness occurred and could not be correlated with pre-optimally harvested fruit treated with ethylene.

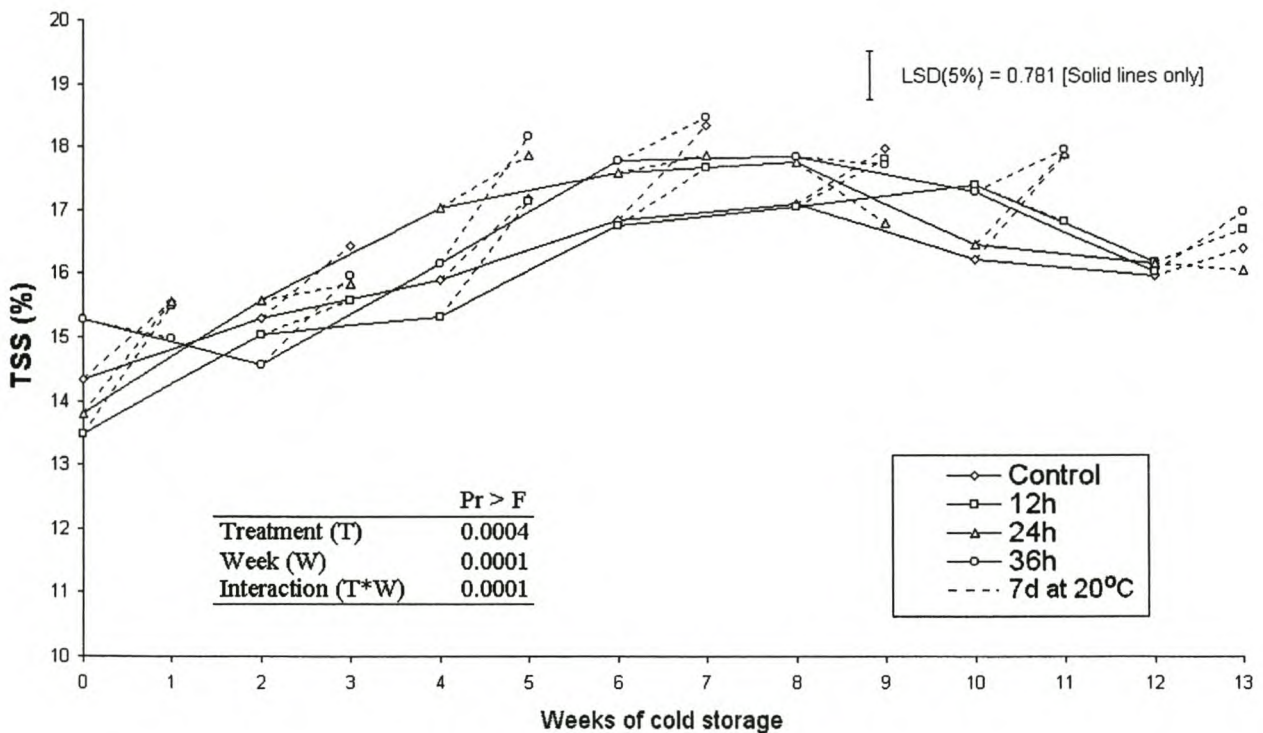Figure 9: Firmness (kg) of pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
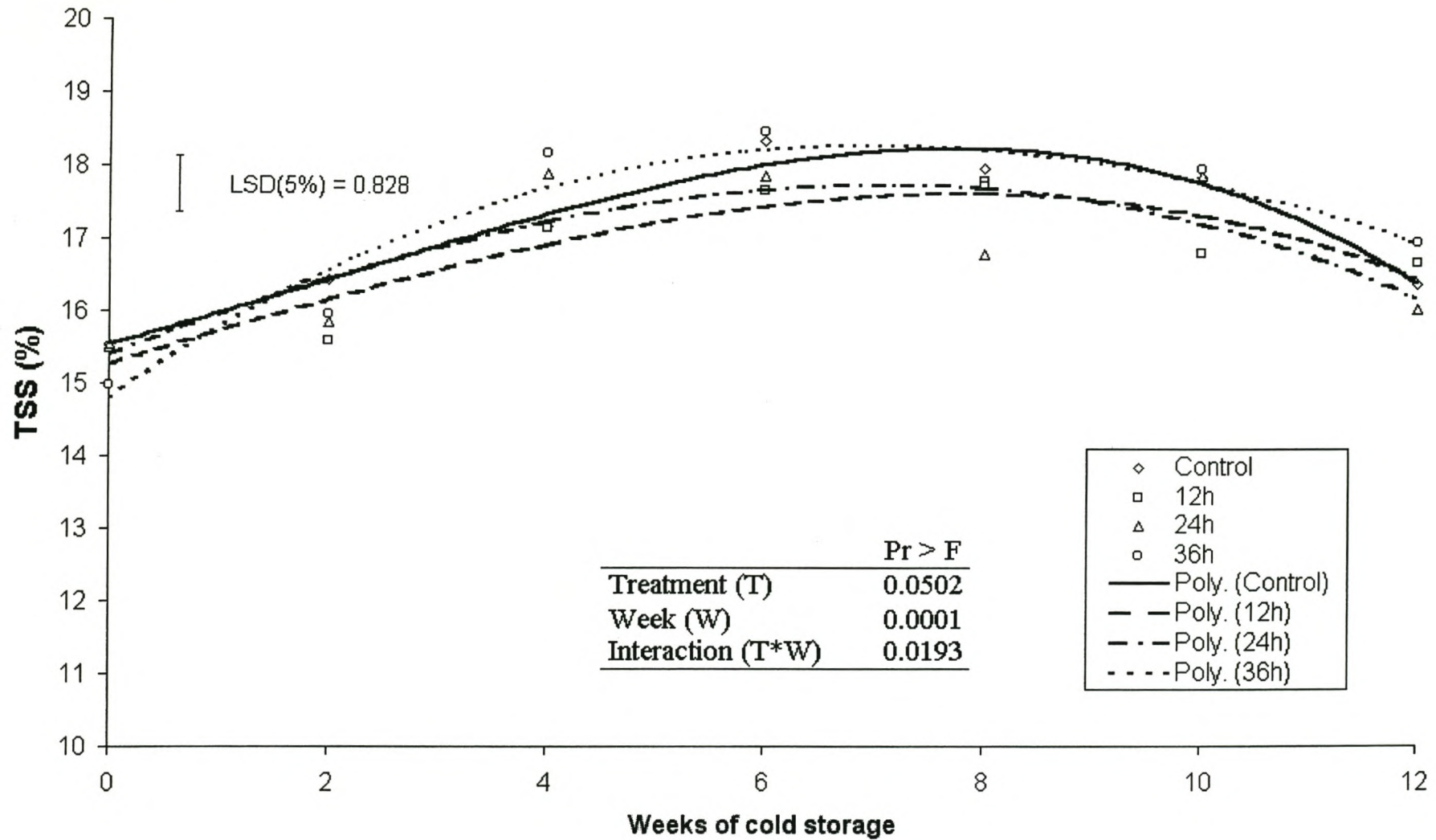
Figure 10: Firmness (kg) of pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9535; R^2(12h) = 0.9532; R^2(24h) = 0.9192; R^2(36h) = 0.8985.

Total soluble solids (TSS) and titrable acidity (TA)

Significance levels indicate strong interactions in TSS between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 11), and stored for seven days at 20°C (Figure 12). Fruit treated with ethylene for 24 and 36 h and stored at –0.5°C had a higher TSS than 12 h and control from week 4 until week 10, but this difference was not significant. When fruit were held for seven days at 20°C after periods of cold storage no differences between treatments could be found (Figure 12).

Strong interactions existed in TA between ethylene treatments and weeks for fruit in cold storage (Figure 13), and after seven days at 20°C (Figure 14). Although significant differences existed in TA of pre-optimally harvested fruit in cold storage (Figure 13), no clear trend could be found. No differences between treatments could be seen when fruit was held at 20°C for seven days (Figure 14).
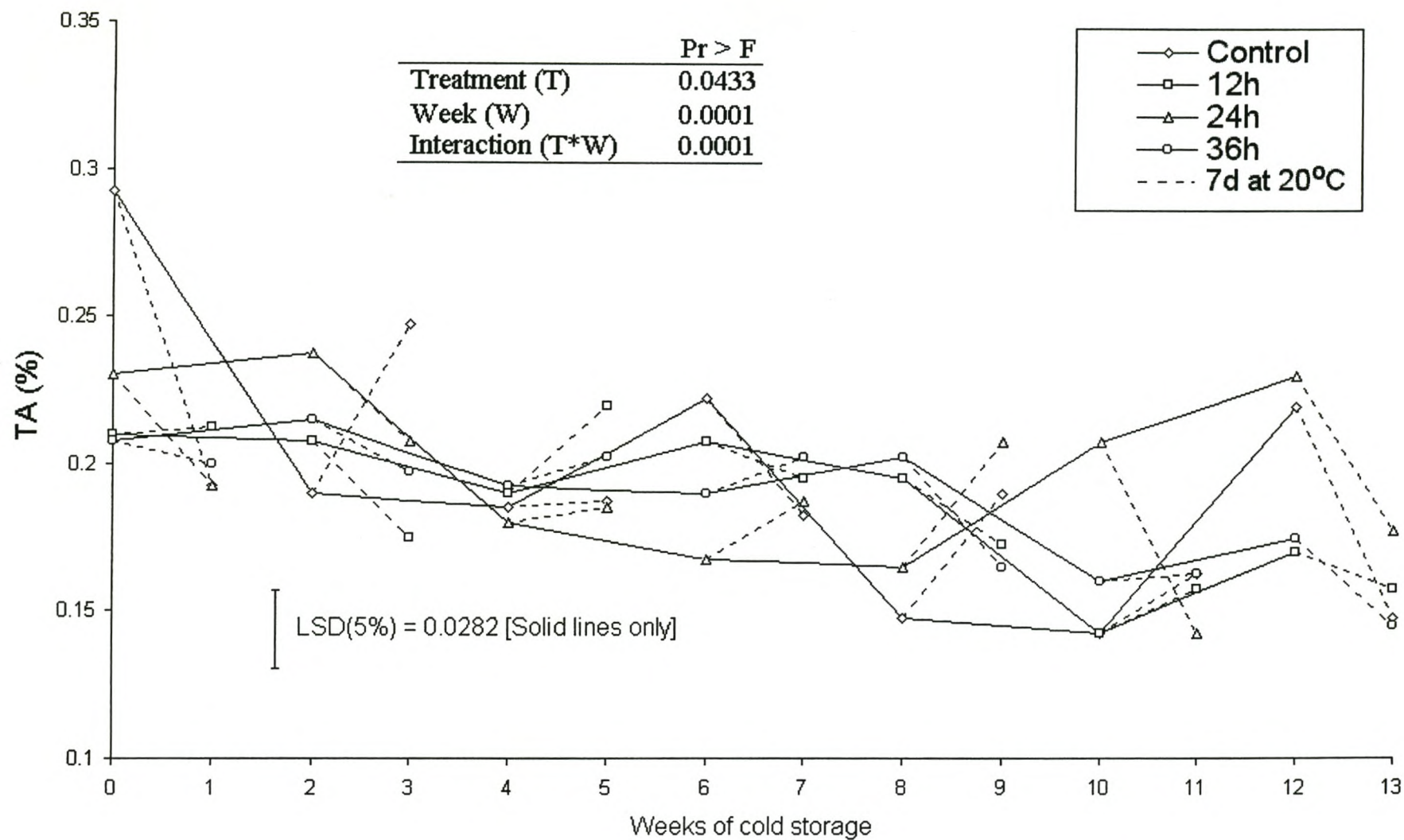

Figure 11: TSS (%) of pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
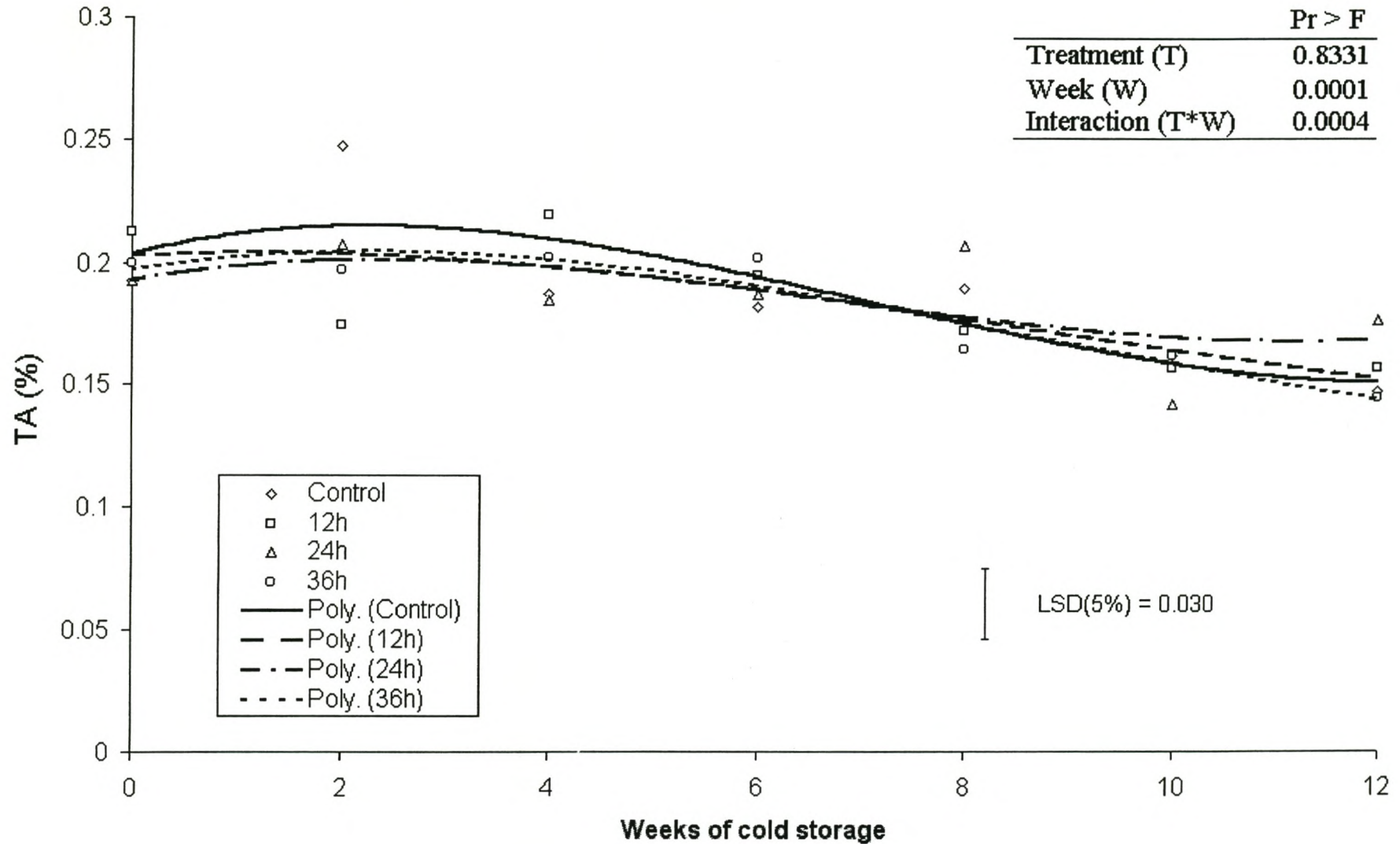
Figure 12: TSS (%) of pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9668; R^2(12h) = 0.8391; R^2(24h) = 0.6753; R^2(36h) = 0.8998.

Figure 13: TA (%) of pre-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

Figure 14: TA (%) of pre-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.6491; R^2(12h) = 0.6089; R^2(24h) = 0.3504; R^2(36h) = 0.9067.

OPTIMUM HARVEST

Ground colour

Significance levels indicate strong interactions in ground colour between ethylene treatments and weeks for optimally harvested fruit in cold storage (Figure 15), and after seven days at 20°C (Figure 16). The control and 12 h ethylene treatment differed significantly, at the 5% level, from the 24 and 36 h ethylene treatments for fruit immediately out of cold storage (Figure 15). This could also be seen when fruit was held for seven days at 20°C (Figure 16), with the only difference being that in this case the 12 h treatment differed from the control as well. The longer ethylene treatments (24 and 36 h) had a more pronounced yellowing effect on ground colour in fruit held in cold storage, and fruit held for seven days at 20°C, than the 12 h ethylene treatment. This was clearly seen when fruit was taken out of cold storage and held for seven days at 20°C (Figure 16). Here, 24 and 36 h ethylene treatments attained the critical value of 3.5 after 4 weeks of cold storage, while the 12 h treatment and control only reached the same value after 8 weeks. This indicates a shortening of the prerequisite cold storage to 4 weeks when optimally harvested 'Forelle' pears are treated with ethylene for periods of 24 h and longer.
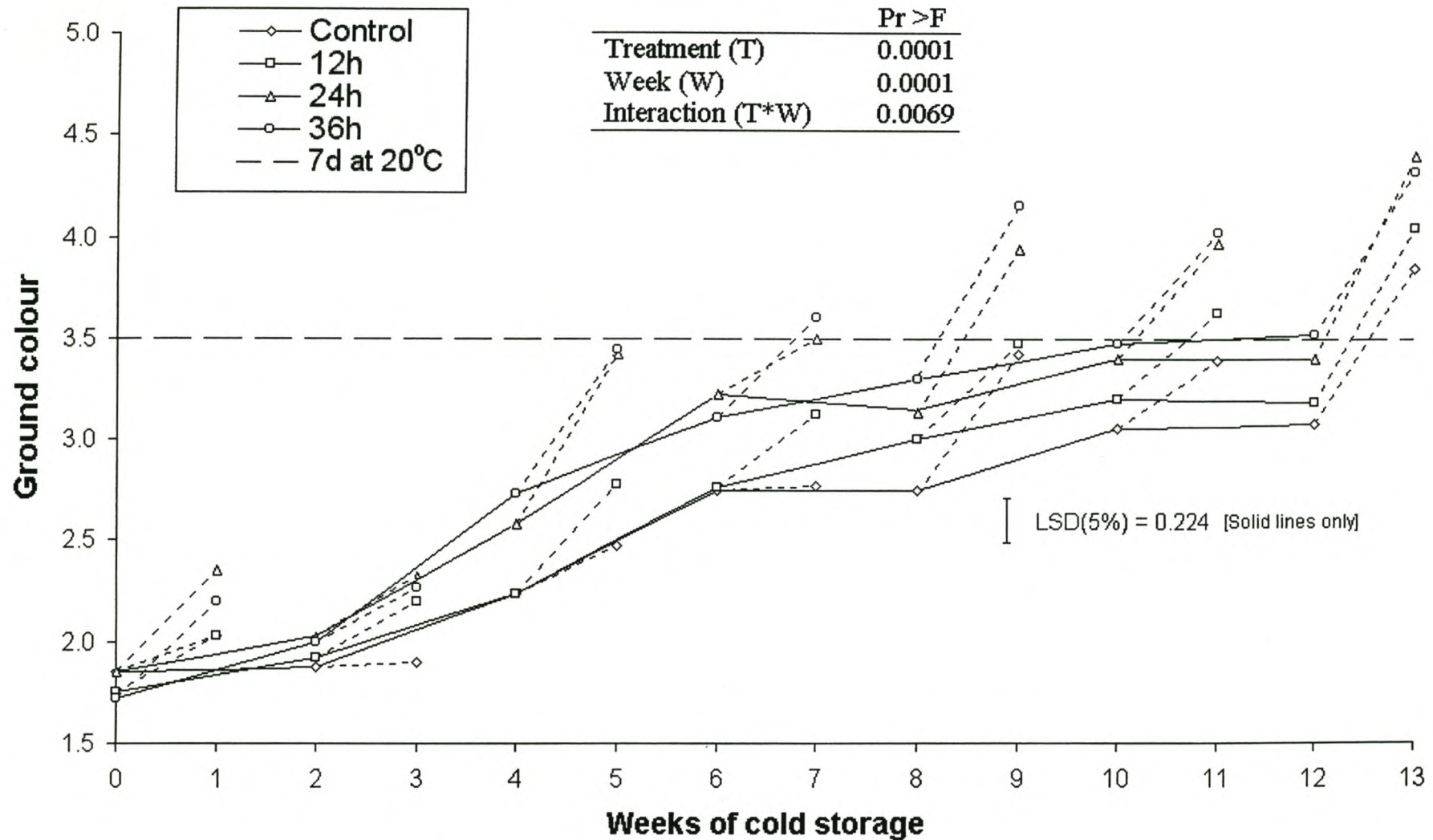
Figure 15: Peel ground colour of optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days. The line at 3.5 represents the critical minimum value for marketing and/or consumption.
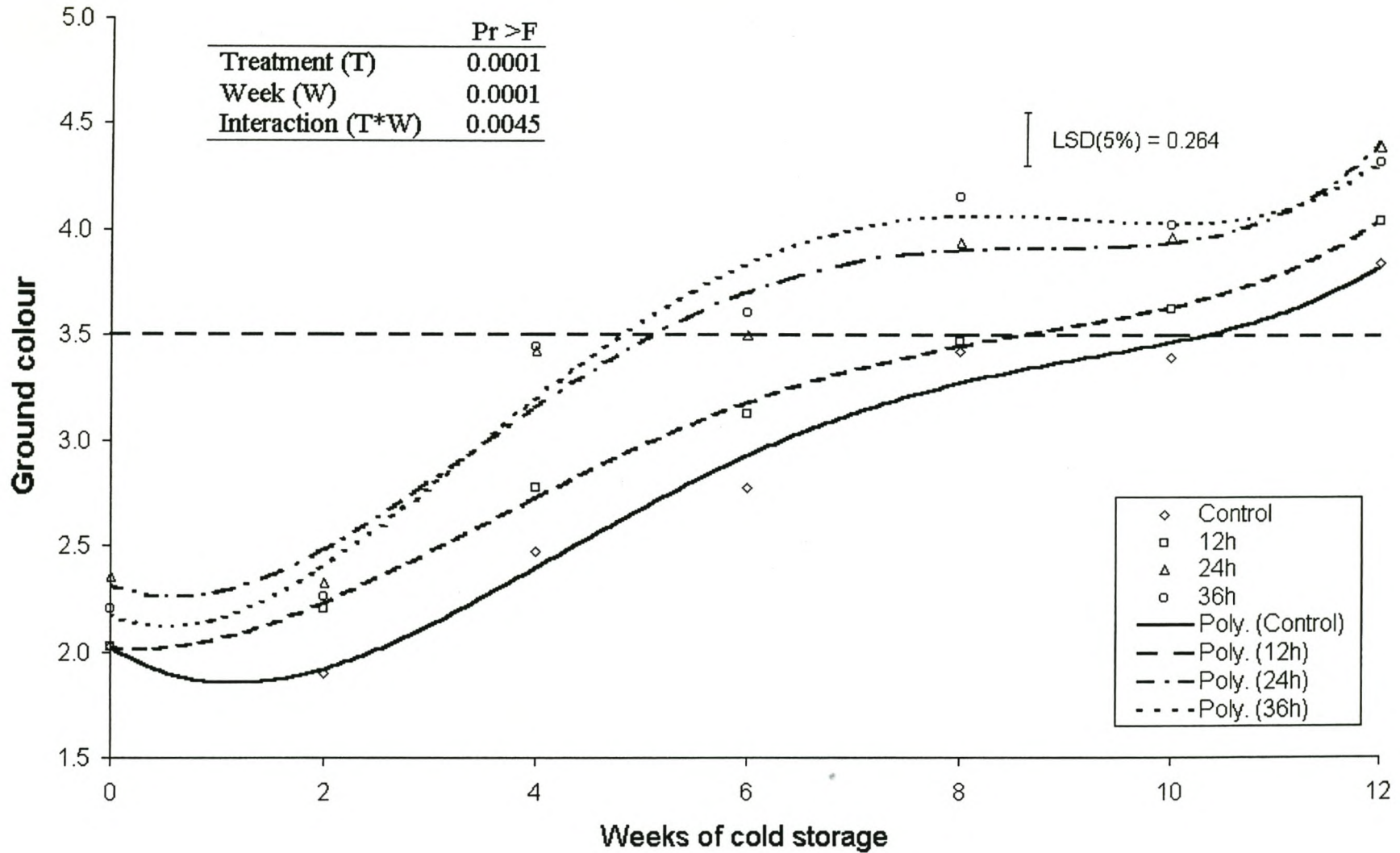
Figure 16: Peel ground colour of optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. The line at 3.5 represents the critical minimum value for marketing and/or consumption. R^2(Control) = 0.9829; R^2(12h) = 0.9980; R^2(24h) = 0.9639; R^2(36h) = 0.9682.

Fruit colour

Fruit colour measured objectively by the colorimeter support the results obtained using the colour chart. Significance levels indicate strong interactions in L* values between ethylene treatments and weeks for fruit in cold storage (Figure 17), and after seven days at 20°C (Figure 18). Treatment of optimally harvested 'Forelle' pears with ethylene had a significant effect on the lightness of fruit (Figure 17). Control fruit had, throughout the storage period, the lowest L* values, indicating a darker (green) colour. Control fruit after being held for seven days at 20°C still had the lowest L* value, indicating a darker (green) colour.

There were strong interactions in chroma between treatments and weeks for optimally harvested pears in cold storage (Figure 19), and when fruit was held for seven days at 20°C (Figure 20). Although no clear trend can be seen in fruit in cold storage (Figure 19), 24 and 36 h ethylene treated fruit had significantly lower C* values than 12 h and control fruit after 6 weeks of cold storage and seven days of 20°C (Figure 20). This is in accordance with results obtained with pre-optimally harvested fruit.

Hue angle for pre-optimally harvested fruit showed strong interactions between treatments and weeks for fruit in cold storage (Figure 21), and fruit held for seven days at 20°C (Figure 22). Treatment of optimally harvested 'Forelle' pears with ethylene had a significant yellowing effect (Figure 21 and 22), with 24 and 36 h ethylene treatments having significantly lower hue angles than the 12 h treatment and control fruit, when fruit was held for seven days at 20°C. No significant differences could be found between control fruit and 12 h ethylene treated fruit, and between the 24 and 36 h ethylene treated fruit. Interestingly the pronounced 'greening' effect found in pre-optimally harvested fruit had decreased considerably in the optimally harvested fruit. The significant decrease in hue angle of 24 and 36 h treated fruit held for seven days at 20°C began after 6 weeks of cold storage. This was 2 weeks earlier than in pre-optimally harvested fruit. After 10 weeks the effect of cold storage resulted in a drop in hue angle of control fruit.
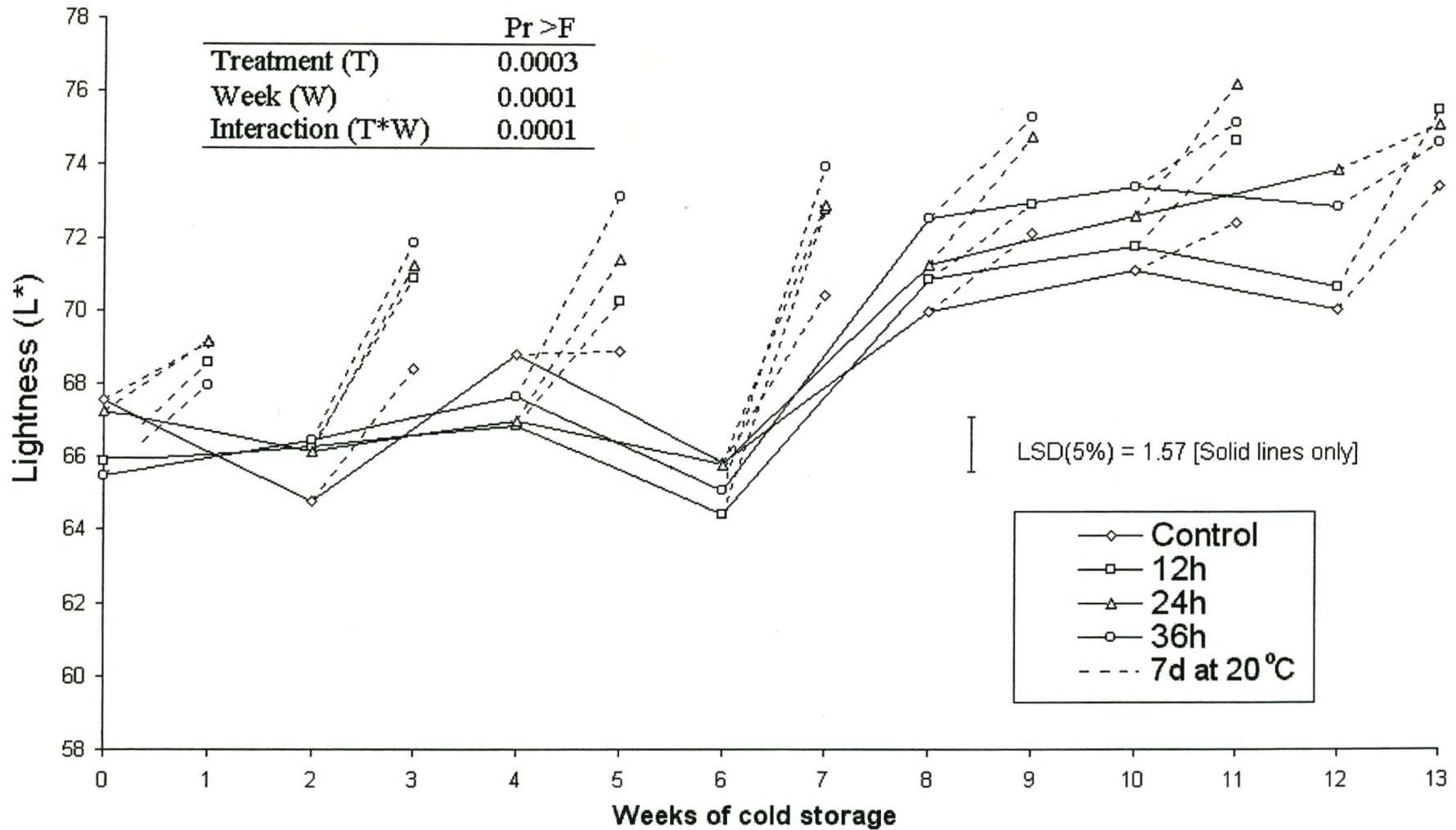
Figure 17: Lightness (L*) of peel in optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
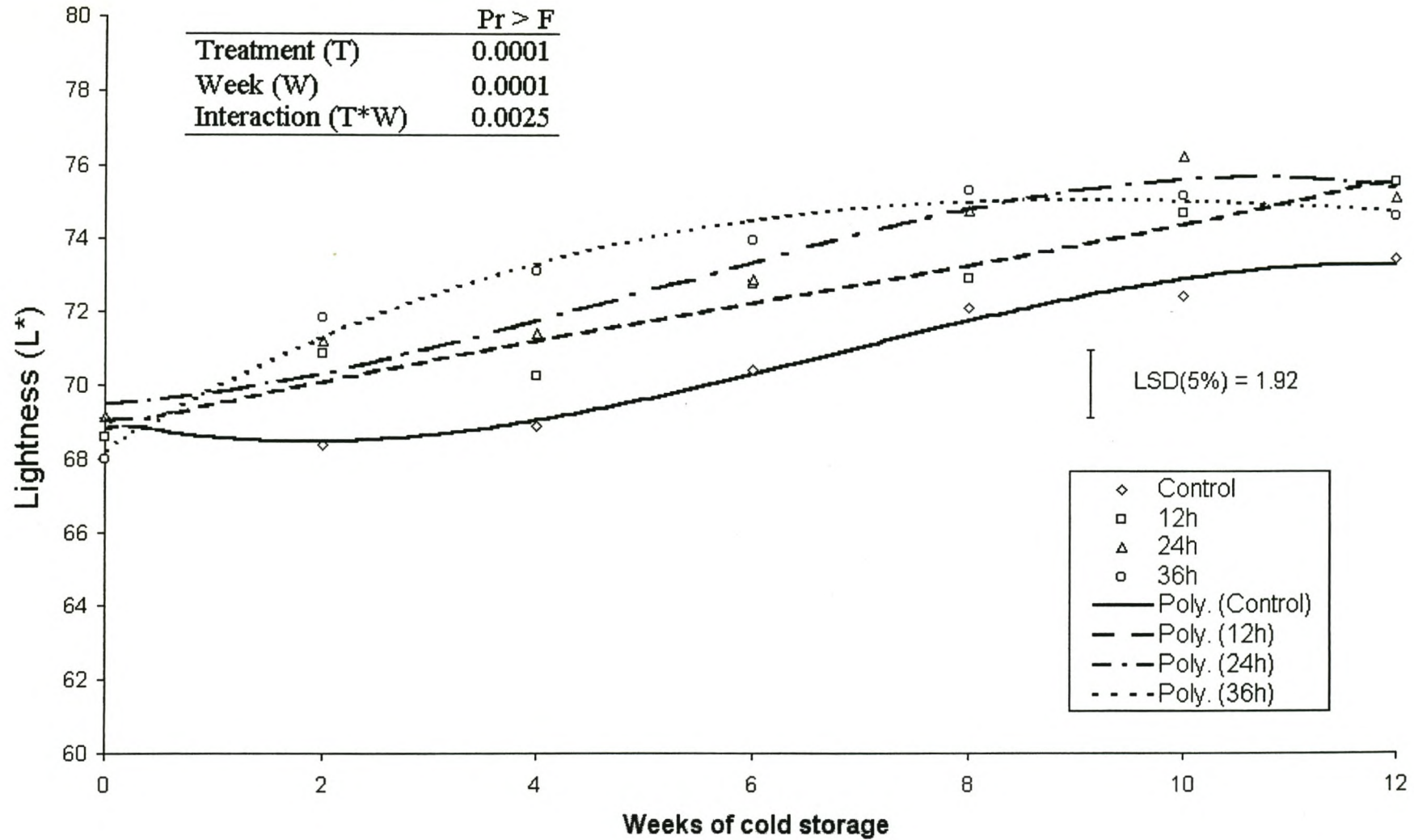
Figure 18: Lightness (L*) of peel in optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9233; R^2(12h) = 0.9429; R^2(24h) = 0.9309; R^2(36h) = 0.9736.
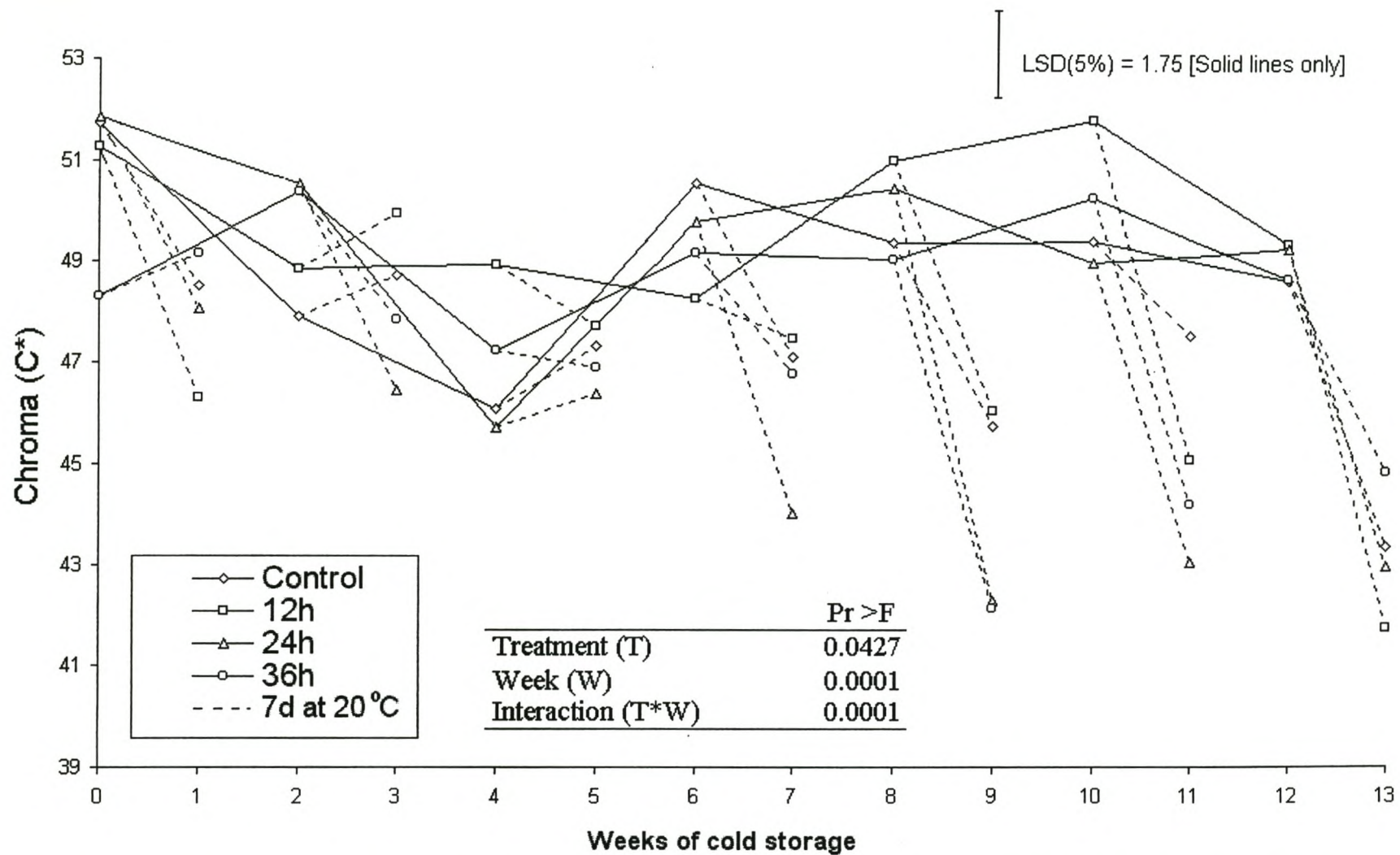
Figure 19: Chroma (C*) of peel in optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
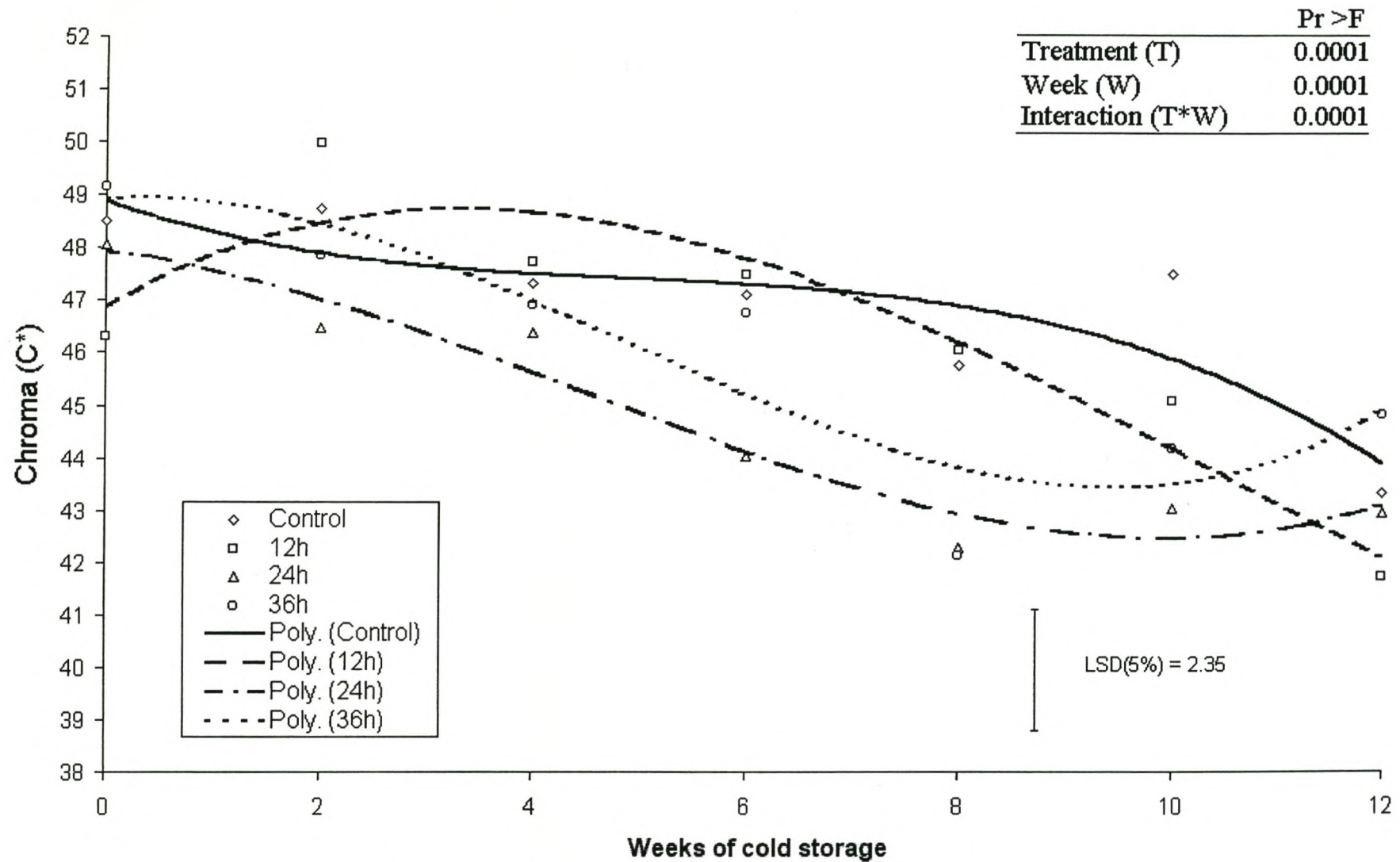
	Pr >F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

Legend:
◇ Control
□ 12h
△ 24h
○ 36h
—— Poly. (Control)
— — Poly. (12h)
— · — Poly. (24h)
· · · · Poly. (36h)

LSD(5%) = 2.35

Y-axis: Chroma (C*)
X-axis: Weeks of cold storage

Figure 20: Chroma (C*) of peel in optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.7478; R^2(12h) = 0.8860; R^2(24h) = 0.9419; R^2(36h) = 0.8204.
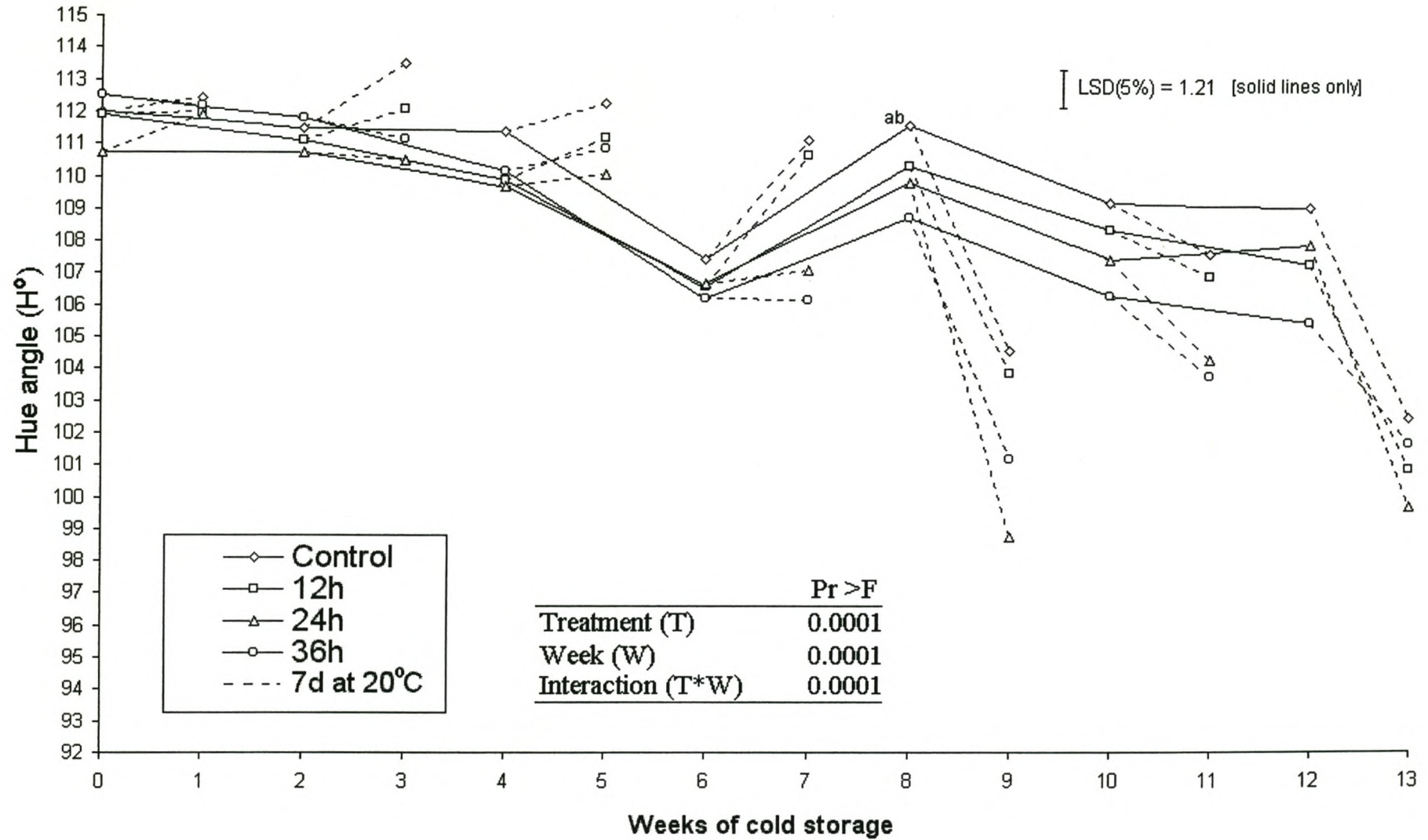
Figure 21: Hue angle (H°) of peel in optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
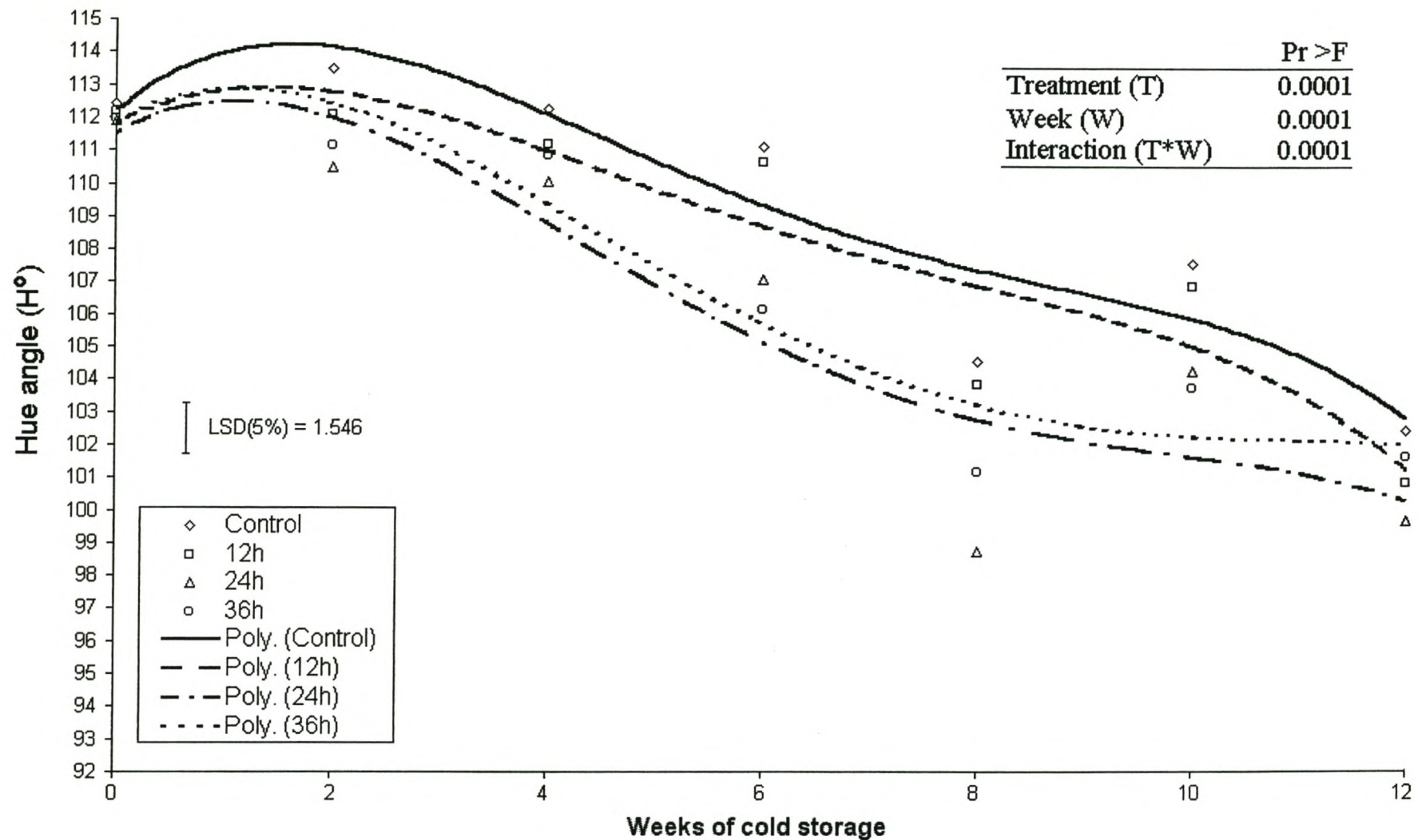
Figure 22: Hue angle (H°) of peel in optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R²(Control) = 0.8668; R²(12h) = 0.8541; R²(24h) = 0.8137; R²(36h) = 0.9191.

Flesh firmness

Significance levels indicate strong interactions in firmness between ethylene treatments and weeks for fruit in cold storage (Figure 23), and for fruit stored for seven days at 20°C (Figure 24). Optimally harvested 'Forelle' pears held in cold storage were not adversely affected by ethylene. This can be seen in that the ethylene treated fruit were at some stages, even firmer than control fruit (Figure 23). Ethylene treated 'Forelle' pears that are picked at optimal maturity, can thus be stored at –0.5°C equally as long as untreated fruit without danger of a loss in firmness. Ethylene treated fruit, however, softened faster than control fruit when fruit were taken out of cold storage and held for seven days at 20°C (Figure 23 and 24). The major decrease in firmness began after 4 weeks of cold storage, with the 24 h ethylene treatment having significantly the greatest drop, followed by the 36 h treatment 2 weeks later. After 8 weeks, cold storage began to have an effect and control fruit softened equally as much as ethylene treated fruit. My personal observation was that the minimal wooliness that occurred could not be correlated with optimally harvested fruit treated with ethylene.
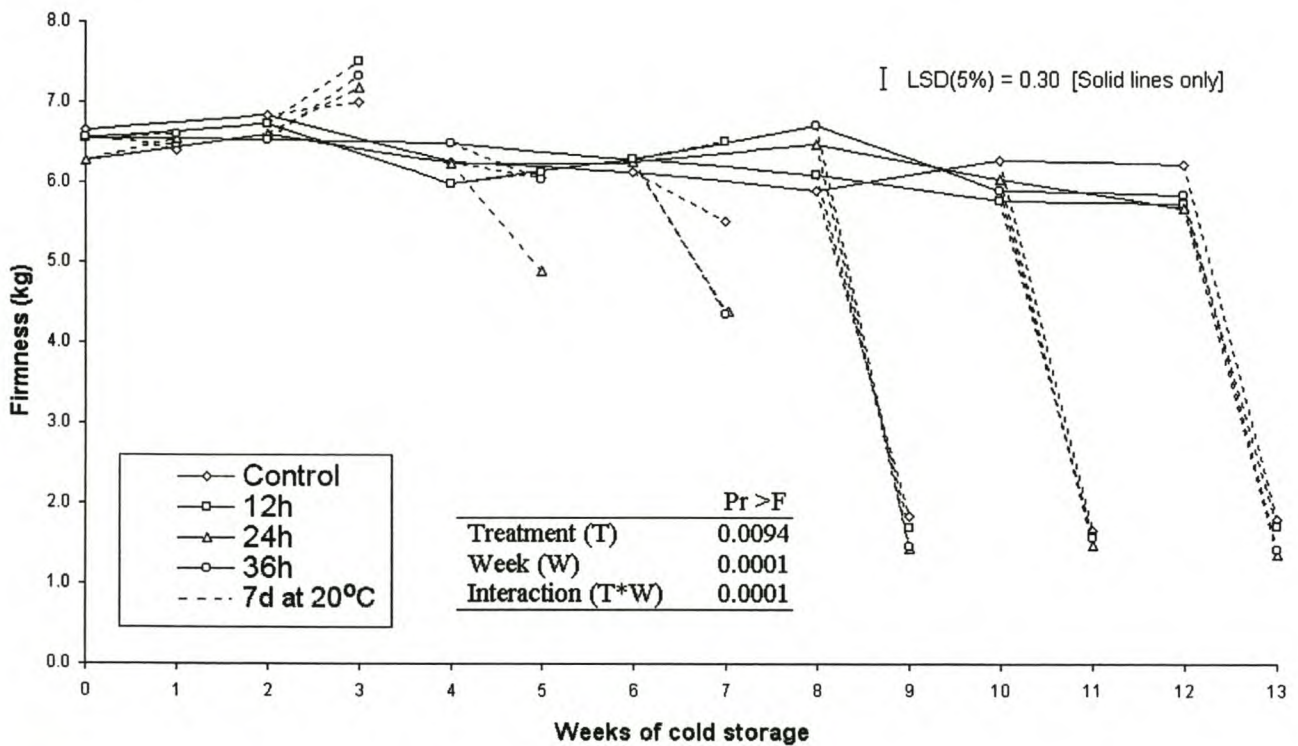

Figure 23: Firmness (kg) of optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
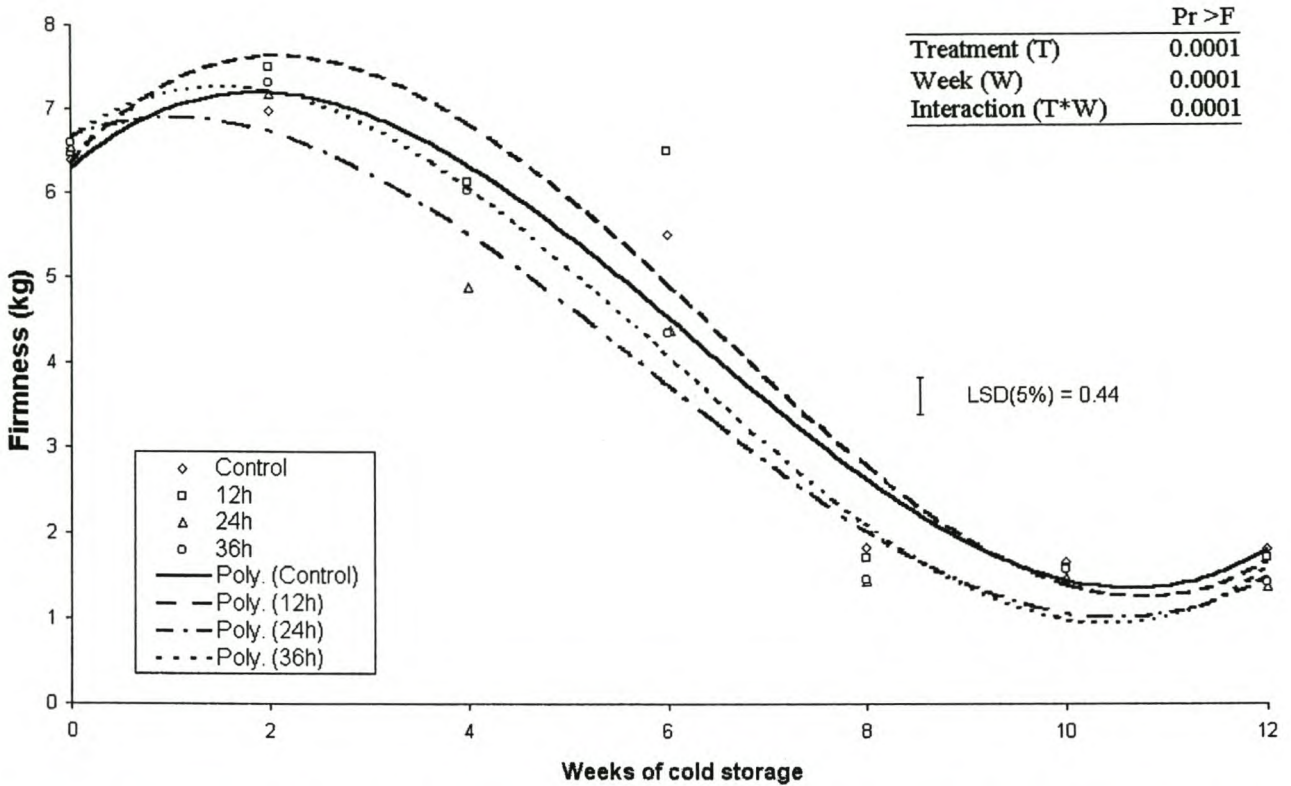
	Pr >F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

Figure 24: Firmness (kg) of optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9502; R^2(12h) = 0.9022; R^2(24h) = 0.9585; R^2(36h) = 0.9790.

Total soluble solids (TSS) and titrable acidity (TA)

Significance levels indicate strong interactions in TSS between ethylene treatments and cold storage periods (weeks) for fruit in cold storage (Figure 25), and after seven days at 20°C (Figure 26). Control fruit, with the exception of week 6, had significantly lower TSS content than ethylene treated fruit over the 12 week cold storage period (Figure 25). When fruit was held for seven days at 20°C after periods of cold storage (Figure 26) it was found that 12 and 36 h treatments had significantly higher TSS content than the 24 h treatment and control.

Although significant differences existed, as well as strong interactions between treatments and weeks, in TA of fruit in cold storage (Figure 27), and after seven days at 20°C (Figure 28), no clear trends could be identified.
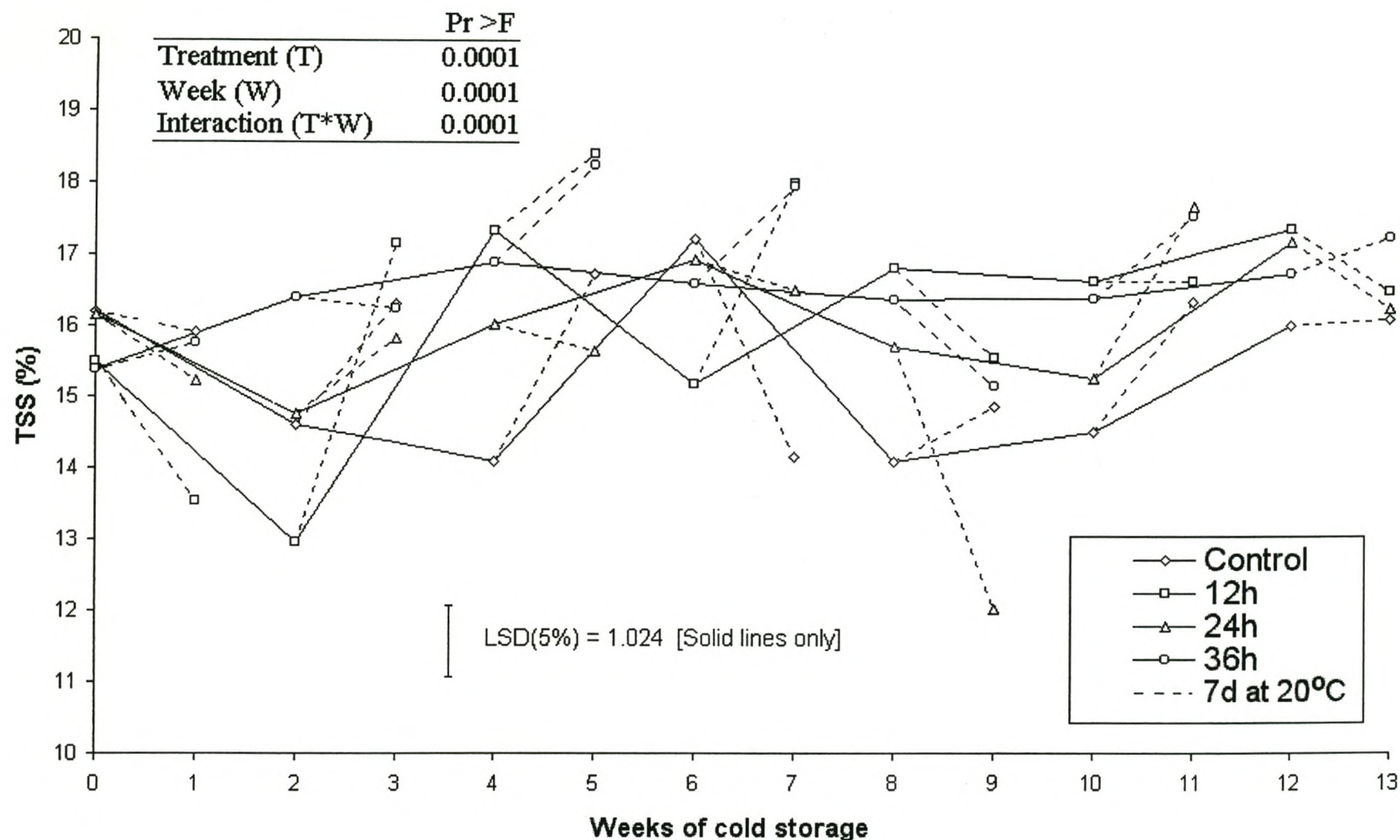
Figure 25: TSS (%) of optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

Figure 26: TSS (%) of optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.6479; R^2(12h) = 0.8988; R^2(24h) = 0.1976; R^2(36h) = 0.3663.

	Pr >F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

LSD(5%) = 0.0264 [Solid lines only]

Legend:
- Control
- 12h
- 24h
- 36h
- 7d at 20°C

Y-axis: TA (%)
X-axis: Weeks of cold storage

Figure 27: TA (%) of optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
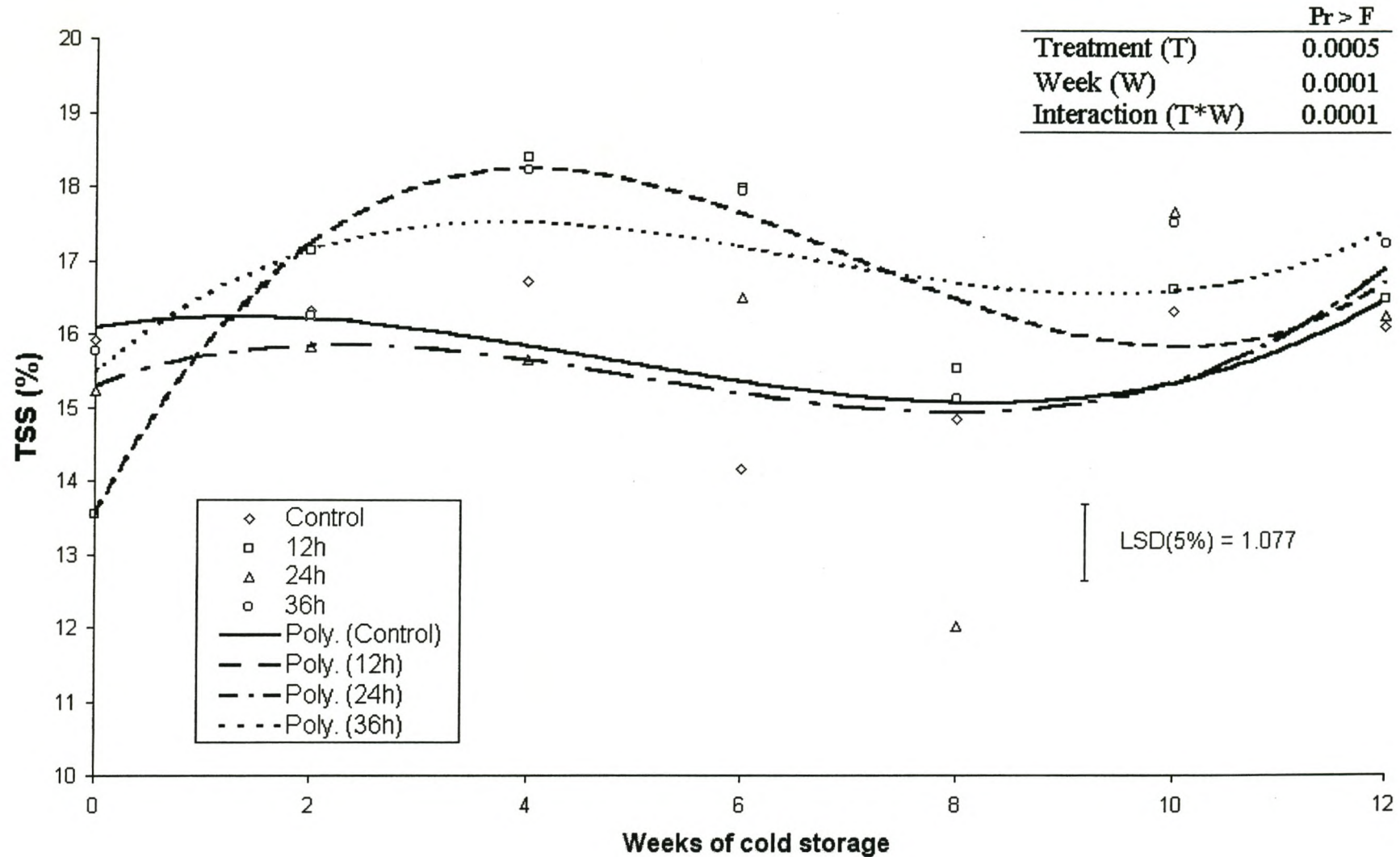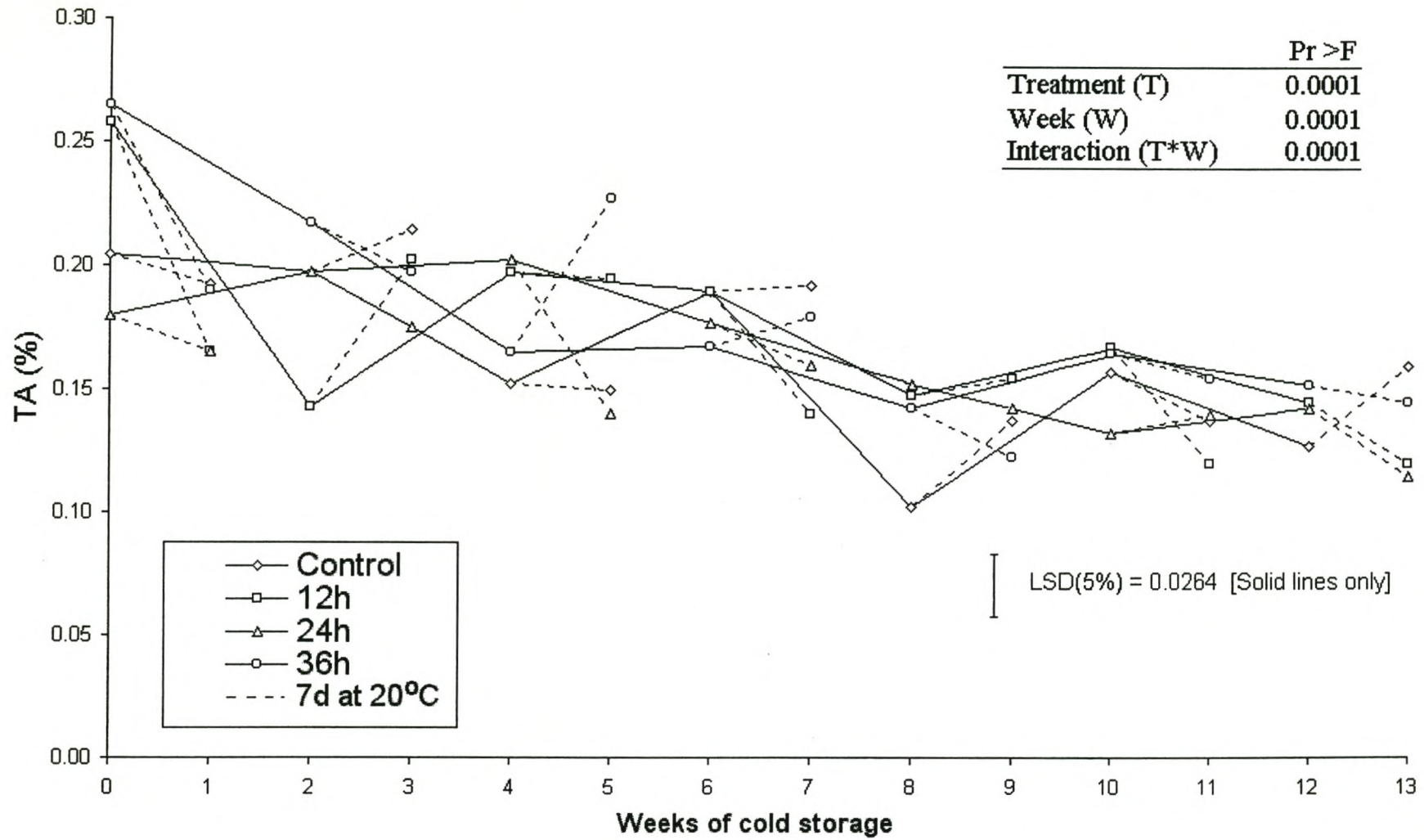
	Pr >F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0001

LSD(5%) = 0.0275

Legend:
◇ Control
□ 12h
△ 24h
○ 36h
—— Poly. (Control)
— — Poly. (12h)
— · — Poly. (24h)
- - - - Poly. (36h)

Weeks of cold storage

Figure 28: TA (%) of optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.5786; R^2(12h) = 0.8822; R^2(24h) = 0.8008; R^2(36h) = 0.7457.

POST-OPTIMUM HARVEST

Ground colour

Post-optimally harvested 'Forelle' pears treated with exogenous ethylene showed strong interactions in ground colour between treatments and weeks when fruit were in cold storage (Figure 29), and when held for seven days at 20°C (Figure 30). The longer ethylene treatments (24 and 36 h) had a more pronounced yellowing effect on ground colour in fruit in cold storage, and when fruit was held for seven days at 20°C, than only 12 h of ethylene. When fruit was taken out of cold storage and held for seven days at 20°C (Figure 30), 24 and 36 h ethylene treatments attained the critical value of 3.5 after 4 weeks of cold storage, while the 12 h treatment and control reached the same value after 8 weeks. This indicates a shortening of the prerequisite cold storage to 4 weeks when post-optimally harvested 'Forelle' pears were treated with ethylene for periods longer than 24 h.

Figure 29: Peel ground colour of post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending fro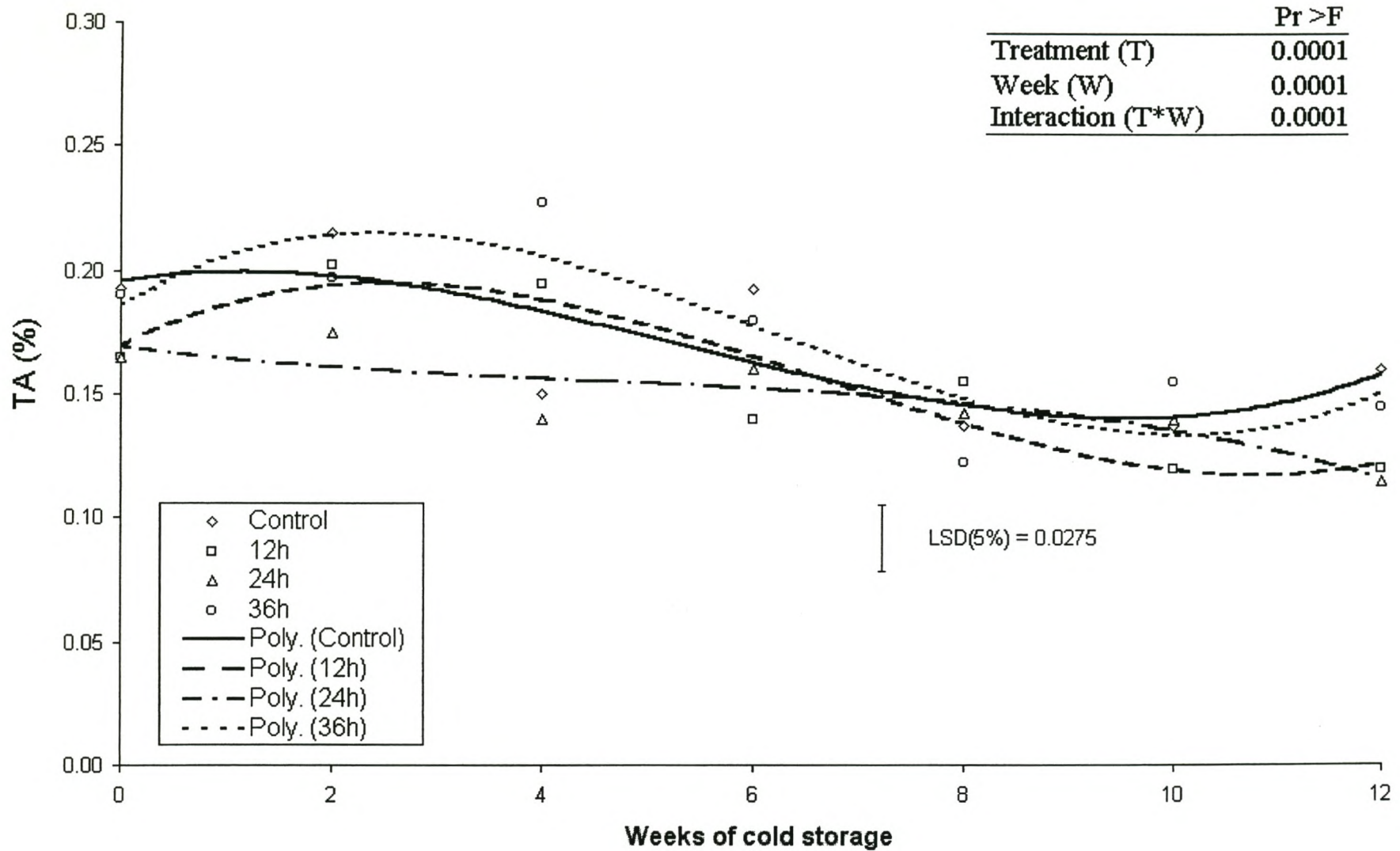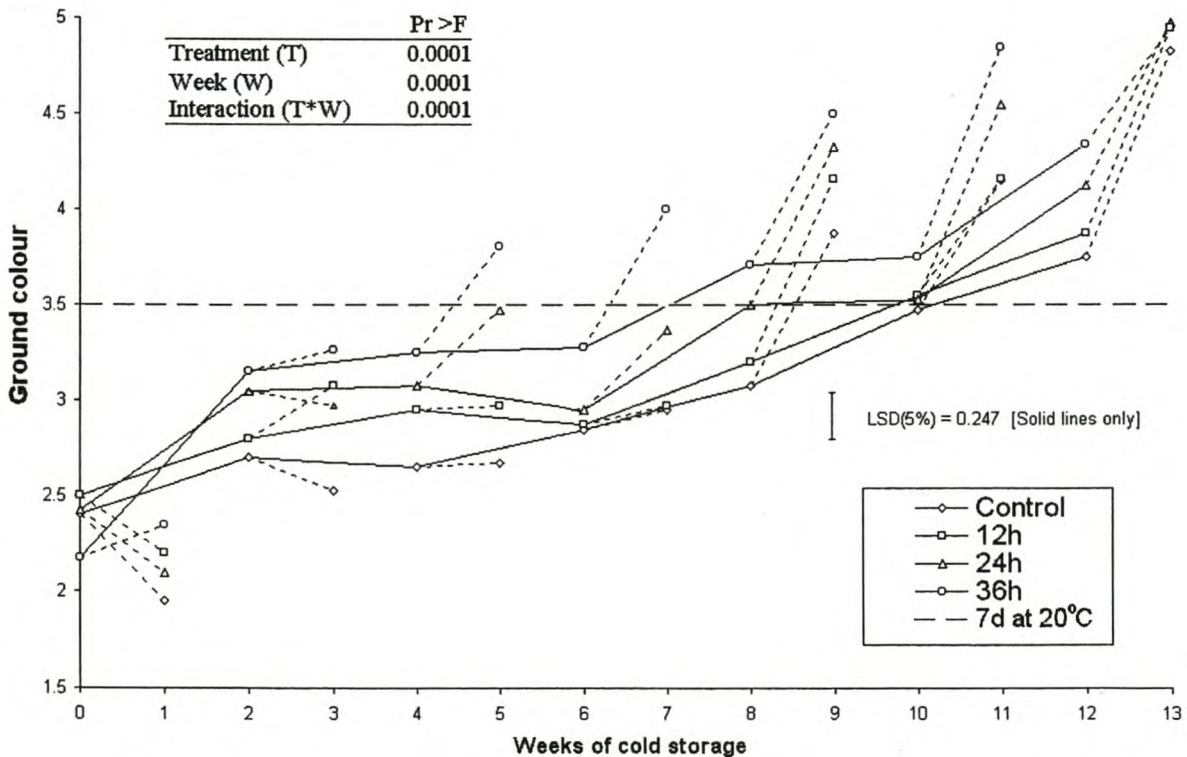m solid lines represent fruit held at 20°C for seven days. The line at 3.5 represents the critical minimum value for marketing and/or consumption.
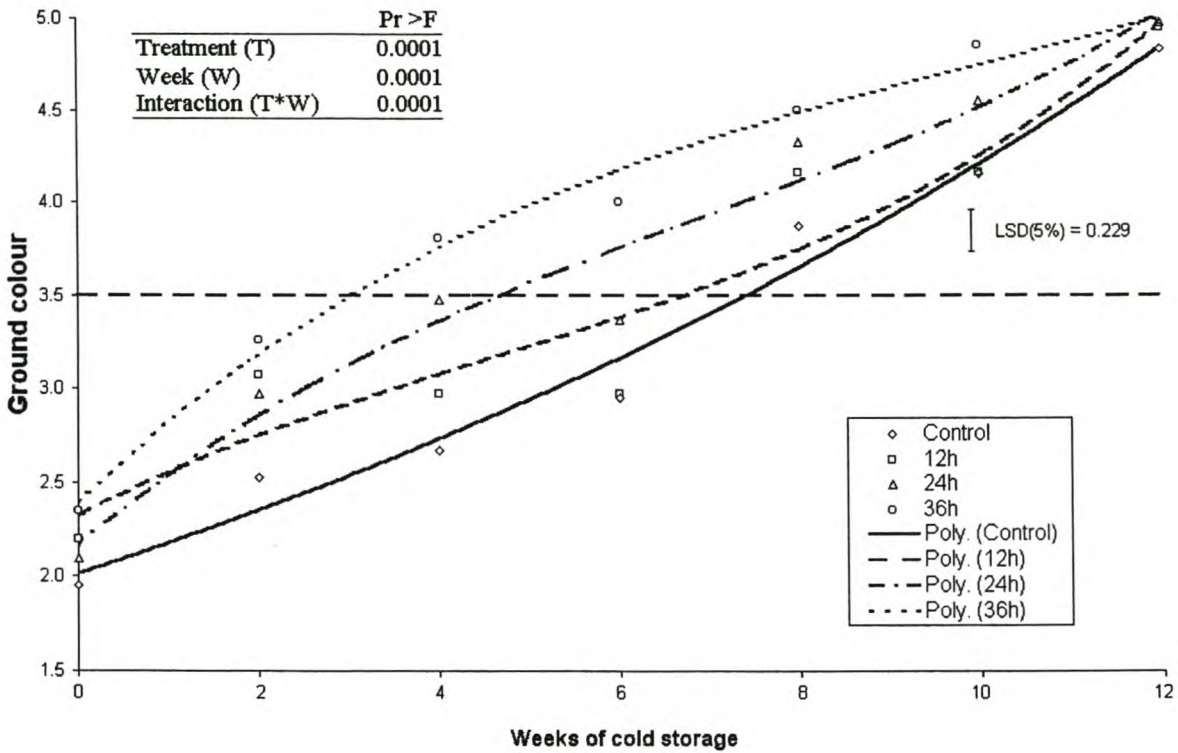
Figure 30: Peel ground colour of post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. The line at 3.5 represents the critical minimum value for marketing and/or consumption. R^2(Control) = 0.9832; R^2(12h) = 0.9329; R^2(24h) = 0.9745; R^2(36h) = 0.9977.

Fruit colour

Fruit colour measured objectively by the colorimeter support the results obtained using the colour chart. There were strong interactions in L* values among treatments and weeks for fruit in cold storage (Figure 31), and fruit held for seven days at 20°C (Figure 32). The treatment of post-optimally harvested 'Forelle' pears with ethylene had a significant effect on the L* value of colour when fruit was held for seven days at 20°C (Figure 31), but no distinct trend could be found in fruit held in cold storage (Figure 32). Control fruit held for seven days at 20°C had a significantly lower L* value for the first 4 weeks, indicating a darker (green) colour.

There were strong interactions in chroma between treatments and weeks for post-optimally harvested pears in cold storage (Figure 33), and when fruit was held for seven days at 20°C (Figure 34). Ethylene treated fruit had significantly lower C* values than the control fruit during cold storage and after seven days of 20°C (Figure 33). This is in accordance with results obtained

with pre-optimally and optimally harvested fruit.

There were strong interactions in hue angle for post-optimally harvested fruit between treatments and weeks for fruit in cold storage (Figure 35), and fruit held at 20°C for seven days (Figure 36). Treatment of post-optimally harvested 'Forelle' pears with ethylene had a significant yellowing effect (Figure 33) with ethylene treated fruit having significantly lower hue angle values than the control fruit in cold storage, and when fruit was held for seven days at 20°C. A strong positive correlation could be found between treatment length and a decrease in hue angle for post-optimally harvested 'Forelle' pears, with the longest ethylene treatment having the greatest decrease.

The pronounced 'greening' effect found in pre-optimally and optimally harvested fruit decreased even further in post-optimally harvested fruit. The significantly early decrease in hue angle of 24 and 36 h treatments held for seven days at 20°C began after 4 weeks of cold storage (Figure 35 and 36). After 8 weeks, cold storage began to have an effect and control fruit softened equally as much as ethylene treated fruit. This was 4 weeks earlier than pre-optimally harvested fruit, and 2 weeks earlier than optimally harvested fruit.

Figure 31: Lightness (L*) of peel in post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.

Figure 32: Lightness (L*) of peel in post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9787; R^2(12h) = 0.757; R^2(24h) = 0.6255; R^2(36h) = 0.2808.

	Pr >F
Treatment (T)	0.0155
Week (W)	0.0001
Interaction (T*W)	0.0001

LSD(5%) = 2.14 [Solid lines only]

Legend:
- ◇ Control
- □ 12h
- △ 24h
- ○ 36h
- - - - 7d at 20°C
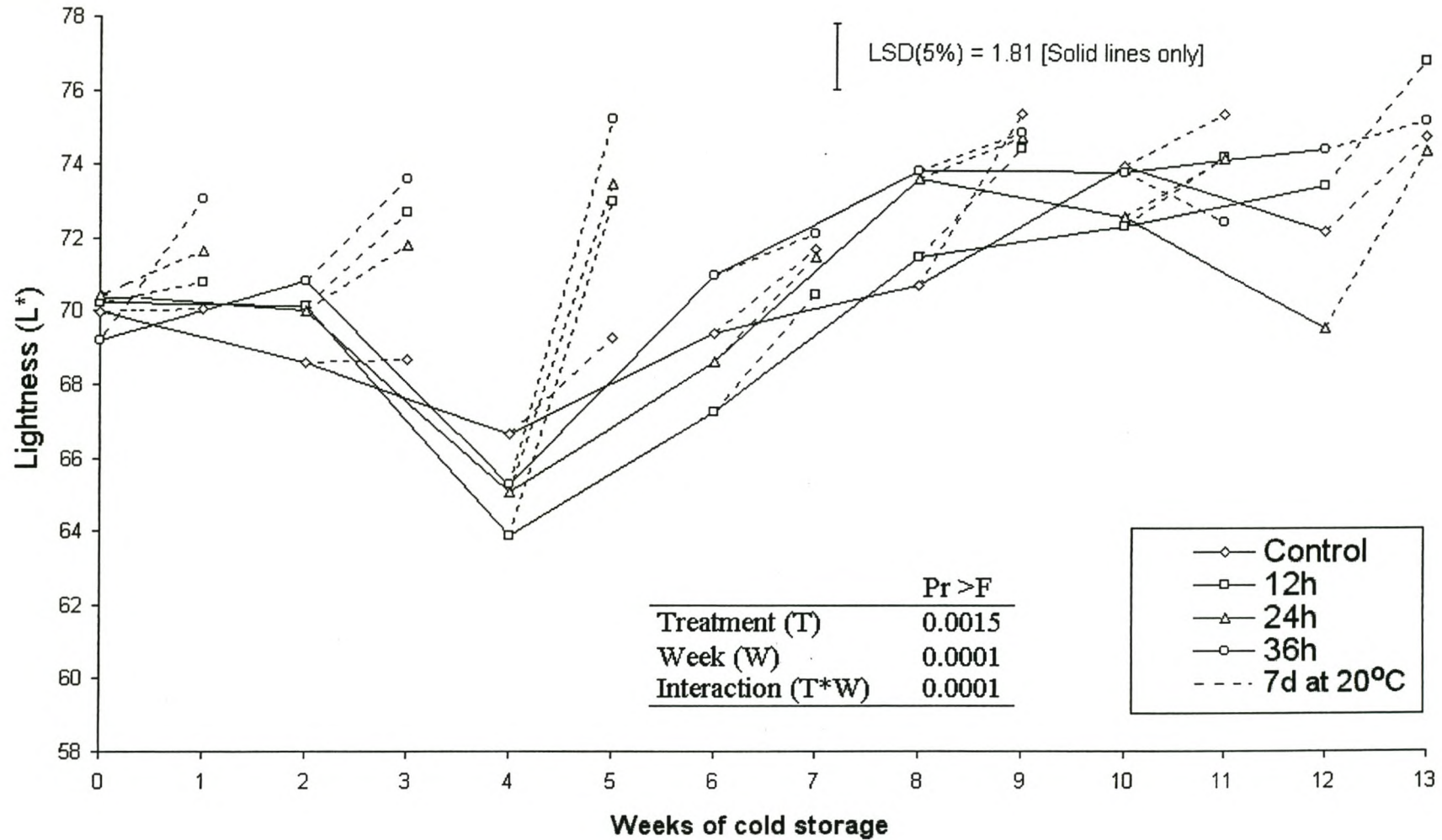
Figure 33: Chroma (C*) of peel in post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
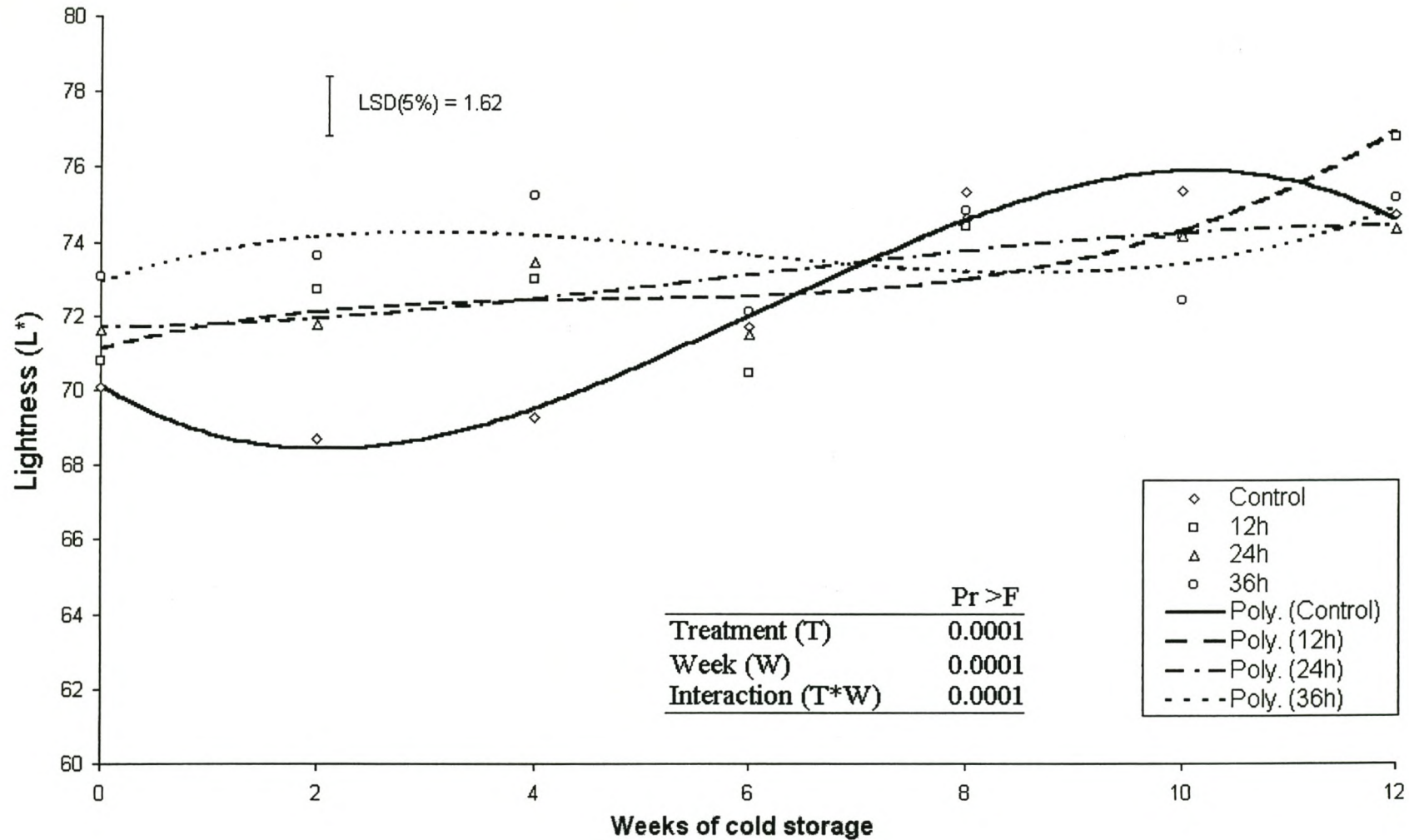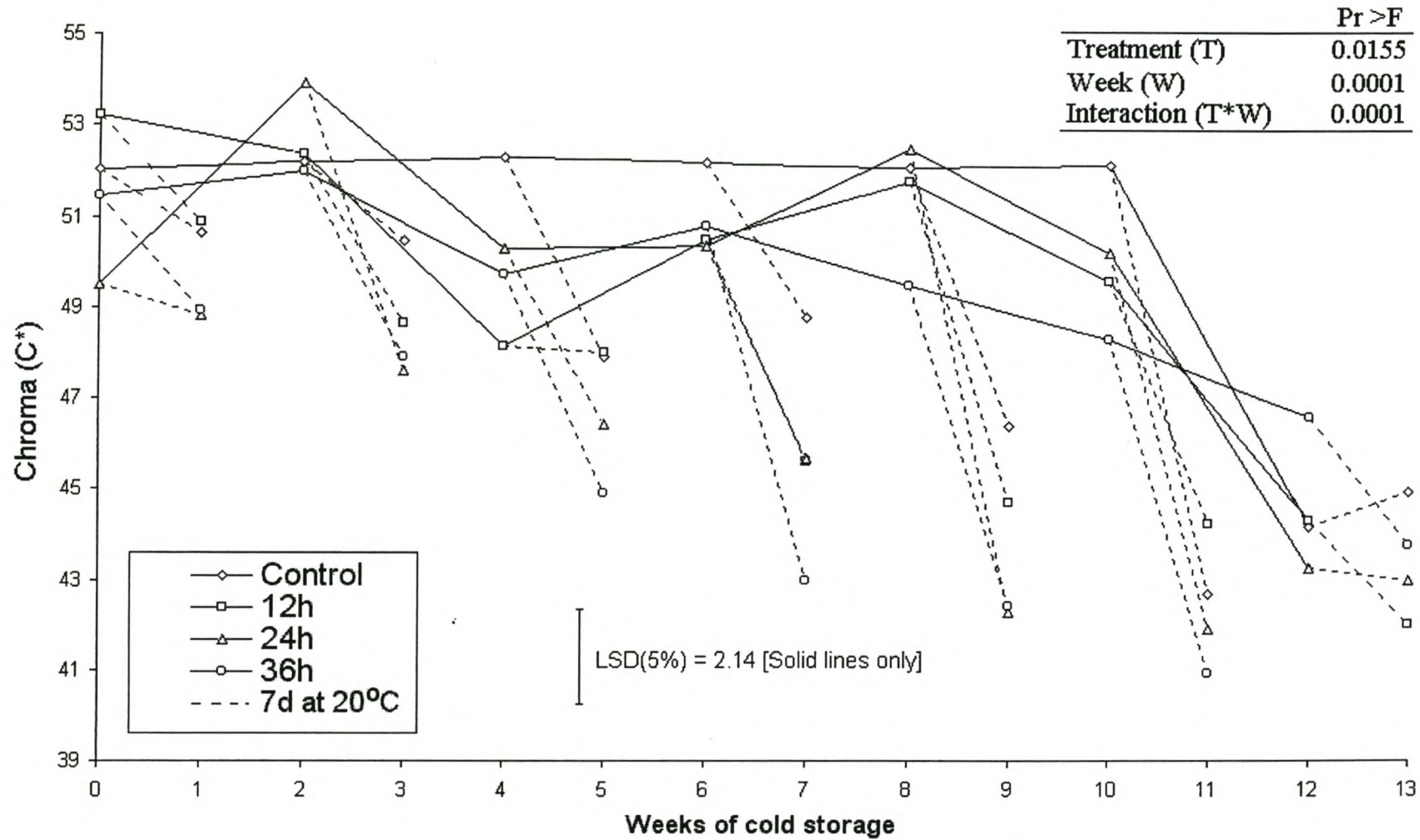
	Pr > F
Treatment (T)	0.0001
Week (W)	0.0001
Interaction (T*W)	0.0002

LSD(5%) = 1.70

Legend:
- ◇ Control
- □ 12h
- △ 24h
- ○ 36h
- —— Poly. (Control)
- — — Poly. (12h)
- — · — Poly. (24h)
- · · · · Poly. (36h)

Axis labels: Chroma (C*) ; Weeks of cold storage

Figure 34: Chroma (C*) of peel in post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.8537; R^2(12h) = 0.9807; R^2(24h) = 0.9573; R^2(36h) = 0.9729.

Figure 35: Hue angle (H°) of peel in post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
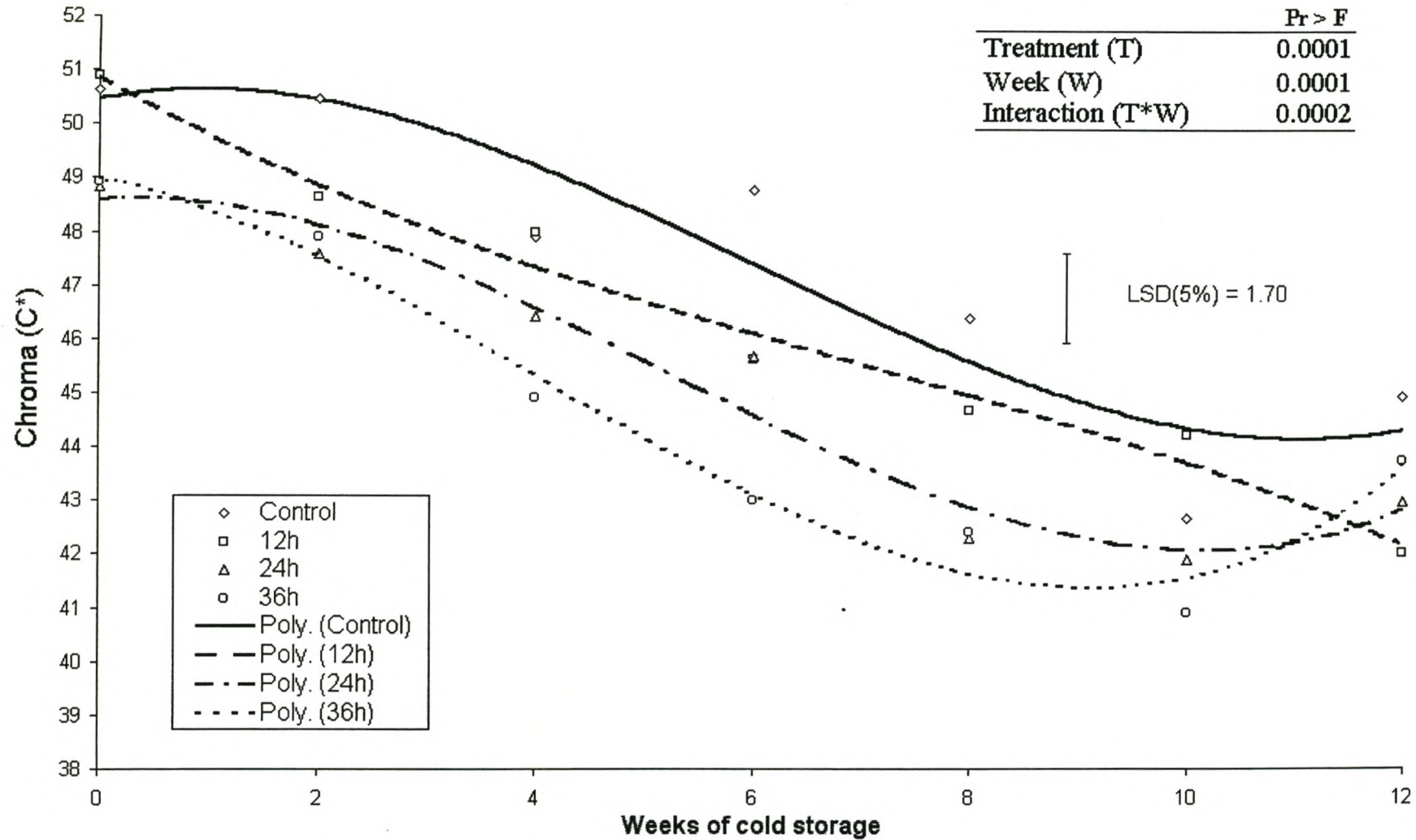
Figure 36: Hue angle (H°) of peel in post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R²(Control) = 0.9971; R²(12h) = 0.9939; R²(24h) = 0.9952; R²(36h) = 0.9544.

Flesh firmness

Strong interactions existed in firmness between treatments and weeks for post-optimally harvested fruit in cold storage (Figure 37), and for fruit held at 20°C for seven days (Figure 38). Post-optimally harvested 'Forelle' pears held in cold storage were adversely affected by the 36 h ethylene treatment (Figure 37). Fruit treated for 24 h and longer soften faster than control fruit at the end of cold storage and when held for seven days at 20°C (Figure 38). Twelve hours of ethylene gassing were insufficient to obtain the significant decrease in firmness achieved by 24 and 36 h of ethylene. The 36 h ethylene treatment softened significantly more than the 24 h ethylene treatment when fruit was held for seven days at 20°C. The ability of 24 and 36 h ethylene treated fruits to have a significant decrease in firmness started after 4 weeks in cold storage, while 12 h and control fruit required 6 weeks of cold storage to achieve a similar decrease. My personal observation was that although wooliness occurred it could not be correlated with fruit treated with ethylene but more with advanced maturity at harvest.
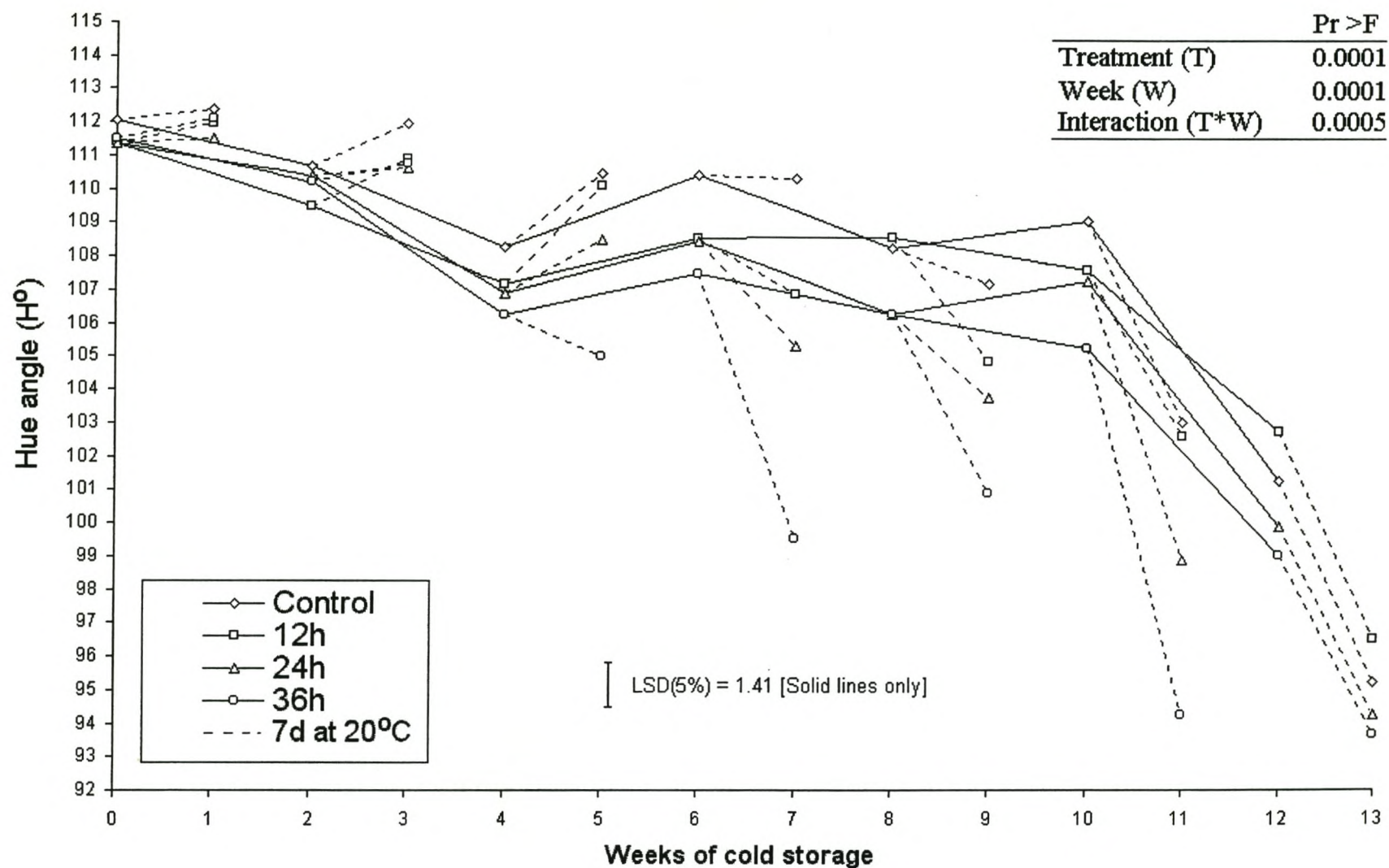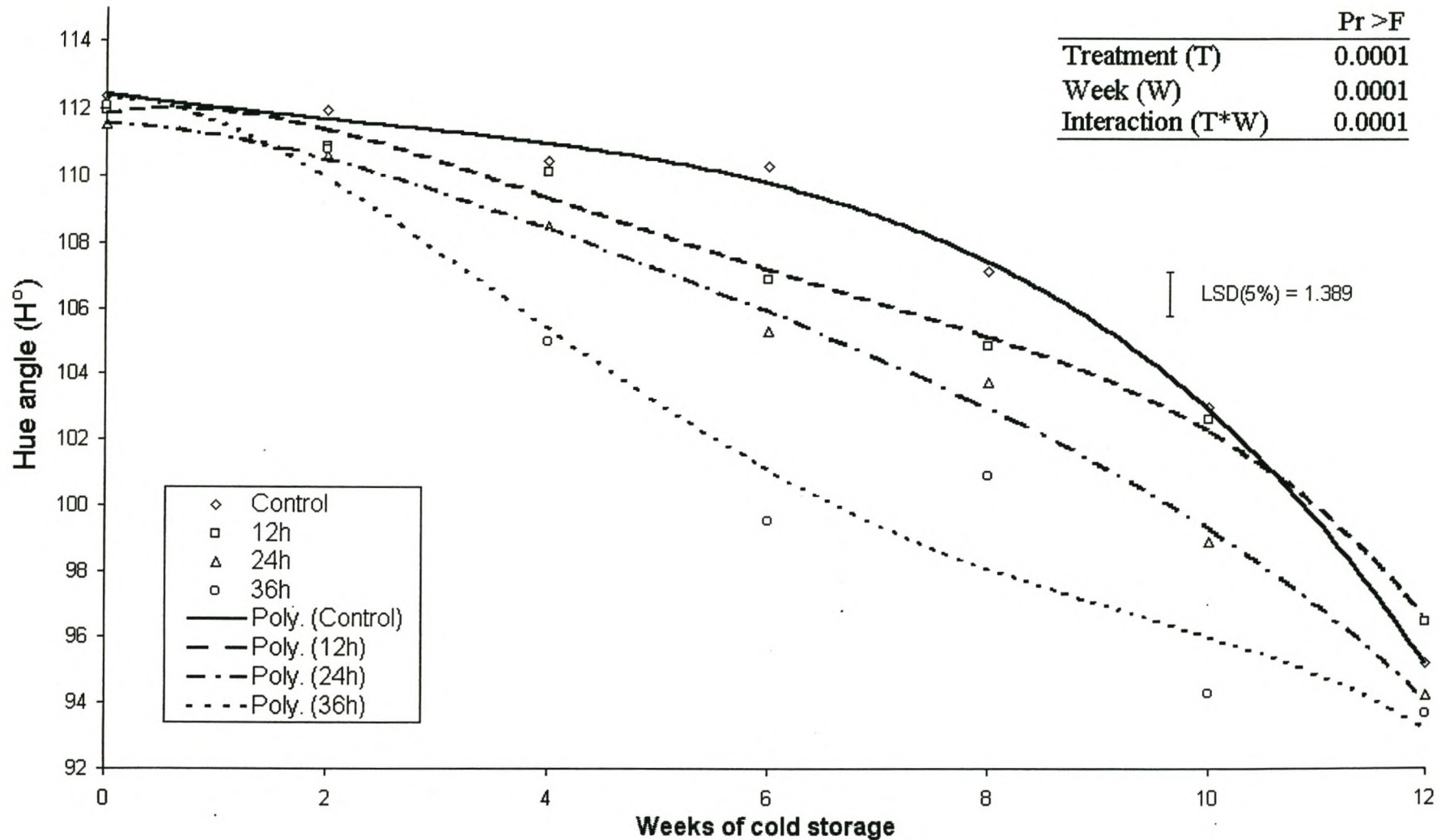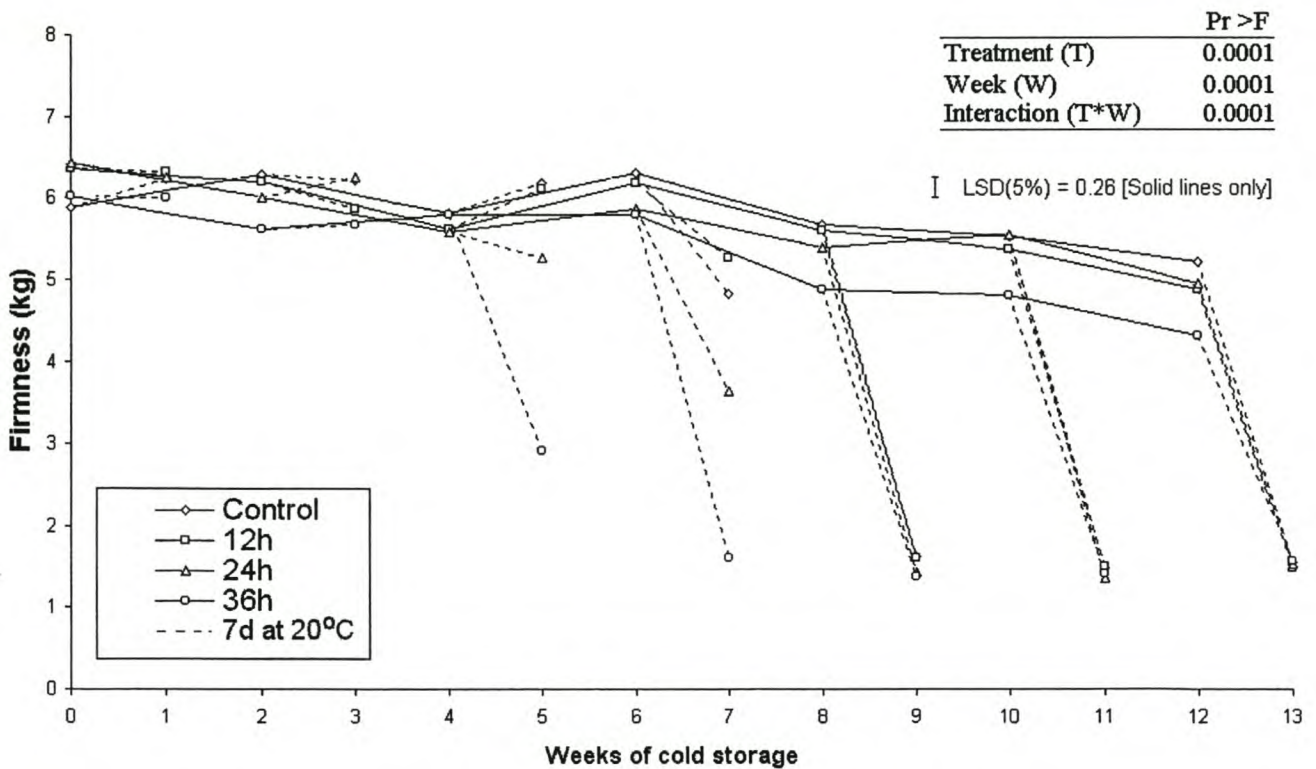

Figure 37: Firmness (kg) of post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven

Figure 38: Firmness (kg) of post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9564; R^2(12h) = 0.9188; R^2(24h) = 0.9884; R^2(36h) = 0.9458.

Total soluble solids (TSS) and titrable acidity (TA)

Strong interactions existed in TSS between treatments and weeks for post-optimally harvested fruit in cold storage (Figure 38), and when held for seven days at 20°C (Figure 39). There existed no visible trends and differences in TSS content for fruit in cold storage (Figure 39), or for fruit held at 20°C for seven days (Figure 40).

Poor interactions could be found in TA between treatments and weeks for fruit in cold storage (Figure 41), and fruit held for seven days at 20°C (Figure 42). Although not significant, control fruit in cold storage (Figure 42) had a higher acid content than ethylene treated fruit. When post-optimally harvested fruit was held at 20°C for seven days, a significant decrease ($P \leq 0.05$) in acid content was found (Figure 42). Control fruit had a significantly higher acid content than ethylene treated fruit. No differences existed between ethylene treatments.

Figure 39: TSS (%) of post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
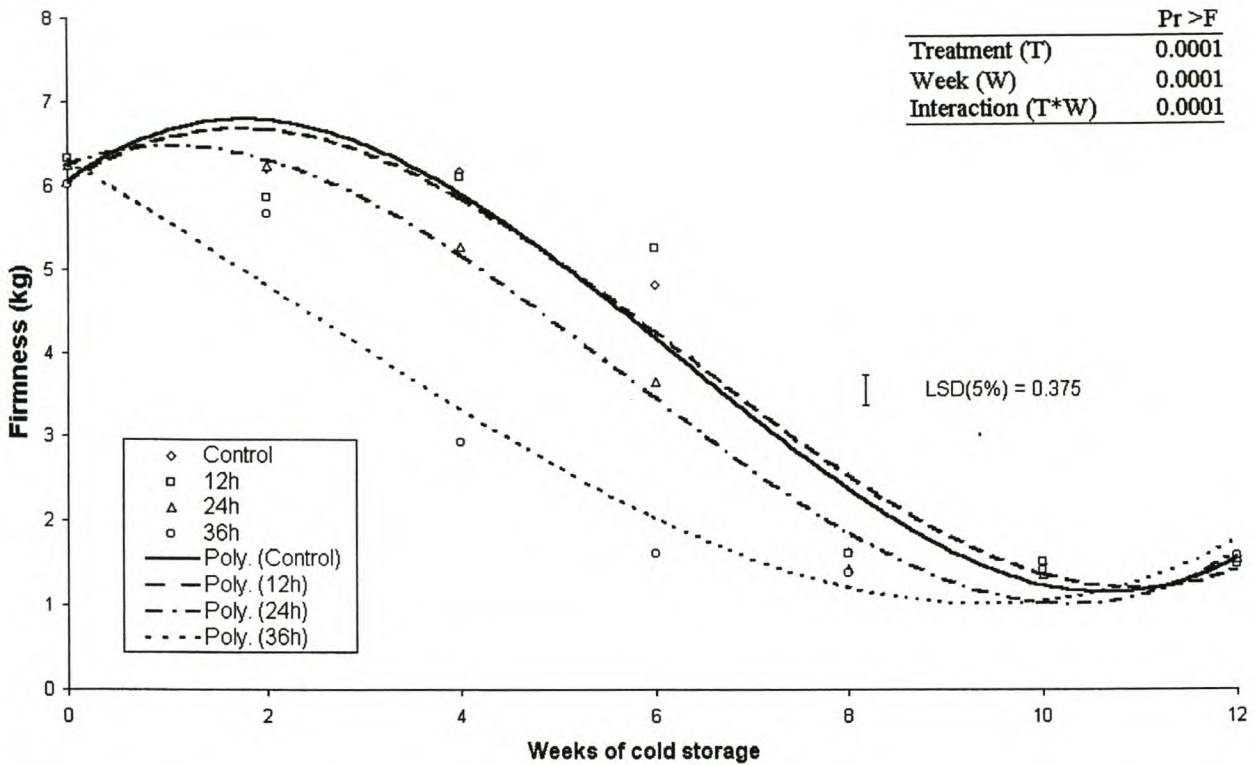
Figure 40: TSS (%) of post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.3501; R^2(12h) = 0.1201; R^2(24h) = 0.7342; R^2(36h) = 0.7408.

Figure 41: TA (%) content in post-optimally harvested 'Forelle' pears after cold storage (-0.5°C) under normal atmospheric conditions (solid lines). Dotted lines extending from solid lines represent fruit held at 20°C for seven days.
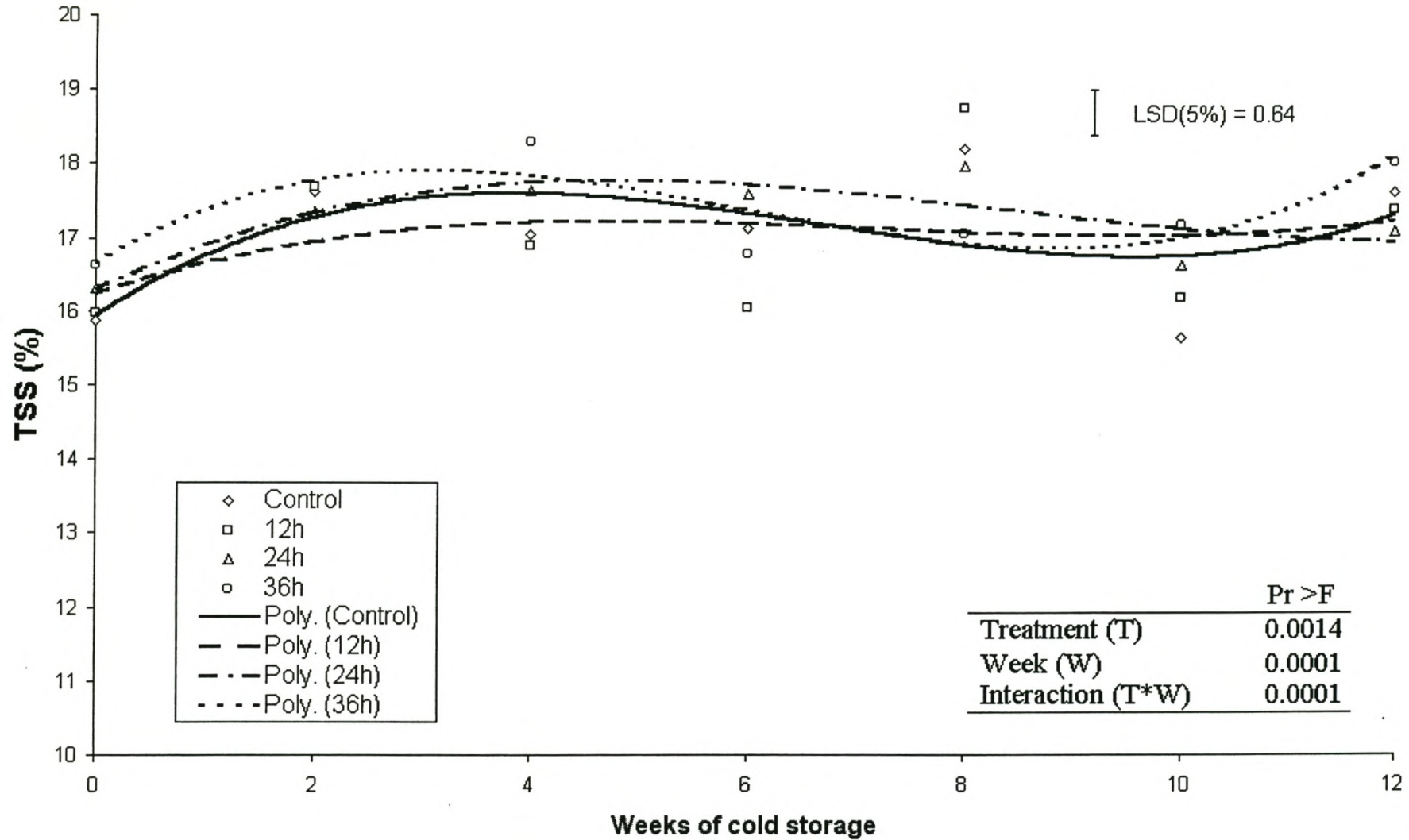
Figure 42: TA (%) of post-optimally harvested 'Forelle' pears held at 20°C for seven days after cold storage for different periods. R^2(Control) = 0.9489; R^2(12h) = 0.9408; R^2(24h) = 0.9281; R^2(36h) = 0.9581.
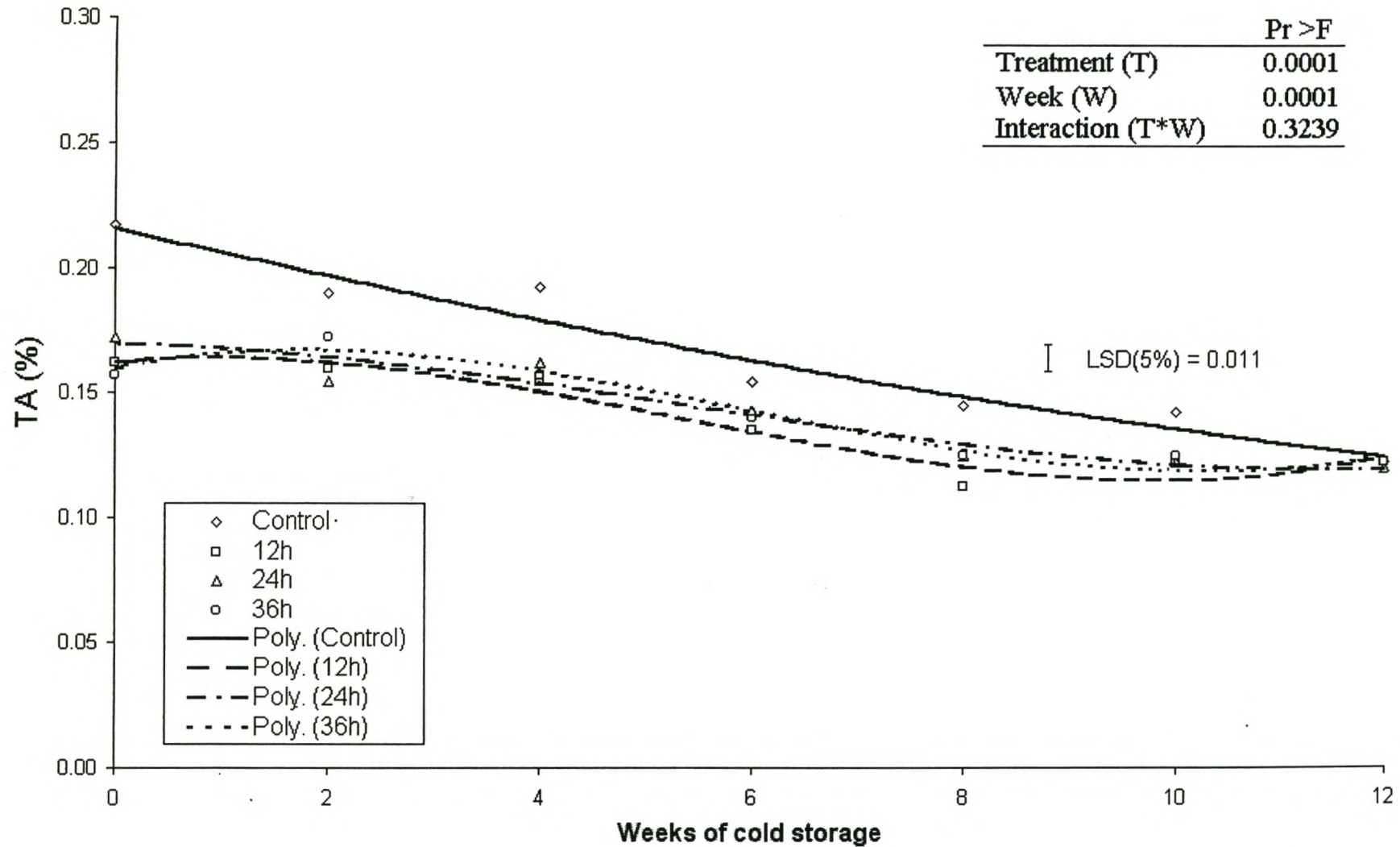
DISCUSSION

'Forelle', one of the most important pear cultivars in South Africa due to its high export value, requires a minimum of twelve weeks of cold storage before being considered marketable (De Vries & Hurndall, 1993). Results obtained by ethylene treatment indicate a potential to reduce this cold storage period, thereby allowing earlier marketing when demand and prices are high.

It can be concluded that harvest maturity of 'Forelle' pears had a great effect on the response to ethylene before storage. A definite correlation between the length of ethylene treatment and the early attainment of a yellow ground colour could be detected at all three harvesting times. It seems that regardless of harvesting time, a minimum of 24 h of ethylene is required to attain a physiological response in ground colour, measured by a colour chart and colorimeter, and firmness measured with a penetrometer. Ethylene treated pre-optimally harvested fruit attained the proper maturity for marketing after about eight weeks of cold storage, while both post-optimally and optimally harvested fruit attained this maturity after four weeks of cold storage. Pre-optimally harvested fruit exhibited a pronounced 'greening' effect as measured by changes in hue angle. This 'greening' effect cannot be explained, but subsided substantially in optimally and post-optimally harvested fruit. It is therefore very important that fruit not be picked immature.

Firmness of 'Forelle' pears held in cold storage was not adversely affected by ethylene at any harvest time. Fruit can thus be stored at low temperatures for as long as untreated fruit without danger of a loss in firmness. Fruit treated for a period longer than 24 h will soften significantly faster on removal from cold storage. Firmness of post-optimally harvested fruit treated with ethylene decreased after a significantly shorter cold storage period than optimally harvested fruit, likewise optimally harvested fruit showed a reduction in firmness with significantly shorter cold storage periods than pre-optimally harvested fruit. My personal observation was that the minimal wooliness that occurred could not be correlated with fruit treated with ethylene but more with advanced maturity at harvest. No clear conclusions can be made from changes in TSS and TA between harvesting times. In a few cases the TSS of treated fruit was significantly higher and the acid content significantly lower, but this trend did not repeat itself in every harvesting time.

Although ground colour measurements indicate that post optimally and optimally harvested, ethylene treated fruit need only four weeks of cold storage to attain the minimum required value (3.5 on the Unifruco colour chart), a properly ripened fruit is accompanied by a yellow skin

colour, soft texture and correct TSS/TA ratio. It would therefore be wise to wait a minimum of six weeks before marketing ethylene treated fruit to ensure that a yellow fruit is accompanied by a favourable palatability.

ACKNOWLEDGEMENTS

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CHAPTER 4: PAPER 3

EFFECT OF PRE-HARVEST APPLICATIONS OF CALCIUM NITRATE, CALCIUM CHLORIDE AND UREA ON RIPENING OF 'PACKHAM'S TRIUMPH' AND 'FORELLE' PEARS AFTER CONTROLLED ATMOSPHERE STORAGE.

Abstract

Physiologically young and physiologically old 'Packham's Triumph' and 'Forelle' orchards were subjected to three foliar treatments one week prior to harvest. Trees were treated with calcium nitrate (0.7%), calcium chloride (0.7%) and urea (750 g.100L^{-1} water) and harvested according to bearing position. Fruit was stored under controlled atmosphere (CA) for a period of 9 months. Deterioration in quality particularly with 'Packham's Triumph' pears during CA storage hampered data collection. However the four treatments had no significant effect on the storage potential of 'Packham's Triumph' and 'Forelle' pears. No significant differences due to bearing position and tree age could be noted.

Keywords: Foliar sprays; Controlled atmosphere; Calcium nitrate; Calcium chloride; Urea

INTRODUCTION

'Packham's Triumph' pears are characteristically yellow, juicy and soft when ripe, but highly susceptible to bruising, and consequently to pathogen attack. This makes fruit difficult to handle and store, and frequently poor quality fruit reaches the consumer. Firmer fruit are less prone to bruising injury during picking, grading, packing and transport. Therefore treatments which potentially lead to firmer fruit at harvest without compromising other aspects of fruit quality are of major importance to fruit growers and warrant investigation. A relatively high percentage of fruit can be lost due to large variations in fruit maturity after storage. This variation in maturity cannot be detected before fruit are stored but after a few months of storage a great deal of fruit stored in CA storage are not fit to be marketed.

Higher calcium levels in fruit are sometimes associated with increased fruit firmness at harvest in apples (Brown et al., 1996). In addition, Poovaiah (1988) found a linear relationship between fruit flesh firmness and calcium status of 'Golden Delicious' apples after four months of cold

storage. Fruit senescence and quality are affected by calcium which can affect intracellular and extracellular processes (Fallahi et al., 1997). According to Faust & Shear (1972) calcium preserves the cellular organization not only by preserving the cell membranes, but also by maintaining nucleic acid and protein synthesis. There is evidence that post harvest quality of pears can be enhanced by treatment with calcium chloride (Meheriuk, 1990; Sugar et al., 1991; Sugar, 1989; Meheriuk & Sholberg, 1990).

Nitrogen, a macronutrient in plants, plays an important role as a component of protein, enzymes, co-enzymes, nucleic acids, chlorophyll, etc. (Stassen, 1980). Urea sprays delay yellowing in green apple cultivars like 'Granny Smith' and 'Newton' and high nitrogen levels have a greening effect on apple peel and flesh colour (Fallahi *et al.*, 1985; Nielsen *et al.*, 1984; Meheriuk *et al.*, 1996; Meheriuk *et al.*, 1992). Excessive nitrogen fertilization, and thus high levels of nitrogen in the fruit during the growing season, has a negative effect on fruit firmness and storage potential (Marcelle, 1995; Bramlage *et al.*, 1980). Urea sprays are recommended on apple and citrus trees to supplement soil application of nitrogen. They are reported not to be effective on stone fruits or on pear (Swietlik & Faust, 1983).

MATERIALS AND METHODS

A physiologically young and physiologically old orchard of both 'Packham's Triumph' and 'Forelle' *(Pyrus communis* L.) pears were selected in the Ceres region, Western Cape, South Africa. Four trees of similar vegetative status and crop load from each site were chosen in an effort to rule out variation due to tree architecture. The trial consisted of four foliar treatments in a complete random block design:

$Ca(NO_3)$ at a concentration of 0.7% applied seven days before harvest.

$CaCl_2$ at a concentration of 0.7% applied seven days before harvest.

Urea at a concentration of 750 g.100 L^{-1} applied seven days before harvest.

Water to serve as a control.

Agral 90 was added to the spraying mixture as wetting agent. Seven days after treatment all fruit were harvested. Harvesting of fruit was done in three classifications:

Bearing terminally on a long shoot

Bearing laterally on one year old bearing wood.

Older bearing units and spurs.

All fruit was harvested from the treated trees and stored under controlled atmospheric (CA) conditions for a period of nine months. After fruit was removed from CA storage, maturity tests were conducted immediately and again after seven days at 20°C.

Peel ground colour was evaluated with a Unifruco colour chart (0.5 = dark green through to 5 = deep yellow). Colour measurements were taken from the non-blushed side with a Nippon Denshoku colorimeter (Handy colorimeter, HR-3000). Colour is reported as a set of L*, C* and H° values. The L* value refers to the lightness of the colour on a scale of 0 to 100 where 0 = black through to 100 = white. The C* value refers to the chroma or intensity of colour, with a higher value referring to a colour of greater intensity. The H° value refers to the hue angle on a scale of 0° = red/purple, 90° = yellow, 180° = blue/green and 270° = blue. (McGuire, 1992). Flesh firmness (kg) was tested with a penetrometer (Southtrade fruit pressure tester, mod. FT 327) with an 8 mm tip. Two measurements were taken from each fruit, at opposite sides, after a piece of skin (roughly 2 cm in diameter) was removed. Total soluble solids (TSS; %) were measured by an digital refractrometer (Brix % Palette PR-100 (0-32%) Refractometer). Titrable acidity (TA; %) was calculated as % Malic acid, by titrating juice from liquidised segments, taken from groups of 6 made from the 30 fruit in each batch, with 0.1 NaOH to a pH of 8.2. ANOVA was done using the SAS System (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

No significant differences were found between treatments for both the cultivars 'Packham's Triumph' and 'Forelle' (Table 1 and 2). Although there were significant differences in some maturity indices, these should not be over-analysed because the trend is not followed in the rest of the indices as a rule. Furthermore it can be concluded no significant differences were evident between bearing positions or between old and young trees.

Table 1: Maturity evaluations for 'Packham's Triumph' pears harvested from three bearing positions on trees from physiologically young and old orchards after foliar sprays. Fruit were evaluated after 9 months CA storage and again after a further seven days at 20°C.

Date	Treatment	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9	CaNO3	3.94 a	70.21 a	45.75 a	105.15 a	16.84 a	1.88 a
months	CaCl2	3.96 a	69.19 a	45.62 a	105.41 a	16.74 a	1.78 a
	Urea	3.68 a	69.59 a	46.69 a	105.92 a	16.83 a	1.99 a
	Control	4.08 a	69.40 a	45.90 a	105.11 a	16.74 a	1.93 a
	LSD(5%)	0.54	2.02	1.20	1.28	0.37	0.56
9 m + 7d	CaNO3	4.64 a	68.33 a	43.87 b	97.08 a	17.01 a	1.24 a
20°C	CaCl2	4.69 a	69.83 a	44.73 ab	96.50 a	17.07 a	1.22 a
	Urea	4.70 a	70.72 a	46.23 a	97.55 a	17.11 a	1.18 a
	Control	4.66 a	68.85 a	44.63 ab	97.53 a	17.07 a	1.15 a
	LSD(5%)	0.32	3.43	2.22	3.11	0.23	0.21

Date	Position	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9	Terminal	3.86 ab	69.81 a	46.03 a	106.05 a	16.80 a	2.11 a
months	Lateral on 1 year	4.15 a	69.93 a	45.91 a	105.51 a	16.80 a	1.81 ab
	Lateral on more than 1 year	3.74 b	69.07 a	46.03 a	104.62 a	16.79 a	1.77 b
	LSD(5%)	0.40	1.26	1.29	1.55	0.16	0.32
9 m + 7d	Terminal	4.60 b	68.14 b	44.11 a	96.97 a	17.05 a	1.22 a
20°C	Lateral on 1 year	4.75 a	68.72 b	44.49 a	96.07 a	17.05 a	1.21 a
	Lateral on more than 1 year	4.65 ab	71.04 a	45.67 a	98.28 a	17.08 a	1.17 a
	LSD(5%)	0.12	2.25	1.59	2.28	0.08	0.10

Date	Tree Age	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9	Old	4.19 a	70.02 a	46.24 a	104.21 b	16.80 a	1.94 a
months	Young	3.64 b	69.19 a	45.74 a	106.56 a	16.79 a	1.85 a
	LSD(5%)	0.38	1.43	0.85	0.90	0.26	0.39
9 m + 7d	Old	4.72 a	69.26 a	45.04 a	96.24 a	17.06 a	1.19 a
20°C	Young	4.62 a	69.47 a	44.54 a	98.06 a	17.06 a	1.21 a
	LSD(5%)	0.23	2.42	1.56	2.19	0.16	0.15

Table 2: Maturity evaluations for 'Forelle' pears harvested from three bearing positions on trees from physiologically young and old orchards after foliar sprays. Fruit were evaluated after 9 months CA storage and again after a further seven days at 20°C.

Date	Treatment	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9 months	CaNO3	3.42 a	67.17 a	44.13 a	102.73 a	18.05 a	6.42 a
	CaCl2	3.32 a	68.02 a	43.73 a	103.83 a	18.12 a	6.19 ab
	Urea	3.32 a	67.47 a	43.79 a	103.36 a	17.73 a	6.25 ab
	Control	3.22 a	68.10 a	43.92 a	105.33 a	17.22 a	6.05 b
	LSD(5%)	0.69	6.52	2.98	7.54	1.47	0.32
9 m + 7d 20°C	CaNO3	4.00 ab	70.04 a	45.10 a	99.73 a	18.62 a	5.14 a
	CaCl2	3.88 c	70.16 a	44.90 a	100.94 a	17.91 a	4.90 a
	Urea	4.07 a	70.36 a	44.19 a	98.67 a	18.04 a	5.06 a
	Control	3.93 bc	71.79 a	43.67 a	101.78 a	17.59 a	5.38 a
	LSD(5%)	0.12	4.69	3.15	4.77	1.23	1.06

Date	Position	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9 months	Terminal	3.48 a	65.97 b	42.18 b	100.81 a	18.17 a	6.47 a
	Lateral on 1 year	3.33 a	69.27 a	44.26 ab	104.43 a	17.68 b	6.06 b
	Lateral on more than 1 year	3.18 a	67.56 ab	44.96 a	105.69 a	17.56 b	6.19 ab
	LSD(5%)	0.37	2.82	2.09	5.32	0.48	0.28
9 m + 7d 20°C	Terminal	3.81 b	69.14 b	43.68 b	99.86 a	18.10 a	5.33 a
	Lateral on 1 year	4.06 a	71.23 a	44.53 ab	100.34 a	18.03 a	5.04 ab
	Lateral on more than 1 year	4.02 a	71.12 a	45.07 a	100.50 a	17.99 a	5.00 b
	LSD(5%)	0.13	1.66	0.90	1.95	0.36	0.32

Date	Tree Age	Ground colour	L	C	H	TSS (%)	Firmness (kg)
After 9 months	Old	3.18 a	65.62 a	42.26 b	101.98 a	18.01 a	6.30 a
	Young	3.46 a	69.68 a	45.45 a	105.56 a	17.56 a	6.16 a
	LSD(5%)	0.49	4.61	2.11	5.33	1.04	0.23
9 m + 7d 20°C	Old	3.94 a	68.78 b	43.82 a	98.54 b	18.35 a	5.39 a
	Young	4.00 a	72.41 a	45.14 a	102.01 a	17.71 a	4.82 a
	LSD(5%)	0.08	3.32	2.22	3.37	0.87	0.75

As data were collected from only one tree per treatment it is very difficult to rule out experimental error and not possible to draw too many conclusions from the results obtained. A further negating factor was that after CA storage a great deal of fruit had already senesced and fruit quality had deteriorated. This was predominantly present in the cultivar 'Packham's Triumph'. Fruit was of a bad quality due to loss of firmness (Table 1), abrasions and pathogen attack. The 9 months of CA storage for especially 'Packham's Triumph' pears may need to be re-examined.

CONCLUSIONS

It can be concluded that foliar sprays have no significant effect on the storage potential of 'Packham's Triumph' and 'Forelle' pears. A great deal of data were lost due to deterioration in quality of mostly 'Packham's Triumph' pears during CA storage. No significant differences due to bearing position and tree age were found.

ACKNOWLEDGEMENTS

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