POSTHARVEST MANIPULATION OF FRUIT COLOUR IN APPLES AND PEARS

BY

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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.

Abstract

Red colour development on bi-coloured apples and pears ensures better prices for producers. The use of postharvest irradiation to improve colour has been successful on apples, and the objectives of this thesis were to optimise conditions during irradiation for apples and to evaluate the effects of irradiation on pears.

'Cripp's Pink' apples responded to postharvest irradiation with high-pressure sodium (HPS) lights by developing a red blush, whereas the response to irradiation with UV B plus incandescent lights was less effective. 'Braeburn' apples held at –0.5°C for 4 or 8 weeks prior to irradiation showed a decrease in hue angle and an increase in anthocyanin concentration after 72 hours of irradiation with HPS lamps. In 'Forelle' pears treated in the same way, neither colour development nor anthocyanin synthesis was affected by irradiation.

'Braeburn' and 'Cripp's Pink' apples picked weekly for 5 weeks until the optimum harvest date were irradiated with HPS lights. A significant increase in fruit colour was only measured in mature fruit of both cultivars.

'Cripp's Pink' apples were harvested from two production areas with different microclimates, namely, Ceres and Grabouw, and stored for 0, 2 or 5 days at –0.5 °C before irradiation for 120 hours at either 6°C or 20°C. Fruit from Ceres that were irradiated immediately after harvest developed better colour at 6 °C than at 20 °C. The differences between fruit irradiated at the two temperatures were no longer significant after 5 days of cold storage prior to irradiation. Fruit from Grabouw consistently developed better colour when irradiated at 6°C than at 20 °C. Colour development

slightly after 5 days of cold storage prior to irradiation. In another experiment, fruit from both areas were stored at –0.5°C for 20 days before irradiation at either 6°C or 6/20°C. The fluctuating temperature regime resulted in decreases in hue angle of 70° and 65° for the fruit from Grabouw and Ceres, respectively. The decreases were smaller (±20°) when fruit were irradiated at 6°C.

The hue angle value of well-coloured 'Cripp's Pink' apples held at 37°C under HPS lights for 144 hours increased from 29.3° to 48.3°, and anthocyanin concentration decreased from 739.9 $\mu g \cdot g^{-1}$ to 283.6 $\mu g \cdot g^{-1}$. Control fruit held at the same temperature in the dark did not show any change in hue angle value or anthocyanin concentration.

'Bon Rouge' and 'Red d' Anjou', two full red pear cultivars, irradiated with HPS lights for 72 hours, showed no significant changes in hue angle. 'Forelle' pears, harvested with or without attached leaves, were irradiated with HPS at two temperature regimes, 20°C and 20°/6°C. The resulting decreases in hue angle were attributed to yellowing and not red colour formation.

In conclusion, the response of apples to postharvest irradiation was affected by maturity and temperature, while pears failed to respond at all.

Na-oes manipulering van vrugkleur op appels en pere

Opsomming

Rooikleurontwikkeling van twee-kleur appels en pere verseker beter pryse vir die produsente. Na-oesbestraling om kleur te verbeter is al suksesvol uitgevoer op appels, en die doelwit van hierdie tesis was om die kondisies vir appels gedurende bestraling te optimaliseer en om die effek van bestraling op pere te evalueer.

'Cripp's Pink' appels het reageer op na-oesbestraling met hoëdruk-natriumligte (HDN) deur ´n rooi blos te ontwikkel, terwyl die reaksie op bestraling met UV-B plus gloeilamplig minder effektief was. 'Braeburn' appels opgeberg by –0.5°C vir 4 of 8 weke voor bestraling het 'n afname in die kleurskakeringswaarde getoon, asook 'n toename in antosianienkonsentrasie na 72 uur se bestraling met HDN ligte. 'Forelle' pere wat dieselfde behandeling ontvang het, het geen kleurontwikkeling en geen antosianienontwikkeling getoon na bestraling nie.

'Braeburn' en 'Cripp's Pink' appels wat weekliks geoes is vir 5 weke tot die optimum oesdatum is bestraal met HDN ligte. Slegs die volwasse vrugte van beide kultivars het 'n betekenisvolle toename in kleur getoon.

'Cripp's Pink' appels is geoes in twee produksie areas met verskillende mikroklimate, naamlik Ceres en Grabouw. Vrugte is opgeberg vir 0, 2 of 5 dae by –0.5°C voor bestraling vir 120 uur by of 6°C of 20°C. Vrugte van Ceres wat onmiddellik na oes bestraal is het beter kleur ontwikkel by 6°C as by 20°C. Kleurontwikkeling by vrugte bestraal by 6 of 20°C het nie verskil wanneer vrugte vooraf opgeberg was by -0.5°C vir 5

dae nie. Vrugte van Grabouw het konstant beter kleur ontwikkel wanneer bestraling by 6°C eerder as 20°C plaasgevind het. In die volgende eksperiment, was beide die vrugte van Ceres en Grabouw vir 20 dae opgeberg by –0.5°C voor bestraling by 6°C of 6°/20°C. Die fluktuerende temperatuur regime het afnames van 70° en 65° in kleurskakeringswaarde getoon vir die vrugte van Grabouw en Ceres, respektiewelik. Die afname was kleiner (±20°) wanneer vrugte by 6°C bestraal is.

'Cripp's Pink' appels wat goed gekleur was en opgeberg is by 37°C terwyl dit blootgestel is aan HDN ligte vir 144 uur, het 'n toename van 29.3° tot 48.3° getoon vir die kleurskakeringswaarde, en antosianienkonsentrasie het afgeneem van 739 $\mu g \cdot g^{-1}$ tot 283.6 $\mu g \cdot g^{-1}$. Die kontrole vrugte opgeberg by dieselfde temperatuur in die donker het geen verandering in beide die kleurskakeringswaarde of die antosianienkonsentrasie getoon nie.

'Bon Rouge' en 'Red d' Anjou', twee volrooi peerkultivars, is bestraal met HDN ligte vir 72 uur en het geen betekenisvolle verandering in kleur getoon nie. 'Forelle' pere, geoes met of sonder 'n aangehegte stingelsegment, is bestraal met HDN ligte by twee verskillende temperatuur regimes, nl. 20°C of 20/6°C. Die afname in kleurskakeringswaarde is aan vergeling toegeskryf eerder as aan rooikleurontwikkeling.

Ter opsomming, die reaksie van appels op na-oes bestraling is beïnvloed deur rypheid en temperatuur, terwyl pere geen reaksie getoon het nie.

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Contents

1. Introduction

In apples a good red skin colour plays an important role in market value, since consumer experience has determined that within a cultivar a redder fruit tastes better. Consequently red cultivars are preferred, and better-coloured fruit sell at higher retail prices (Mancinelli, 1983; Saure, 1990). Colour development is not usually a problem in apples grown in cooler, temperate zones. The opposite holds for apples grown in warmer regions where higher temperatures contribute significantly to poor colour development (Blankenship & Unrath, 1988).

Red skin colour is mainly due to anthocyanin glycosides, known collectively as anthocyanins (Mancinelli, 1983; Macheix *et al.*, 1990; Saure, 1990; Lancaster, 1992). In addition to their role as attractants, anthocyanins and other flavonoids accumulate in the epidermis, and help filter UV radiation, hence protecting the photosynthetic tissue (Beggs *et al.*, 1986; Schmelzer *et al.,* 1988; Stapleton, 1992; Koes *et al.*, 1993; Lister *et al.,* 1994; Kerckhoff & Kendrick, 1997). Currently, it is accepted that light (Downs *et al.*, 1965; Beggs *et al.,* 1986; Arakawa, 1988), temperature (Creasy, 1968; Tan, 1979; Faragher, 1983; Saure, 1990; Lancaster, 1992) and senescence (Chalmers *et al.*, 1973) regulate anthocyanin biosynthesis and accumulation. Levels of phenylalanine ammonia lyase (PAL) activity mediate this regulation.

Although the intensity and quality of the red skin colour is directly related to the anthocyanin concentration, the final apple skin colour as we perceive it, is determined by the interaction of anthocyanin molecules with other compounds. These compounds include chlorophyll, carotenoids, other flavonols (Lancaster, 1992; Lancaster *et al.*, 1994; Dong *et al.*, 1995), and colourless phenolic compounds (Mazza & Miniati, 1993). The flavonols and proanthocyanins do not contribute significantly to overall coloration but may be important in enhancing anthocyanin coloration by copigmentation (Lancaster *et al.*,

1994). The background yellow/green colour is due to the plastid pigments, chlorophylls and carotenoids (Lancaster, 1992; Lancaster *et al.*, 1997), whose concentration should, ideally, be low just prior to harvest, to remove any masking effect on the visibility of anthocyanins (Viljoen, 1996).

2. Anthocyanin biochemistry

2.1 Structure

Plant colour is due to three large pigment groups: chlorophylls, carotenoids and flavonoids (Brouillard *et al.,* 1997). Anthocyanins are classified as water soluble flavonoids (Mazza & Miniati, 1993), and are found in the vacuole (Mancinelli, 1983; Lancaster, 1992). Accumulation of anthocyanin is often limited to the epidermal and subepidermal cell layers and this is true in most apples and pears (Macheix *et al.*, 1990). The general pattern of anthocyanin distribution in red pears involves a nonpigmented epidermis and one or two additional nonpigmented layers, lying above two to five layers containing anthocyanins (Dayton, 1966). The total amount of flavonoids in apples increases throughout the season as fruit surface area increases and is present at 10-30 $g \cdot kg^{-1}$ dry mass (Lister *et al.*, 1994).

Flavonoids are compounds which possess a basic C_{15} (C_6-C_3-C_6) skeleton. The various flavonoid classes arise due to differences in the oxidation level of the central C_3 pyran ring. An exception to the latter being found in chalcones where the C_3 is open (Brouillard, 1982; Macheix *et al.,* 1990). Based on the degree of oxidation on the C_3 unit, flavonoids are divided into subclasses such as flavonols, flavanones, isoflavonoids and anthocyanins. Each type of flavonoid may undergo further modification(s) such as hydroxylation, methylation, acylation, glucosylation or rhamnosylation, resulting in the enormous diversity and colour of flavonoids found in nature (Koes *et al.*, 1993).

The carbon atoms in the flavan skeleton originate from two pathways: in ring A the carbon atoms are derived from a head to tail condensation of acetate units (Siegelman & Hendriks, 1958a; Beggs *et al.*, 1986), and in ring B the carbon atoms C2, C3 and C4 are derived from a phenylpropane unit. The phenylpropane residue derives from p-coumaric acid, itself formed via the shikimate pathway (Goodwin & Mercer, 1983). Anthocyanins consist of a 3-ring structure and have a positive charge delocalized over the entire structure (Markham, 1982).

There are six major anthocyanidins that contribute to fruit pigmentation namely pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Macheix *et al.*, 1990). Differences between individual anthocyanins are the number of hydroxyl groups and degree of methylation of these hydroxyl groups; the nature and number of sugars attached to the molecule and position of attachment; the nature and number of aliphatic or aromatic acids attached to the sugar and the physiochemical environment in which they are viewed (Brouillard, 1983; Mazza & Brouillard, 1990). Anthocyanidins may be glycosylated, usually at the 3, 5, or 7 positions on the A-ring and heterocyclic C-ring; they are then referred to as anthocyanins (Lancaster, 1992). These may be synthesised in response to many environmental factors such as light, cold stress, pathogen attack, mechanical damage, developmental-genetic factors, age, nutritional status and water conditions (Mancinelli, 1983; Gianfagna & Berkowitz, 1986; Dong *et al.,* 1995).

At present all anthocyanins isolated from apples have been derivatives of cyanidin (Lancaster, 1992). Cyanidin 3-galactoside has been identified as the major anthocyanin of apples (Mazza and Miniati, 1993; Timberlake and Bridle, 1971). Currently cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, and cyanidin 7-arabinoside have been identified as minor components in apples (Francis, 1970). Elucidation of the full anthocyanin complement in apples, as in pears, is still to be achieved. Francis (1970)

and Macheix *et al.* (1990) concluded that the two major pigments in apples and pears are identical, namely cyanidin 3-galactoside and cyanidin 7-arabinoside. Compared with other fruit, the concentration of anthocyanins in pear peels is distinctly lower (Mazza & Miniati, 1993).

2.2 pH

Most natural anthocyanins appear red at low pH, colourless at intermediate pH, and blue at higher pH in aqueous media (Brouillard, 1983; Mazza & Miniati, 1993). In acidic (pH>2) or neutral media, four anthocyanin structures exist in equilibrium: the flavylium cation AH^+, the quinonoidal base A, the carbinol pseudobase B, and the chalcone C (Mazza & Miniati, 1993).

In a strongly acidic aqueous media the anthocyanin exists predominantly as the red flavylium cation (AH^+). The two acidic hydroxyl groups present at positions 4' and 7' contribute to its change in colour. One loses a proton around pH 4 and the second close to pH 7 to form the purple/blue quinonoidal bases (neutral A and negatively charged A^-, respectively). These are two fast acid-base reactions (Figure 1) (Brouillard *et al.,* 1997).

In this aqueous environment, water molecules bind to the flavylium cation at pH levels near 2, forming the colourless hemiacetal (B), also known as carbinol or psuedobase. The flavylium ion can be restored by acidification (Figure 1) (Brouillard *et al.,* 1997).

These slower pseudo-acid-base reactions (covalent hydrations), are eventually followed by reversible central-ring openings leading to the formation of two isomeric chalcones (C). In the chalcone form, the anthocyanin may irreversibly degrade (Brouillard *et al.,* 1997).

2.3 Copigmentation

In vivo, stabilising mechanisms exist which strongly favour formation of coloured structures. The most efficient of these mechanisms is copigmentation (Mazza & Miniati, 1993). A copigment can be defined as a molecule which itself has no colour, but when placed in a pigment-containing solution, usually intensifies and may modify the initial colour of the solution (Brouillard *et al.*, 1997). Intramolecular copigmentation occurs when the copigment is part of the anthocyanin molecule, and intermolecular copigmentation occurs when a separate compound acts as the copigment (Mazza & Miniati, 1993). The main effect of a copigment is to produce, in association with almost all anthocyanins, an increase in colour intensity (hyperchromic effect) and a shift in the wavelength of the maximum absorbance towards higher wavelengths (bathochromic shift), giving slightly more purple to blue colours under certain conditions (Mazza & Miniati, 1993).

At pH<1, copigmentation has a negligible effect on colour. From pH around 1 to neutrality, copigmentation can produce colour enhancement and variation, depending on pigment and copigment structures and concentrations, temperatures and the nature of the medium where it takes place, with aqueous solutions producing the more spectacular results (Brouillard *et al.*, 1997; Mazza & Miniati, 1993).

Theoretically, apple coloration could be enhanced by copigmentation between cyanidin glycosides and quercetin glycosides. This stabilisation is actually minor, because changes in the hue of apple skin is more likely to result from the visual blending of chlorophyll, carotenoids and anthocyanins than from differences in copigmentation (Lancaster *et al.*, 1994).

3. Biosynthesis

3.1 Biosynthetic pathway

The flavonoids are derivatives of cinnamic acid, formed through the deamination of phenylalanine. The early steps in the conversion of phenylalanine to derivatives of cinnamic acids, by the enzyme PAL, which is common to the biosynthetic pathways of cinnamic esters, flavonoids and lignins (Lancaster, 1992), is known as the general phenylpropanoid pathway (Mancinelli, 1983; Beggs *et al*, 1986; Lancaster, 1992; Koes *et al.*, 1993). Malonyl-CoA is synthesised by carboxylation of acetyl-CoA, a central intermediate in the Krebs tricarboxylic acid cycle (Koes *et al.*, 1993).

In the formation of the various flavonoid classes, competition exists for the common chalcone precursor (Beggs *et al.*, 1986). The first specific flavonoid structure arises by condensation of one molecule of 4-coumaroyl-CoA with three molecules of malonyl-CoA, catalysed by the enzyme flavanone synthase (chalcone synthase). The product of this reaction, yellow coloured tetrahydroxy-chalcone (Dong *et al.,* 1995), originates from two different pathways of primary metabolism (Koes *et al.*, 1993). Chalcone is converted into naringenin flavanone (a flavanone with the basic structure of flavonoids) by the enzyme chalcone flavanone isomerase (CHI) (Mancinelli, 1983; Beggs *et al*, 1986; Lancaster, 1992; Dong *et al.,* 1995). This is an important enzyme, because flavanones are the intermediates for many flavonoids (Beggs *et al*, 1986).

Through 3' hydroxylation of the naringenin flavanone by the enzyme flavanone 3-hydroxylase, dihydroflavonol is formed (Grisebach, 1982). Quercetin glycosides are produced by the action of flavanone 3-hydroxylase in the conversion of dihydrokaempferol to dihydroquercetin (Lancaster, 1992). Dihydroquercetin is an important intermediate in the anthocyanin synthesis pathway. Catalysed by dihydroquercetin reductase (DFR), dihydroquercetin is transformed into flavan-3,4-diol

(leucocyanidin), which is the precursor for anthocyanidin or procyanidin synthesis (Ju *et al.*, 1995b).

Cyanidin is formed from leucocyanidin and thereafter cyanidin is glycosylated. Currently the reaction step(s) for the formation of anthocyanin from leucoanthocyanin are unknown and the enzyme is loosely referred to as anthocyanidin synthase (Lancaster, 1992). UDPgalactose:flavonoid-3-o-galactosyltransferase (UFGalT) is the last enzyme involved in anthocyanin synthesis in apples and responsible for attaching the galactose to cyanidin (Ju *et al.,* 1997).

3.2. Regulation of anthocyanin synthesis

Anthocyanin biosynthesis is developmentally regulated. Anthocyanin concentration is high in juvenile fruit during the time of intense cell division, decreases during fruit growth, and then increases during fruit ripening (Saure, 1990; Lancaster, 1992; Ju *et al.,* 1995b, Curry, 1997). The anthocyanins produced during early fruit development are identical in structure to those in ripe fruit (Lancaster, 1992). The occurrence of anthocyanin biosynthesis under a variety of environmental conditions, would indicate endogenous control. Anthocyanin formation appears to be suppressed in immature fruit, possibly as a result of endogenous gibberellins (GA) (Saure, 1990).

Chalmers *et al.* (1973) stated that the increasing capability of mature fruit to form anthocyanin compared to immature fruit, is not based on an increased endogenous rate of synthesis, but rather in the potential of the degradation of anthocyanin. The rate of degradation of anthocyanin in immature fruit is as rapid as it is formed. In mature fruit the rate of degradation is less than the rate of synthesis.

Due to the low phytochrome content of apples, anthocyanin formation in mature apples is dependent on photosynthetic activity (Downs *et al.,* 1965; Mancinelli and Rabino, 1984). It has been shown that inhibition of photosynthesis is effective in reducing the rate of anthocyanin synthesis (Downs *et al.,* 1965). This indicates that the synergistic effect of red light with UV (312 nm), is dependent on photosynthetic activity (Arakawa, 1988).

As a result of the above-mentioned observation by Mancinelli and Rabino (1984), Saure (1990) speculated that anthocyanin formation in young fruit does not depend on photosynthetic activity. This is based on the following line of reasoning: (1) young apples exhibit high meristematic activity; (2) meristematic tissues tend to be rich in phytochrome; (3) young apples are therefore assumed to be rich in phytochrome too.

4. Light

Light is a key regulatory factor in apple skin reddening (Chalmers & Faragher, 1977a; Tan, 1979; Arakawa *et al.*, 1985; Saure, 1990). The importance of light in the production of red colour, is accentuated by the absence of colour on the fruit that are in the inside of the tree canopy (Saure, 1990). This colour is not shared between cells, but direct irradiation of each single cell in the apple skin is needed to produce an even and overall red colour. This suggests that there is no communication between cells with respect to the distribution of colour in apple skin (Kootstra *et al.*, 1994).

The action of light on anthocyanin synthesis can be explained by two reactions involved. The first is the High Irradiance Response (HIR) reaction (Downs *et al.*, 1965). Plant responses that require (a) high irradiance levels for long periods, (b) different incident energy levels at different action spectra and (c) that have action spectra that are atypical of the commonly observed phytochrome responses, are known as the HIR reactions (Downs *et al.*, 1965; Mancinelli, 1983; Beggs *et al.*, 1986; Saure, 1990; Salisbury & Ross,

1992). Most phytochrome reactions are saturated by energy levels of red light equal to as little as 200 $J \cdot m^{-2}$ (this is less than 1 % of the energy provided by 1 minute of full sunlight). The HIR require at least 100 times more energy (Salisbury & Ross, 1992). Thus, in inducing anthocyanin production, high light intensities are required to establish the HIR reaction, and is then followed by the second reaction, which is a subsequent low-energy photoreaction, establishing phytochrome in its P_{fr} form (Downs *et al.,* 1965).

4.1 Photoreceptors

4.1.1 Phytochrome

Phytochrome is a homodimer of two identical polypeptides and attached to these polypeptides is a prosthetic group, called a chromophore. It is the chromophore, not the protein that absorbs the light that causes phytochrome responses (Mancinelli, 1985). There are two major types of phytochrome: type 1 and type 2. Type 1 predominates in etiolated seedlings, whereas type 2 predominates in green plants. These two types differ slightly in circumference, as well as the absorption spectra. Seedlings grown in total darkness contain 10 to 100 times as much total phytochrome as seedlings grown in light. As an organ/seedling develops either in light or in darkness, the different types of phytochrome develops. An abundance of type 1 (obtained in darkness) allows organs/seedlings to intercept very weak light and develop into normal green plants, and thus lose most of their type 1 phytochrome as more type 2 is synthesised (Furuya, 1989).

Phytochrome can consists in two forms: the physiologically inactive red-absorbing form P_r and the physiologically active far red absorbing form P_{fr}. Red light (R) converts P_r to P_{fr}, and P_{fr} in turn is converted to P_r both by far red (FR) as well as dark reversion. The absorption spectrum for purified phytochrome has a maximum in red wavelengths at about 666 nm for the red absorbing P_r form and at about 730 nm for the far red P_{fr} form (Mancinelli, 1985). Phytochrome regulates the abundance of its own mRNA by reversible

production and destruction when exposed to red or far red light. Production of P_{fr} by red light results in a decrease in transcription of the phytochrome genes. The transcription is remarkably rapid (within 5 minutes from exposure to red light). Thus phytochrome not only regulates numerous events, including colour development, during plant development, but also regulates the expression of its own genes (Colbert, 1988).

The conversion of phytochrome from the inactive P_r to the active P_{fr} form by absorbing light, causes *de novo* synthesis of the enzymes that catalyse the flavonoids (Beggs *et al.* 1986, Bruns *et al.,* 1986; Schmelzer *et al.,* 1988; Saure, 1990; Salisbury & Ross, 1992; Lancaster, 1992; Ju *et al.*, 1995b).

The expression of the HIR depends upon the presence of the physiologically active and unstable form of phytochrome (P_{fr}) (Siegelman and Hendricks, 1958b; Downs *et al.,* 1965; Mancinelli, 1984; Saure, 1990). Arakawa (1988) confirmed this with his experiments on postharvest colour formation of apples.

4.1.2 Cryptochrome

The second photoreceptor involved in anthocyanin production is cryptochrome (Mancinelli, 1983; Beggs *et al.*, 1986; Saure, 1990; Mancinelli *et al.*, 1991). Blue light (BL) was found to be significantly more effective in enhancing anthocyanin production in cabbage and tomato seedlings, than RF (a mixture of R and FR containing no BL), which indicates the involvement of cryptochrome. Since RF excites phytochrome, but not cryptochrome, and blue light excites both receptors, the difference in anthocyanin production between blue light and RF treatments that maintain the same state of phytochrome can be reasonably attributed to an involvement of cryptochrome in the response to blue light (Mancinelli *et al.*, 1991). The nature of cryptochrome is unknown.

It could be a flavin derivative, but carotenoids may also be involved in mediating the action of UV/BL radiation (Mancinelli, 1983).

4.1.3 UV-B receptor

Mancinelli (1980) postulated the possible involvement of a third receptor, called the UV-B receptor. Experiments by Arakawa (1988) confirmed that a specific photoreceptor for UV-B was involved. Irradiation with far-red (FR) light did not influence the effect of UV-B on anthocyanin synthesis, suggesting that the effect of UV-B was independent of phytochrome. The peak of action for this receptor is 300 nm with little effectiveness above 320 nm (Beggs *et al.*, 1986).

As mentioned before, it is suggested that light (including UV-B) stimulates the synthesis of the enzymes involved or increases the concentration of the substrates needed, but Yatsuhashi and Hashimoto (1985) suggested that UV light stimulates large amounts of the UV photoreceptor.

4.1.4 Coactions

Coaction is where one photoreceptor appears to modify the response to another, resulting in a response greater or less, than the sum of the responses to each photoreceptor alone (Beggs *et al.*, 1986). There is some evidence for the interaction between phytochrome and cryptochrome in the photoregulation of anthocyanin production under prolonged irradiation. Hypotheses on photoreceptor interactions suggest that P_{fr} might be required for the expression of the cryptochrome-mediated effects of blue light. Thus the state of phytochrome might be a limiting factor for the expression of cryptochrome mediated reactions. On the other hand, cryptochrome might establish of enhance the sensitivity of the responding system to P_{fr} (Mancinelli *et al.*, 1991).

The unknown nature of the UV-B photoreceptor and cryptochrome, and the fact that the UV- and BL excite not only these two photoreceptors, but also phytochrome, complicate studies on photoreceptor involvement and interaction in the mediation of responses to UV and BL (Mancinelli *et al.*, 1991).

4.2 Spectral sensitivity

The spectral sensitivity of the HIR varies with plant species and three general response groups have been identified:

Group 1: Peaks of action are found in the UV-BL, R and FR regions (Mancinelli, 1983).

Group 2: The main peak of action is in the R, but the UV-BL region is also effective, whereas action in the FR is either absent or minimal. FR can even have an inhibitory effect (Mancinelli 1983; Mancinelli & Rabino, 1984; Saure, 1990).

Group 3: There is action only in the UV-BL region (Mancinelli, 1983).

In post harvest irradiation of apples, peaks of action have been found in the (a) ultraviolet (UV-A, 320-400 nm), (b) blue (BL, 400-480 nm) (Bishop & Klein, 1975) and (c) red (R, 600-690 nm) regions of the spectrum. Recently it has been shown that (d) UV-B (290-320 nm) is also active in stimulating anthocyanin production (Chalmers & Faragher, 1977a; Mancinelli, 1983).

The following table summarises the responses of various apple and pear cultivars to the different action spectra of artificial light. Lights were applied postharvest, unless specified.

Cultivar	Action spectra	Reference	Remark
Jonathan, Rome Beauty, Arkansas	650 nm (red)	Siegelman & Hendriks (1958a), Downs *et al.* (1965)	
McIntosh	400 nm (blue), 650 nm (red light) additive	Bishop & Klein (1975)	
Jonathan	254 nm (UV-C)	Chalmers & Faragher (1977a)	
Matsu (yellow-coloured)	White light alone, white light (red) + UV-B (312 nm)	Arakawa *et al.* (1986)	No response to white light and slight effect to white + UV-B
Starking Delicious, Fuji	White light (red) + UV-B (312 nm)*	Arakawa *et al.* (1986)	Starking Delicious higher anthocyanin production than Fuji. UV-B (312nm) produced 4 times the anthocyanin compared to white light alone.
Starking Delicious, Fuji	White light	Arakawa *et al.* (1986)	Starking Delicious higher anthocyanin production than Fuji
Jonathan	UV-B	Arakawa *et al.* (1985)	
Attached McIntosh	400-480 nm (blue)	Procter & Creasy (1971)	
Attached McIntosh	Far red light (700-775 nm)	Procter & Creasy (1971)	No effect
Royal Gala	Red + UV-B or UV-B alone	Arakawa *et al.* (1985)	
Royal Gala	Red, blue and UV-A light alone	Arakawa *et al.* (1985)	Slightly effective
Sensation Red Bartlett	600 nm (red) and 400-500 nm (blue)	Dussi *et al.* (1995)	400-500 nm gives darker, redder pears

From the table, it is clear that combinations of certain action spectra can have synergistic effects on colour development. This can be explained by an example of the combination of white light and UV-B light that stimulates anthocyanin production synergistically up to the highest energy flux of white light tested. This synergistic effect is actually that of the UV and the red region of white light, and with the combination of this lights, four times the amount of anthocyanins was produced compared to the use of only white light (Arakawa *et al.*, 1986).

Mancinelli (1984) found that light pretreatments changed the spectral sensitivity on the continuous irradiation response of cabbage, rye and tomato seedlings. Blanke (1989) found that the peel of young fruits absorbed more light than that of mature fruits. Red peel absorbed more light below 620 nm than green peel and compared with natural sunlight, the light absorbed by the peel was richer in blue-green light and poorer in red light.

4.3 Responses to different light intensities

4.3.1 Initiation

The extent of the response to irradiation is a function of duration and fluence rate of the exposure. Increasing the light intensity has a promotive effect on anthocyanin biosynthesis. The formation of anthocyanin is considerably higher in continuous light than under intermittent light cycles of 14 hours light and 10 hours dark (Faragher, 1983). The minimum energy requirement for anthocyanin synthesis and responsiveness to light, varies considerably with cultivar, and changes during the season (Procter, 1974; Arakawa, 1988). For example 'Starking Delicious' and 'Jonagold' had a lower light threshold than 'Tsuragu', 'Fuji', 'Mutsu' and 'Golden Delicious' (Arakawa *et al.*, 1986). Procter and Creasy (1971) found that energy levels of 5 $W \cdot m^{-2}$ for 48 hours were

adequate to initiate pigment synthesis in attached 'McIntosh' apples. Thus a minimum energy of 100 $J \cdot cm^{-2}$ is needed to initiate anthocyanin synthesis in attached fruit. A threshold of about 3.2 $W \cdot m^{-2}$ is required before anthocyanin synthesis occurred in detached apples (Siegelman & Hendriks, 1958a).

4.3.2 Lag phase/induction period

Siegelman and Hendriks (1958a) observed that green peel of detached mature apples of various cultivars required a preliminary irradiation period of about 20 hours before the onset of anthocyanin formation. The induction period, during which anthocyanin is not formed, can be interpreted as the time required to increase the concentration of a substrate to a level permitting the steady state formation of anthocyanin at a rate proportional to irradiation intensity. The duration of the lag phase also varies depending on the cultivar. The lag phase for 'McIntosh' apples is 12 to 15 hours (Procter & Creasy, 1971).

Procter & Creasy (1971) and Bishop and Klein (1975) showed that continuous fluorescent light did not shorten the induction period for anthocyanin synthesis in detached or attached 'McIntosh' apples. Keeping apples in the dark before irradiation reduced the induction period by 40%, but the same amount of anthocyanin was formed compared to apples that were immediately irradiated (Siegelman & Hendricks, 1958a).

4.3.3 Pretreatments

In seedlings of cabbage, rye and tomato, exposure to light pretreatments brought about an enhancement of the inductive R-FR reversible response and a decrease in the response to continuous irradiation. The extent of the R-FR reversible response is larger in light-pretreated than in dark pretreated systems. Small, but detectable quantities of

anthocyanin are formed during a dark incubation period following a short exposure to light (Mancinelli, 1983).

The different effect of light pretreatments on inductive and HIR responses is difficult to explain. Mancinelli (1984) suggested that the decrease of the HIR response is partly the consequence of the reduction of the phytochrome level. A possible explanation is that the action of P_{fr} under inductive conditions might be different from the action of P_{fr} under HIR conditions. The light-dependent enhancement of the inductive response is a result of changes in availability of 'receptors' for P_{fr} and in 'responsiveness' of cell functions on which phytochrome acts. Anthocyanin production in cabbage leaf disks and *Spirodela* requires photosynthetic activity and the light-dependent enhancement of the inductive response might be a consequence of an increase in the level of the precursors required for anthocyanin production (Mancinelli & Rabino, 1984).

4.3.4 Linear period

The induction period/lag phase is followed by a linear phase. The anthocyanin production of this linear phase is a function of time of irradiation at constant irradiance and energy flux (Siegelman & Hendricks, 1958a; Bishop & Klein, 1975). The duration of the linear period can be a further 100 hours (Chalmers & Faragher, 1977a) for whole apples and is 40 to 60 hours in apple skin sections (Siegelman & Hendricks, 1958a).

4.3.5 Light intensities of linear period

It seems that short treatments (10 minutes) of UV light prior to light exposure increased the accumulation of anthocyanin in whole fruit to about twice the levels of the control (Faragher, 1983). Different energy levels are needed for different action spectra/lights. Appreciable synthesis of cyanidin 3-galactoside in the apple skin, requires 7 $J \cdot cm^{-2}$ incident energy at the action maximum near 650 nm (red light) and about three times as

much energy in the region of the subsidiary maximum between 430 and 480 nm (blue) (Downs *et al.,* 1965). The amount of anthocyanin formed varies linearly with intensity from 8.54-31.64 $W \cdot m^{-2}$ (Siegelman & Hendriks, 1958a).

The wavelength of light used and the cultivar, is important to determine saturation levels. The production of anthocyanin in 'Jonathan' apples became saturated at about 30 $W \cdot m^{-2}$, when irradiated with white light, and at 17 $W \cdot m^{-2}$ when irradiated with UV-B light (Arakawa *et al.*, 1985). Saturation appeared at 14.5 $W \cdot m^{-2}$ (13°C) in 'Anna' apples when irradiated with cool-white fluorescent light (Saks *et al.*, 1990). Arakawa *et al.* (1986) found different saturation levels for the different cultivars when irradiated with different wavelengths of light (red or white or UV light) ('Fuji', 'Starking Delicious' & 'Mutsu'). The synergistic effect of white light and UV-B, increased up to 44 $W \cdot m^{-2}$, which is the highest fluence rates of white light tested. The combination of white light and UV light, is much more effective than with white light alone, despite the lower intensity used (Arakawa *et al.*, 1985). With increasing UV-B fluence, the epidermal layer is destroyed (Tevini *et al.*, 1981).

4.3.6 Period following irradiation

Mancinelli (1984) concluded that anthocyanin continues to accumulate during a 24 hours period following exposure to light, while Siegelman and Hendriks (1958a) stated that anthocyanin synthesis proceeded in the dark for about 2 days after removal from irradiation. Temperature and duration of the dark period following irradiation have considerable influence on the total amount of anthocyanin formed.

4.3.7 Irradiation of skin discs

Anthocyanin synthesis in skin disks is much higher than in whole fruit skin. In 'Fuji' especially, it is about 7 times as high as in whole fruit skin at 17 $W \cdot m^{-2}$ of white light.

Therefore the latent potential of this cultivar to produce anthocyanin under white light can be fully expressed by wounding (Arakawa *et al.*, 1986). Wounding stimulates anthocyanin production as a result of higher concentrations of ethylene, but this effect differs with cultivar. In wounded tissue, UV light treatment does not increase anthocyanin accumulation or PAL activity (Chalmers & Faragher, 1977b).

4.4. Effect of light on enzymes

Although PAL is not the only enzyme involved in the regulation of anthocyanin synthesis, it is a critical enzyme in controlling the flavonoid synthesis in many plant systems (Chalmers and Faragher 1977b; Arakawa *et al.,* 1986; Blankenship and Unrath 1988; Saure, 1990; Lister *et al.,* 1994; Ju *et al.* 1997). An increase in PAL activity is usually followed by an increase in anthocyanin level, indicating that PAL activity is necessary for the accumulation of anthocyanin (Chalmers and Faragher, 1977b; Tan, 1980). The stimulation of PAL synthesis by light is mediated via the phytochrome system. In apple, PAL activity has been found only in those parts of the skin that were exposed to the sun (Saure, 1990). Virtually no PAL activity and no anthocyanin synthesis were found in attached fruit without light/constant darkness (Chalmers and Faragher, 1977; Tan, 1979; 1980). On the other hand, Blankenship and Unrath (1988) found that the skin of 'Starkrimson Delicious' gradually developed colour even when the PAL level was very low. They also found that the PAL activity was comparable in the cultivars 'Starkrimson Delicious' and 'Golden Delicious', during maturation despite their great difference in capacity for red colour formation.

PAL contributes by regulating concentrations of precursors available for anthocyanin synthesis (Ju *et al.,* 1995a). Its primary products from feedback control could modulate PAL activity, and both PAL activity and the concentration of the precursors might regulate the rate of anthocyanin accumulation. Accumulation of anthocyanin in red apples is likely

to involve additional enzymes between leucocyanidin and cyanidin glycosides, and these may be the key light-inducible enzymes involved in apple skin reddening (Lancaster, 1992; Ju *et al.,* 1997).

Again, the irradiation periods differ between the different cultivars to acquire the maximum activity of the enzymes involved in anthocyanin synthesis. PAL, chalcone synthase and chalcone isomerase (CHI) (leading from phenylalanine to p-coumaroyl CoA) reached maximal activity at about 15 hours after irradiation started (Grisebach, 1982; Beggs *et al.,* 1986). PAL activity in 'Starking Delicious' and 'Fuji' reached a maximum after 24 hours of irradiation, again higher under UV and white light than under white light alone. PAL activity was stimulated more in disks than in whole fruit. When skin disks were kept in the dark, PAL activity increased slowly for at least 72 hours. The enzymes catalysing the formation of a chalcone from p-coumaroyl CoA and three malonyl CoA and later steps in the flavonoid pathway reached their maximum a few hours later (Beggs *et al.,* 1986).

PAL activity in 'Royal Gala' apple skin increases in a linear fashion (10 to 20-fold) over a 3-day irradiation period (Dong *et al.*, 1995). Chalmers and Faragher (1977b) described similar observations in 'Jonathan' apples, except that they observed a decline in PAL activity, which could be the result of the 15 minutes irradiation period in comparison to the 3 day period. After a lag phase of less than 10 hours, PAL activity increased to a maximum at 30 hours and then declined. CHI activity reached a maximum after 1 day of irradiation and stayed constant during the next 2 days of irradiation (Dong *et al.*, 1995). Chalmers and Faragher (1977a) studied the interaction between CHI and UV-light and found that in whole fruit, CHI applied as a dip (concentrations greater than 1 $g \cdot ml^{-1}$) prior to exposure to light, promoted anthocyanin accumulation. The amount of UV light and

CHI necessary to obtain the synergistic response decreased as the CHI concentration increased.

Although whole fruit skin shows a close correlation between anthocyanin synthesis and PAL activity, in skin disks, PAL activity was far higher than in whole fruit skin, but anthocyanin synthesis did not show a similar increase in PAL activity. In some cultivars which do not normally produce red colour, such as 'Matsu' and 'Golden Delicious', white light induced PAL activity, but no anthocyanin accumulated (Arakawa *et al.*, 1986). Thus, although anthocyanin production is associated with an increase in PAL activity, PAL activity may occur without any anthocyanin synthesis. This is understandable, since PAL is active in the biosynthesis of a wide range of phenylpropanoid compounds (Lancaster, 1992).

4.5. Other effects of artificial light on apples and pears

Other characteristics of the action of UV-B and irradiation under increasing fluence rates, are the reduction of the fresh weight and the change of the amounts of chlorophylls, carotenoids and galactolipids of the chloroplasts. The fresh weight fell proportionally with the chlorophylls and carotenoids, and the flavonoid content of barley leaves rose in parallel to the increasing UV-B fluence rates. Of importance is the 50% increase in flavonoids concentration in response to illumination (Tevini *et al.,* 1981).

Carotenoids protect the chlorophylls from destruction and therefore are less damaged when irradiated. The biosynthetic pathway of chlorophyll might be more influenced by UV-B than that of carotenoids. The ratio of chlorophyll a to chlorophyll b rises in UV-irradiated plants, and UV-B radiation stress inhibits the biosynthesis of chlorophyll b more than chlorophyll a (Tevini *et al.*, 1981).

Another effect of irradiation by UV-B is water loss, possibly as a result of alterations to the epidermis. After application of UV-B there is also a rise in the water-soluble proteins synthesised and it is still unknown whether the biosynthesis of all proteins and their amino acids is stimulated. It is possible that only the synthesis of the aromatic amino acids is enhanced, and they are precursors for the biosynthesis of flavonoids (Tevini *et al.*, 1981).

The ability of apples previously stored in air, to synthesise anthocyanin under light decreased sygmoidally with storage time. Apples from CA storage showed no time-dependant decrease in synthetic ability, but, if transferred to regular storage, they exhibited the same decline as did apples from regular storage. Apples may maintain considerable capacity for anthocyanin formation after 6 months of storage or even longer (Bishop & Klein, 1975).

5. Other factors affecting synthesis

In addition to light there are three other factors contributing to colour development. First, the temperature must be high enough and of sufficient duration in the daylight hours to catalyse anthocyanin biosynthesis. Secondly, colour development will vary depending on the stage of fruit maturity. Anthocyanin is produced at a much lower rate after the climacteric phase is entered. Thirdly, a period of cold temperature stimulates anthocyanin production. A few nights of temperatures in the range of 2 to 5°C followed by warm sunny days promotes red colour development (Curry, 1997).

5.1. Temperature

There is a relationship between anthocyanin formation and temperature. Low temperatures enhance, and high temperatures inhibit anthocyanin synthesis (Saure, 1990). Temperature affects anthocyanin synthesis via the regulatory enzyme PAL

(Faragher, 1983). Creasy (1968) suggested that low temperature influences a reaction occurring early in the formation of anthocyanin rather than at a later stage in synthesis. Temperature does not appear to affect PAL levels via an effect on ethylene production or ripening. PAL and ethylene levels change in opposite directions in response to a change in temperature. The effect of temperature varies for fruit on the tree and for fruit after harvest and it depends on maturity, light intensity and quality, cultivar and the stage of fruit development (Faragher, 1983; Arakawa, 1991; Mazza & Miniati, 1993).

5.1.1 Preharvest temperatures

As mentioned above, low temperatures enhance anthocyanin synthesis by increasing the rate of synthesis in exposed tissue, as a result of higher concentrations of PAL at lower temperatures (6°C) (Tan, 1980; Lancaster, 1992). Low temperatures also permit synthesis in poorly exposed skin (Creasy, 1968). Lancaster (1992) suggested that low temperature reduced the loss of sugars in the skin through respiration, resulting in increased photosynthate available for anthocyanin biosynthesis. Low day and night temperature causes the highest anthocyanin accumulation and Macheix *et al.* (1990) and Mazza and Miniati (1993) found 12°C and 2°C respectively to be the best temperatures for anthocyanin accumulation in apples. The temperatures vary between cultivars and can be between 15 to 25°C and 10 to 20°C respectively. A constant temperature of 15°C ('Jonagold') (Macheix *et al.,* 1990), as well as 20°C ('Fuji') is also effective (Arakawa, 1991). Each stage of ripening has its own optimum temperature range for red colour development (Saure, 1990). Together with low night temperatures, Naumann (1964) suggested that the optimum day temperature for anthocyanin accumulation was 23°C.

5.1.2 Postharvest temperatures with irradiation

Temperature affects both the rate of anthocyanin production, and the length of the induction period before anthocyanin synthesis (Siegelman & Hendriks, 1958b). Low

temperature (6°C) both in light and dark conditions stimulated the accumulation of PAL (Tan, 1979). The PAL level in 'Red Spy' apples that received alternating 6/18°C and 6/25°C treatments in light was 20 % higher than that in apples that received light at constant 18°C and 25°C, respectively (Tan, 1979; 1980). In poorly exposed skin at low light levels, low temperatures increase the efficiency of anthocyanin synthesis, although they do not reduce the obligate light requirement (Saure, 1990).

Red colour development with irradiation was tested at different temperatures. Fastest colour development occurred after one day of illumination at a temperature of 20°C (Saks *et al.,* 1990; Dong *et al.*, 1995). Induction of red pigmentation is slowest at low temperatures (2°C), therefore the induction of colour development was slower at 13°C, but fruit colour resulted in a degree of redness that did not differ significantly from that of the fruit illuminated at 20°C (Saks *et al.,* 1990).

The levels of PAL activity induced by light are inversely proportional to temperature (Faragher, 1983). Optimum temperatures for anthocyanin accumulations in 'Jonathan' apples, when irradiated with white light, were 12°C in unripe apples and 16 to 24°C in ripe apples. This can be explained by the PAL levels being higher at low than at high temperature and higher in ripe than in unripe apples (Faragher, 1983). Arakawa (1991) found that the optimum temperature for inducing anthocyanin synthesis increased from 15°C to 25°C as fruit ripens. Faragher (1983) concluded that fruit ripening has a greater effect in colour formation than does low temperature. Saure (1990) indicated that during ripening a change occurred from promotion to inhibition of anthocyanin formation at low temperatures, both in attached and detached fruit. The loss of inhibition by high temperatures, and the loss of promotion at low temperatures was enhanced in apples that were picked earlier. The riper the apple, the higher the optimal range of temperature for colour formation following illumination.

In 'Jonathan', the optimum temperature for anthocyanin accumulation increased clearly from 15°C to 25°C in non-bagged and from 20°C to 25°C in bagged fruit as it ripened. In 'Fuji' and 'Jonagold', 20°C and 15°C, respectively, were the optima for anthocyanin synthesis in both non-bagged and bagged apples (Arakawa, 1991).

5.1.3 PAL-IS

In apple skin, PAL is regulated by a PAL-inactivating system (PAL-IS) (Macheix *et al.,* 1990; Mazza & Miniati, 1993). PAL-IS accumulates while PAL activity declines and anthocyanin synthesis stops, when in constant darkness. The initial rate of PAL-IS accumulation is higher under high temperature, but as this temperature decreases, the PAL-IS declines also at a faster rate after its peak value has been reached (Tan, 1980). The concentration of PAL-IS decreases after exposure of the fruit to light (Macheix *et al.,* 1990). In constant light, the activity of PAL-IS is lower in the apples that received alternating 6/20°C treatment than the apples that received constant 25°C. Apples held at constant darkness, show no PAL activity and no anthocyanin accumulation, but PAL-IS increases with time (Tan, 1980). The PAL-IS of whole apples held in light and dark conditions is 3.5 times higher than that in apple skin disks after 44 hours of incubation at 18°C (Tan, 1979).

5.2. Ethylene

Wounding, UV-light, maturity and cycloheximide stimulate anthocyanin production, most likely via a common factor, ethylene (Chalmers and Faragher, 1977a). Ethylene promotes anthocyanin formation and also increases the activity of PAL (Camm & Towers, 1973). During fruit ripening of some cultivars ethylene increased and the concentration of anthocyanin increased more than 5-fold (Chalmers *et al.,* 1973). Temperature influences the development of ethylene production in some apple and pear cultivars (Knee *et al.*,

1983), but Blankenship (1987) showed that night temperatures does not affect the onset or rate of ethylene production.

UV light from biocidal lamps (254 nm) stimulates ethylene production (Chalmers & Faragher, 1977a), but Arakawa *et al.* (1985) found that there was no increase in ethylene production following irradiation with UV 312 nm, although anthocyanin synthesis increased. Under field conditions, 254 nm radiation is absent, but the fluence rate of the UV-B is considerable. Therefore the effective UV region in sunlight inducing large amounts of anthocyanin may be the UV-B region. It appears that the stimulation of anthocyanin synthesis with sunlight is not mediated by ethylene. Saks *et al.* (1990) concluded that 'Anna' apples could reach satisfactory red pigmentation without increasing the ripening of fruit. Fruit illumination did not accelerate the ripening beyond that of commercially harvested fruit held in darkness for 3 days at 20°C.

On the other hand, Faragher and Brohier (1984) stated that there is a close correlation between increases in PAL activity and the potential for anthocyanin accumulation during ripening, where ethylene plays an important role. In ripe apples, ethylene treatment has no significant effect on the rate of the anthocyanin accumulation or PAL activity. In ripe apples, ethylene increases the final anthocyanin concentration. Ethylene treatment of unripe apples increased the rate of anthocyanin accumulation 3- to 4-fold and PAL levels 3- to 6-fold (Faragher & Brohier, 1984). Gomez-Cordoves *et al.* (1996) also concluded that ethephon treatment increased the concentration of anthocyanin in the skin over the ripening period.

Enclosing 'Fuji' apples in paper bags two months after full bloom delayed the increase in internal ethylene concentration at the onset of fruit ripening, and increased the respiration

rate early in the bagging period (Tan *et al.*, 1998). Thus time of bagging fruit determines onset of ethylene production during fruit ripening.

5.3 Sugar

Glucose, galactose, arabinose, xylose and rhamnose are the sugars involved in anthocyanin synthesis (Beggs *et al.*, 1986). It seems that the red side of apples contains more sugar than the green side. Magness (1928) proved that after long periods of irradiation, apples with more sugar produced more anthocyanin than apples with less sugar.

The decrease in anthocyanin concentration after the initial peak in the meristematic phase, could be a result of a shortage of sugars due to the conversion of sugar to starch. The result of this competition for sugar during synthesis of carbohydrates, could decrease anthocyanin synthesis. Alternatively, a drop in light intensities as the canopy increases, could also decrease anthocyanin synthesis. As soon as the fruit mature, the levels of sugars, e.g. galactose would be sufficient to favour anthocyanin synthesis (Chalmers *et al.* 1973). The amount of sugar available for anthocyanin formation just after the meristematic phase is an interesting question as it reflects the number of unanswered questions about the involvement of sugar. As mentioned earlier, Lancaster (1992) suggested that low temperatures reduce the loss of sugars in the skin through respiration, resulting in increased photosynthate, which would flow through to anthocyanin biosynthesis.

Downs *et al.,* (1965) concluded that sucrose, either stored or supplied, rather than direct photosynthetically derived compounds, supplies the carbon substrate for the high-energy photoreaction that is required for appreciable synthesis of anthocyanin in apple skin. The increase of anthocyanin synthesis that concurs with a decrease in chlorophyll could arise

from an increased sugar supply. Uota (1952) concluded from temperature treatments that sugar *per se* was not an important factor in the synthesis of anthocyanin pigment as there seemed to be no correlation between sugar content and colour development at the time of fruit maturity.

Many researchers showed an increased anthocyanin concentration in apple peel or apple discs with illumination, but this could also have been as a result of ethylene production stimulated by the wounding action of peeling (Siegelman & Hendriks, 1958a). Giafagna & Berkowitz (1986) were able to reverse the reduction of anthocyanin formation in dark-pretreated skin discs of 'McIntosh' apples into promotion when glucose was added. The effect of sucrose in enhancing anthocyanin production is more pronounced under UV-A than under blue, red and white light (Mancinelli & Rabino, 1984; Giafagna & Berkowitz, 1986).

6. Conclusions

The two important variables involved in colour development are temperature and light, and both variables can be used to manipulate pre- and postharvest colour development. Many practices are adopted to improve preharvest colour, and these cultural practices could affect the postharvest responses. It could be financially viable to improve red colour development of poorly coloured fruit after harvest. To manipulate postharvest colour it is necessary to understand the biochemistry of anthocyanins. More research is required before all the processes involved in the photoregulation of anthocyanin production and other HIR responses are understood. The concept of using artificial light in conjunction with optimum temperature regimes and the optimum developmental stage of the fruit, to improve red colour to a required export level, would be beneficial to the industry. This concept requires considerably more research before becoming a commercially feasible solution. At this point it is clear that apples colour after harvest, but

sufficient anthocyanin synthesis still requires a radiation exposure time that is economically impractical. The factor that accounts for the long irradiation period is the approximately 20 hours required for induction of synthesis. The ideal is to make the irradiation concept useable to the fruit producer, by gaining knowledge of the cultivar specifications with respect to irradiation, such as inductive period, saturation levels, irradiation intensities, types of lights used, etc. This information can be used to identify the variables that can be manipulated for increased accumulation of anthocyanin.

Some of the variables still to be investigated are the optimum temperatures, which, in conjunction with light, stimulates synthesis. Determining the temperature and light levels that inhibits PAL-IS accumulation, could also help to promote anthocyanin production. Also important is the level of photosynthate that is available for anthocyanin production, and possible changes in pH during postharvest treatments that may affect anthocyanin expression. Another factor involved in the visibility of anthocyanins is chlorophyll masking. Chlorophyll degradation is therefore an additional variable to investigate.

There is very little published information on anthocyanin physiology in pears. There is no information available on the pattern of synthesis, the requirements for light at different stages of development to develop acceptable colour, and the ability of pears to form colour after harvest.

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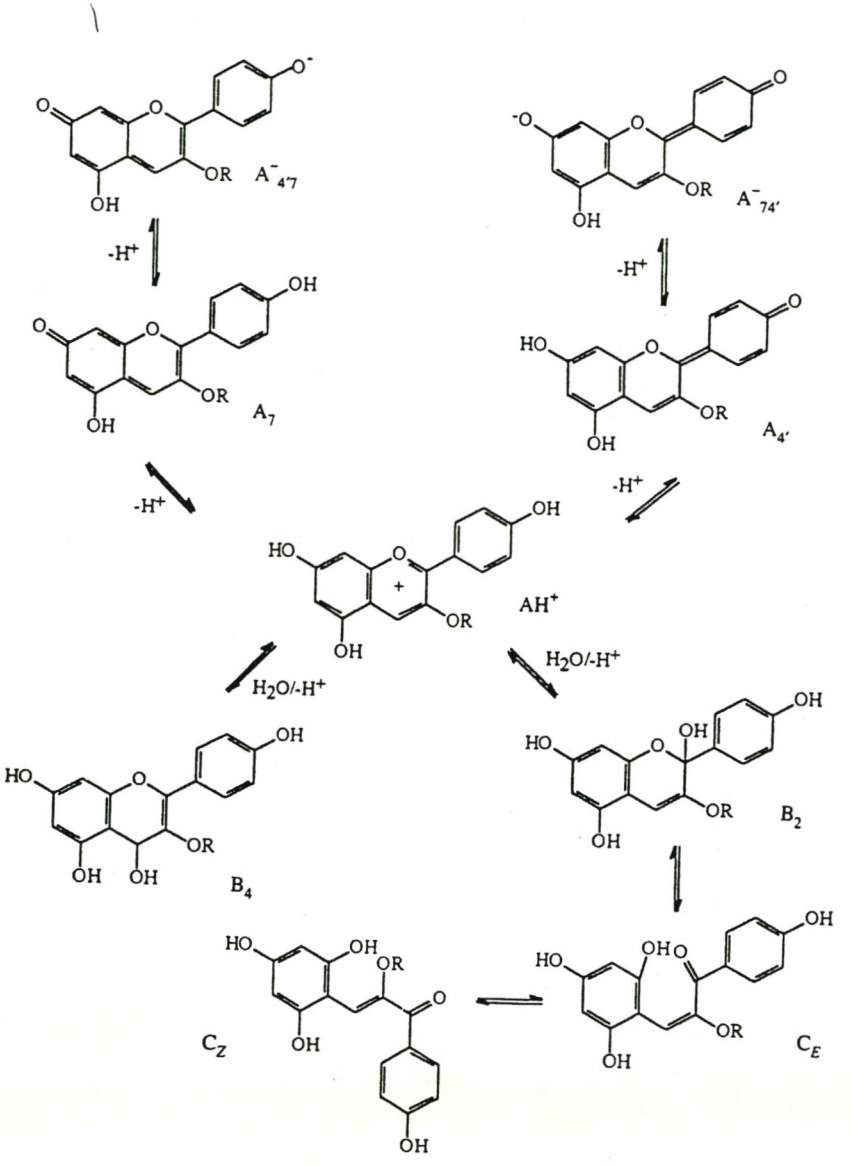

Fig. 1 Structural transformations of pelargonidin 3-glucoside. R stands for a glucosyl group (see Brouillard *et al.*, 1997).

PAPER 1: Postharvest irradiation affects colour development of apples and pears.

Abstract

Two apple cultivars and a pear cultivar were irradiated after harvest to stimulate anthocyanin production and improve skin colour. 'Cripp's Pink' apples were irradiated immediately after harvest. UV-B + incandescent light was compared to sodium light, and fruit were irradiated for 0, 24, 48 and 72 hours. According to the decrease in hue angle, the sodium light was more effective in increasing skin colour, than UV-B + incandescent light. Sodium light resulted in an increase of at least three times the initial anthocyanin concentration, compared to the doubling of the anthocyanin concentration after irradiation with UV-B + incandescent light. 'Braeburn' apples and 'Forelle' pears were stored for 4 and 8 weeks before being irradiated with sodium light for 0, 24, 48 and 72 hours. In 'Braeburn' apples there was a visible decrease in the hue angle and a significant increase in anthocyanin concentration. The effect on hue angle and anthocyanin concentration was more pronounced in the fruit stored for 4 weeks compared to those stored for 8 weeks. In 'Forelle' pears, however, the decrease in hue angle was associated with a change in skin colour from green to yellow and not an increase in anthocyanin concentration. Thus, applying sodium light postharvest had a positive effect on red colour development of 'Braeburn' apples, but not 'Forelle' pears. The postharvest irradiation of fruit had no significant effect on the firmness of both apples and pears. When UV-B + incandescent light was used, no significant increase in water loss was measured, compared to the significant linear increase with sodium light.

Introduction

High quality apples are produced in colder regions in the world, but fruit production has extended into warmer areas (Blankenship, 1987). South Africa is considered one of the warmest apple growing areas in the world, and as a consequence problems are experienced with red colour development. Fruit colour has long been a valued attribute, both commercially and aesthetically (Lancaster, 1992), and contributes to the marketability of fruit (Mancinelli, 1983; Saure, 1990).

Red skin colour is mainly due to anthocyanin pigments (Mancinelli, 1983; Macheix *et al.*, 1990; Saure, 1990; Lancaster, 1992). There are two peaks of anthocyanin production in apples. The first occurs in young fruit during cell division, and the second takes place during maturation of the fruit. Anthocyanin concentrations increase three to five fold during the month prior to harvest. During this time, chlorophyll decreases to a quarter of the original concentration, and carotenoids increase fourfold (Gorski & Creasy, 1977; Lancaster, 1992).

Temperature and light are the two most important factors influencing anthocyanin production (Downs *et al.*, 1965; Beggs *et al.,* 1986; Blankenship, 1987, Arakawa, 1988). Since little can be done to reduce temperature, the emphasis is on improving light interception in orchards by pruning and training (Ferree *et al.*, 1993). There has also been some success in improving red colour development both on the tree and after harvest, using artificial light, without affecting firmness (Siegelman & Hendricks, 1958; Procter *et al.*, 1971; Arakawa *et al.*, 1985; Mancinelli, 1985; Arakawa, 1988, Saks *et al.*, 1990; Saure, 1990; Dong *et al.*, 1995; Dussi *et al.*, 1995).

Light-dependent anthocyanin production requires prolonged exposures to relatively high fluence rates of visible (white light: 380 to 780 nm) and near visible (UV light: 280 to 380 nm) radiation for a definite expression (Mancinelli, 1985). This is the so-called high irradiance response (HIR). An interaction between phytochrome and

cryptochrome is involved in the photoregulation of the HIR and anthocyanin production (Mancinelli *et al.*, 1991). Arakawa (1988) also found evidence for a specific photoreceptor for UV-B light.

The spectral sensitivity of anthocyanin production under continuous irradiation is controversial (Mancinelli, 1980). All the research indicates that a range from UV B (280 to 320 nm) to red light (680 to 780 nm) can increase anthocyanin production (Siegelman & Hendricks, 1958; Arakawa *et al.*, 1985). Simultaneous irradiation with white light and UV-B light stimulates anthocyanin production synergistically. This synergism is actually between the red region of white light and the UV-B. It seems from recent studies that red light alone, is only slightly effective in stimulating anthocyanin production, but the effect of UV-B is considerable, even without the synergistic action of red light (Arakawa *et al.*, 1985).

White light is usually obtained from white fluorescent lamps, UV light from UV fluorescent lamps and red fluorescent lamps are used to obtain red light. Far red light is generated by an infrared tungsten bulb (Arakawa, 1988). Filters can be used to eliminate wavelengths not required (Arakawa *et al.*, 1985; 1986; Arakawa, 1988; Saks *et al.,* 1990; Dong *et al.,* 1995).

The objectives of this experiment were firstly, to study the postharvest improvement of colour in bi-colour or blushed cultivars of apple and pear, using artificial lights; secondly, to determine whether storage at –0.5°C before irradiation would affect the response; and thirdly, to investigate the response to different kinds of lights.

Material and methods

Plant material: 'Forelle' pears, and 'Braeburn' and 'Cripp's Pink' apples were obtained from three different farms in the Koue Bokkeveld area near Ceres in the Western Cape, South Africa (latitude: 33°10'S; longitude: 19°20'E; altitude: 800 to 900

m). The Western Cape has a Mediterranean climate with cool, wet winters and hot, dry summers. Fruit were picked randomly from the inside of the trees.

Irradiation treatments: Since preliminary experiments indicated that the UV-B and incandescent light used by Arakawa *et al.* (1986) were not very effective, it was decided to compare UV-B plus incandescent light (UV-B+I) with high pressure sodium light (HPS) on 'Cripp's Pink' apples. In the first experiment, 'Cripp's Pink' apples were irradiated immediately after harvest with either UV-B (280 nm; FL 20 SLB /18 chemical) plus incandescent light (380 to 720 nm, Osram), or a 400 W HPS light (Philips SON-T, 253 mW/W). The light intensities were measured with a Decagon light meter (AccuPAR version 4.1, Decagon devices, Pullman, Washington, U.S.A) approximately 1 m from the light source. The light intensity of the UV-B+I could not be measured, but 4 x 40 W UV-B plus 4 x 60 W Osram bulbs were used, and the light intensity that reached the fruit from the HPS light was 22 to 24 $W \cdot m^{-2}$. Fruit were exposed to different irradiation periods, namely, 0, 24, 48 and 72 hours. Twenty-four fruit were used per treatment, with 6 replicates comprising 4 fruit each.

The second experiment was performed on 'Braeburn' apples. Two irradiation cycles were used. In the first cycle the fruit were irradiated 4 weeks after harvest using a 400 W HPS light. The second cycle was performed 8 weeks after harvest, again using a 400 W HPS light. The light intensity was the same as in experiment 1. The two different cycles (4 and 8 weeks after harvest) were used to determine whether fruit has the ability to form colour from artificial irradiation after a period of cold storage (-0.5°C). The same irradiation periods were used as in the first experiment. Again six replicates of four fruit per replicate were used per treatment.

In experiment 3, 'Forelle' pears were irradiated with a 400 W HPS light. The light intensity was the same as in experiment 1 and 2 and the same two irradiation cycles

and periods were used as in experiment 2. Fruit were divided in 6 replicates comprising 4 fruit for every treatment.

In case of exposures less than 72 hours, fruit were removed from the growth chamber and again stored at –0.5°C. All experiments were conducted in the growth chamber set at 18°C. The lights were positioned at the top of the growth chamber, with a perspex layer between the lights and the fruit. Fruit were placed randomly in the growth chamber. After irradiation fruit were peeled.

Colour measurements: Circles were marked on the fruit and external colour was measured in the marked area. A colorimeter (model NR-3000, Nippon Denshoku; Kogyo Co. Ltd.) was used and although colour was expressed as chroma, hue angle and lightness value, only hue angle data are presented. Hue angle (h° = arctangent [b*/a*]) refers to the angle formed by a line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992). Colour measurements were done after every irradiation period.

Anthocyanin analyses: After irradiation fruit were accidentally stored for ± 2 weeks at –0.5°C before the fruit were peeled with a potato peeler. The peel was frozen at –80°C, and subsequently lyophilised. The peel was ground to a fine powder with a Maulinex grinder (type A 10; IKA ® Werke, GMBH & Co. KG., Janke & Kunkel, Str 10 D- 79219, Staufen).

Anthocyanin extraction was based on the procedure of Siegelman & Hendricks (1958). Fifteen ml of a 1% HCl in methanol solution was added to 500 mg of sample and left for 18 hours at 5°C in the dark. Following the extraction period, the samples were centrifuged for 10 minutes at 12 000 g_n. Absorbance of the supernatant was measured at 530 nm on a spectrophotometer (Beckman Instruments Operation, DU®

Series 64, 2500 Harbor Boulevard, Fullerton, Ca. 92631-3100), with 1% HCl in methanol as the blank. A standard curve was obtained with idaein chloride (cy 3-gal) (Carl Roth GmbH and Company, Germany). Anthocyanin concentration was expressed as $\mu g \cdot g^{-1}$ dry peel.

Flesh firmness: An additional eight fruit were included in every treatment to determine the effect of the irradiation on firmness, since this is a destructive analysis. A penetrometer (mod. FT 327; 3 to 27 lbs.; J. R. Janisch, Dacres Avenue, Epping, Industry 2, Goodwood, S.A.) was used to determined the firmness of the fruit. An 8-mm tip was used to determine the firmness of pears and a 11-mm tip was used with apples.

Water loss: The four fruit per 6 replicates were weighed before and after irradiation treatment. This was done to determine the water loss of the fruit during the irradiation treatment.

Data analysis: The data were analysed with the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

Results and Discussion

Experiment 1

Cripp's Pink: 'Cripp's Pink' apples subjected to UV-B+I, showed a small decrease in hue angle (ΔHue = -4.2) after 72 hours of irradiation, although the difference was significant (P = 0.0397) (Table 1). In contrast, the HPS lamps resulted in a decrease in hue angle of 30 after 72 hours (P = 0.0001). Arakawa *et al.* (1985) found that high fluency rates of white light provided from xenon lamps were unable to produce large amounts of anthocyanin on 'Jonathan' apples. When UV-B light, provided by a fluorescent lamp which had an emission peak at 312 nm, was combined with the white light, anthocyanin production was synergistically stimulated and increased even

to the highest fluency rates of white light tested. In experiment 1, results showed that HPS light enhances colour development better than the UV-B+I light. The UV-B light in our trial was also produced by a fluorescent lamp. These data contradict the results of Arakawa *et al.*, (1985). The final hue angle values (after 72 hours) of the fruit exposed to UV-B+I and HPS lamps, showed a significant difference in the decrease between the different lights used (P = 0.0001) (Table 1). It is therefore clear that to achieve a drop in hue angle in 'Cripp's Pink' apples, HPS light is more effective. The perspex layer between the light source and the fruit could have influenced the efficacy of both the UV-B+I and HPS light. Thus, a possible explanation for the poor results from the UV-B+I light could be that the perspex layer absorbed the UV light. This, and the difficulty of accurately measuring UV-B light, especially when combined with another light source (Caldwell & Flint, 1994), were some of the technical limitations in this research. Therefore, it is possible that the intensity of the UV-B too low, or that the combination of the two intensities was not effective.

The difference between the two sets of lights was emphasised when anthocyanin concentration was measured (Table 1; Figure 1). Anthocyanin concentration increased from 85.6 $\mu g \cdot g^{-1}$ up to 155.9 $\mu g \cdot g^{-1}$ after 72 hours of irradiation with UV-B+I. Anthocyanin concentration tended to increase more slowly under UV-B+I than under HPS light (Figure 1). In the case of the latter, the anthocyanin concentration increased linearly over time. The initial anthocyanin concentration of 95.6 $\mu g \cdot g^{-1}$ doubled to 174.0 $\mu g \cdot g^{-1}$ after 48 hours of HPS light, and tripled after 72 hours (271.8 $\mu g \cdot g^{-1}$). Thus the decrease in hue angle reflects a change in colour due to an increase in anthocyanin production. It is clear that certain action spectra can have different effects on colour development and anthocyanin production. Arakawa *et al.*'s (1986) example of the combination of white light and UV-B light that stimulates anthocyanin production synergistically up to the highest energy flux of white light tested, is again contrary to these results (Table 1). They showed that the UV light plus the red region of white light produced four times the concentration of anthocyanin

compared to the use of only white light (Arakawa *et al.*, 1986). 'Cripp's Pink' apples produced nearly three times the initial amount of anthocyanin under HPS light, whereas with UV-B+I light the anthocyanin increase was less than twice the initial concentration (Table 1). The increased anthocyanin concentration was linear for both sets of lights, but the increase was significantly faster for HPS lights ($P = 0.0056$) (Figure 1).

It was also clear from Table 1 that the hue value decreased and the anthocyanin concentration increased with time irradiated. The first reaction in photocontrol of anthocyanin is the HIR reaction, which requires long irradiation periods at high light intensities (Downs *et al.*, 1965). Siegelman & Hendricks (1958) proposed that the anthocyanin production of the linear period is a function of time of irradiation at constant irradiance and energy flux and the duration of the linear period can be a further 100 hours (Chalmers & Faragher, 1977a). Only 72 hours of HPS light were needed to get a suitable red colour development on 'Cripp's Pink' apples. Light intensities of 22 to 24 $W \cdot m^{-2}$, were sufficient for colour development. Siegelman & Hendricks (1958) stated that 5 $W \cdot m^{-2}$ is the threshold for the initiation of anthocyanin synthesis.

Firmness and water loss were two postharvest parameters that were measured to determine whether irradiation after harvest affected fruit quality. The initial firmness of the fruit was 8.1 kg, and there was no significant change in firmness between fruit subjected to any of the irradiation treatments used (Table 1). These results were similar to those of Saks *et al.* (1990) who found no differences in fruit ripening between those fruit that developed colour under artificial light and red fruit from the commercial harvest, in spite of some stimulation of ethylene production during illumination. However, some types of light sources may stimulate ethylene production and therefore affect flesh firmness, because according to Chalmers and Faragher (1977b), UV light provided from biocidal lamps (254 nm) stimulates ethylene

production and anthocyanin synthesis of whole apple fruit. However, in whole fruit experiments with UV-B (312 nm) irradiation, no excessive increase in ethylene production was found following irradiation, whereas anthocyanin concentration was increased. Our results also shows no difference in change in firmness between UV-B+I or the HPS light (P = 0.2112).

A likely effect of irradiation with UV-B is water loss, possibly as a result of alterations to the epidermis (Tevini *et al.*, 1981). When irradiated with UV-B+I there was no significant loss (P = 0.7686) in weight after 72 hours, but with the use of HPS light there was a linear increase in water loss with time of irradiation (P = 0.0001) (Table 1). However, the loss never exceeded 1.1% of the overall weight.

Experiment 2

Braeburn: 'Braeburn' apples stored at -0.5°C for 4 weeks showed a significant decrease of 23 in hue angle (P = 0.0001) after 72 hours of irradiation with HPS light (Table 2). The decrease in hue angle of the 'Braeburn' apples stored for 8 weeks at -0.5°C prior to irradiation with HPS light was also significant (P = 0.0001) after 72 hours, but the decrease was not as large (14.7°). There was a linear increase in anthocyanin concentration from 95.2 $\mu g \cdot g^{-1}$ to 211.2 $\mu g \cdot g^{-1}$ after a 72 hours irradiation period, for the fruit previously stored for 4 weeks, while those apples stored for 8 weeks had a smaller increase (122.8 $\mu g \cdot g^{-1}$ to 172.4 $\mu g \cdot g^{-1}$) (Figure 2).

It is accepted that light and temperature play major roles in colour development (Downs *et al.*, 1965; Beggs *et al.,* 1986; Arakawa, 1988; Saure, 1990; Lancaster, 1992). Attached fruit need a period of low temperature (<20°) before colour development can occur (Creasy, 1968; Curry, 1997). The decrease in hue value of the fruit stored for 4 weeks, indicates that the cold requirement was met, although fruit stored for 8 weeks were not as red after irradiation. A possible reason for this may be

that fruit loses its ability to produce colour after harvest with an increase in storage time. Bishop & Klein (1975) found that the ability of apples previously stored in air, to synthesise anthocyanin under light decreased sygmoidally with storage time. Arthur (1932) found that fruit did not colour after a period of storage (approximately 7 to 8 weeks). My results contradict his findings, possibly because of cultivar specific responses. Fruit stored for 4 weeks had a linear increase in anthocyanin production from the start of irradiation, whereas the fruit stored for 8 weeks prior to irradiation, anthocyanin concentration only increased after 48 hours of irradiation. It was also clear that as the storage period progresses there is a change from a green to a more yellowish background, as a result of chlorophyll breakdown with ripening. Any red colour that develops is more visible on the yellow compared to the green background (green hue value = 180°; yellow hue value = 90°). Dixon and Hewett (1998) found that the total chlorophyll concentration in apples decreases exponentially with time. Yellowing, which is a function of time, increased slowly from 0 to 5°C, increased exponentially from 5 to 20°C, reached a maximum from 20 to 24°C, and then declined at higher temperatures.

Although the 'Braeburn' apples were cold stored, it seems that 'Cripp's Pink' apples reacted better to postharvest irradiation with HPS light than 'Braeburn' apples. Responsiveness of anthocyanin synthesis to light differs considerably with cultivars (Arakawa, 1988).

The firmness of 'Braeburn' measured initially was 8.4 kg. and the final measurement after the treatments changed to 8.3 kg. Again irradiation treatments had no significant effect on the firmness of the apples (Table 2). 'Braeburn' apples showed a linear increase in water loss with time of irradiation ($P = 0.0960$ and $P = 0.0001$, respectively). However, there was no difference in the amount of water lost for the fruit stored for 4 or 8 weeks ($P = 0.1551$). This loss is possibly a result of a greater vapour pressure deficit at 18°C.

Experiment 3

Forelle: When 'Forelle' pears were irradiated after 4 or 8 weeks of storage, only small changes in hue angle were measured (Table 3). While these decreases were statistically significant for the fruit stored for 8 weeks (P = 0.0001), they were not noticeable to the human eye. For both the 4 and 8 week -stored fruit, there was also no significant increase in anthocyanin concentration after 72 hours of irradiation (P = 0.1055 and P = 0.7906, respectively) (Table 3) (Figure 3). There was a significant difference when storage periods were compared in terms of hue angle (P = 0.0001), but not in anthocyanin concentration (P = 0.8132). The decrease in hue angle was a result of yellowing, rather than red colour development.

The irradiation treatment did not affect the firmness of the 'Forelle' pears (Table 3). The water loss again seemed to have a significant linear trend with irradiation time, for both the fruit stored for 4 and 8 weeks (P = 0.0022 and P = 0.0013, respectively). However, there was no difference between the different storage periods (P = 0.1551). 'Forelle' pears lost more weight with irradiation than the two apple cultivars.

Conclusion

'Cripp's Pink' apples coloured significantly after 72 hours of irradiation with HPS light, while UV-B+I light did not have the same effect on colour development. The HPS light resulted in triple the initial amount of anthocyanin, whereas UV-B+I light resulted in lower amounts of anthocyanin in 'Cripp's Pink' apples. 'Braeburn' apples exhibited the ability to produce colour postharvest after a cold storage period, yet it seems that the ability to form colour decreases as the storage period increases. In contrast to apples, there was no postharvest improvement of colour on pears exposed to HPS light after 4 or 8 weeks of cold storage. HPS light had no visible effect on colour.

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Table 1. The effect of irradiation with either UV-B +I or HPS light on the hue angle, anthocyanin concentration, firmness and water loss of 'Cripp's Pink' apples irradiated immediately after harvest.

'Cripp's Pink'							
Treatment	Irradiation time (h)	Initial hue value	Final hue value	Δ Hue value	Anth. Concent. ($\mu g \cdot g^{-1}$)	Firmness (Kg)	Water loss (%)
UV-B+I	0	84.1	84.7	0.7	85.6	8.0	0.24
	24	75.9	76.0	0.1	113.1	8.6	0.44
	48	78.8	77.7	-1.0	100.6	8.4	0.46
	72	75.0	70.7	-4.2 a	155.9 a	8.1 a	0.93 a
Sign. Level (P)				0.0397	0.0728	0.5670	0.7686
HPS	0	69.3	70.9	1.6	96.6	8.1	0.06
	24	80.0	79.6	-0.4	125.7	8.6	0.07
	48	79.6	63.9	-15.3	174.1	9.1	0.63
	72	86.5	56.5	-30.0 b	271.8 b	8.7 a	1.06 a
Sign. Level (P)				0.0001	0.0001	0.2357	0.0001
Significance (P<0.05)							
UV-B+I vs. HPS				0.0001	0.0002	0.2112	0.1989

Significant differences were calculated both within the treatment as well as between the two treatments (two sets of lights). Significant differences between the two sets of lights were calculated comparing the final values of each treatment after the irradiation treatment. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 2. The effect of irradiation with HPS light on the hue angle, anthocyanin concentration, firmness and water loss of 'Braeburn' apples stored for 4 or 8 weeks at –0.5°C before treatment.

'Braeburn'							
Treatment	Irradiation time (h)	Initial hue value	Final hue value	Δ Hue value	Anth. Concent. ($\mu g \cdot g^{-1}$)	Firmness (Kg)	Water loss (%)
4 weeks	0	108.5	107.9	-0.6	95.2	8.4	0.48
	24	109.3	97.4	-11.7	116.7	8.5	0.56
	48	108.8	94.7	-14.2	131.5	8.4	0.57
	72	108.6	85.4	-23.0 a	211.2 a	8.3 a	0.93 a
Sign. level (P)				0.0001	0.0001	0.5924	0.0960
8 weeks	0	106.4	104.3	-2.1	122.8	8.8	0.13
	24	105.5	102.9	-2.6	106.8	8.5	0.32
	48	106.8	99.5	-7.6	110.3	9.0	0.77
	72	106.5	91.8	-14.9 b	172.4 b	9.0 a	1.03 a
Sign. level (P)				0.0001	0.0001	0.3033	0.0001
Significance (P<0.05)							
4 weeks vs. 8 weeks				0.0001	0.0073	0.1547	0.1551

Significant differences were calculated both within the treatment as well as between the two treatments (two cold storage periods). Significant differences between the two cold storage periods were calculated comparing the final values of each treatment after the irradiation treatment. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 3. The effect of irradiation with HPS light on the hue angle, anthocyanin concentration, firmness and water loss of 'Forelle' pears stored for 4 or 8 weeks at –0.5°C before treatment.

'Forelle'							
Treatment	Irradiation time (h)	Initial hue value	Final hue value	Δ Hue value	Anth. Concent. ($\mu g \cdot g^{-1}$)	Firmness (Kg)	Water loss (%)
4 weeks	0	110.2	107.9	-2.2	151.4	5.9	0.18
	24	105.1	107.9	5.1	132.1	6.0	0.77
	48	108.8	107.4	-1.4	133.0	6.3	1.50
	72	107.4	107.9	0.5 a	171.6 a	6.0 a	3.40 a
Sign. level (P)				0.0992	0.1055	0.3851	0.0022
8 weeks	0	104.3	107.5	3.2	159.5	6.1	0.46
	24	104.9	105.5	0.6	146.8	6.0	0.88
	48	105.0	102.7	-2.3	163.2	6.1	1.78
	72	104.9	100.4	-4.5 b	166.0 a	5.9 a	3.25 a
Sign. level (P)				0.0001	0.7906	0.1657	0.0013
Significance (P<0.05)							
4 weeks vs. 8 weeks				0.0001	0.8132	0.1688	0.1551

Significant differences were calculated both within the treatment as well as between the two treatments (two cold storage periods). Significant differences between the two cold storage periods were calculated comparing the final values of each treatment after the irradiation treatment. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

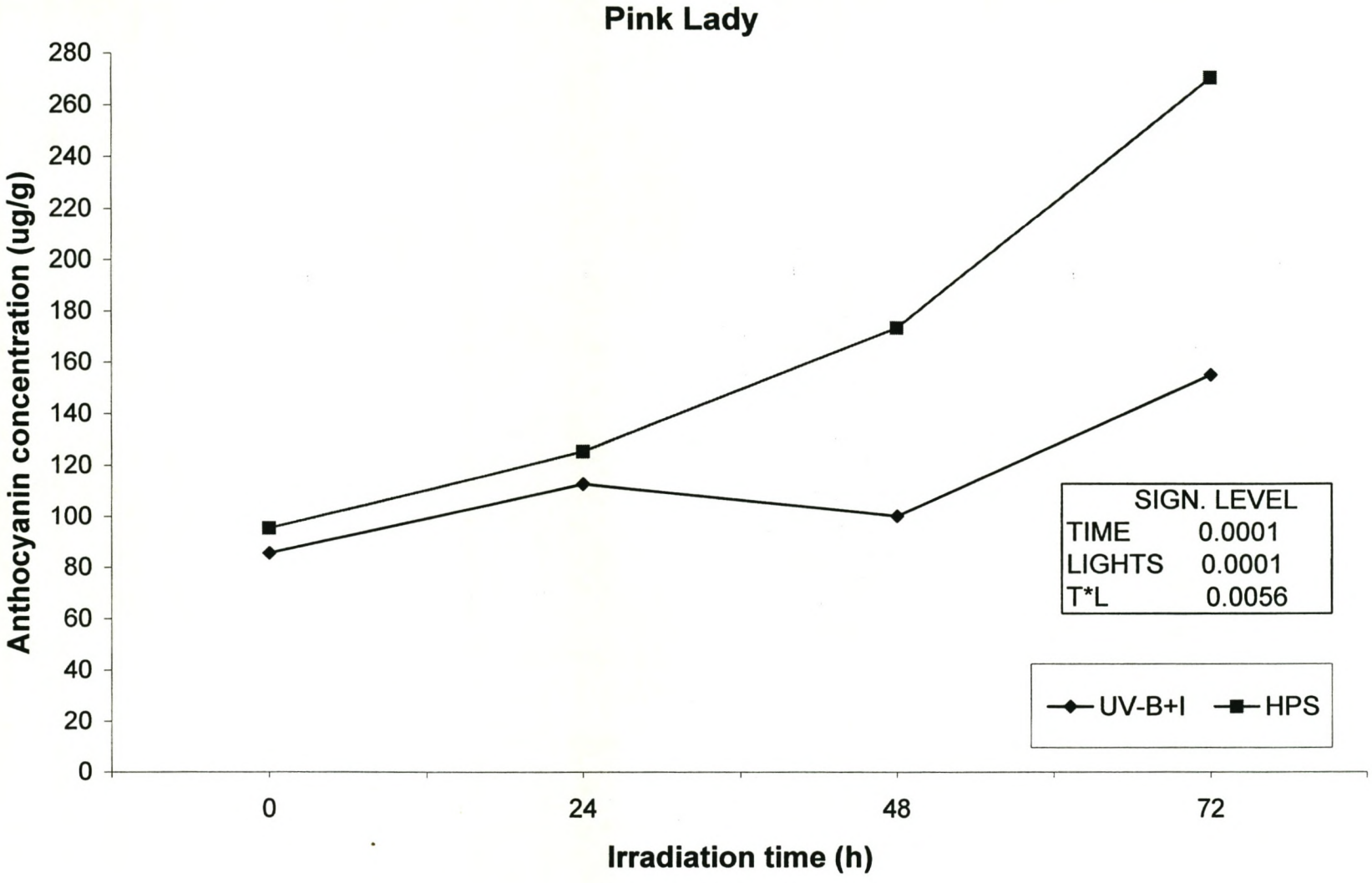

Figure 1: Anthocyanin concentration of 'Cripp's Pink' apples, irradiated immediately after harvest, either with UV-B + incandescent (UV-B+I) light or high pressure sodium (HPS) light.

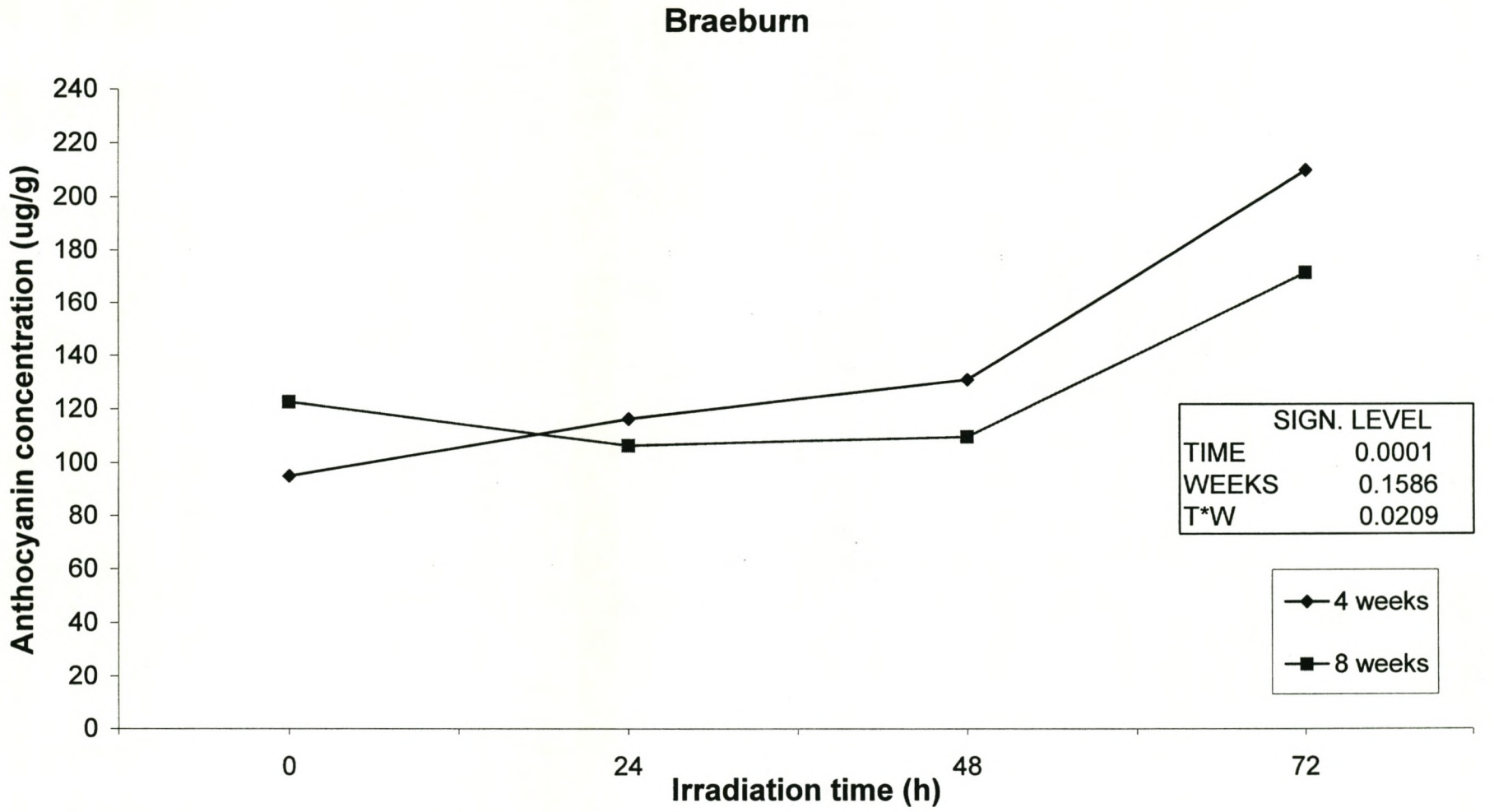

Figure 2: Effect of irradiation with HPS light on the anthocyanin concentration of 'Braeburn' apples, after a storage period of 4 or 8 weeks at -0.5 °C.

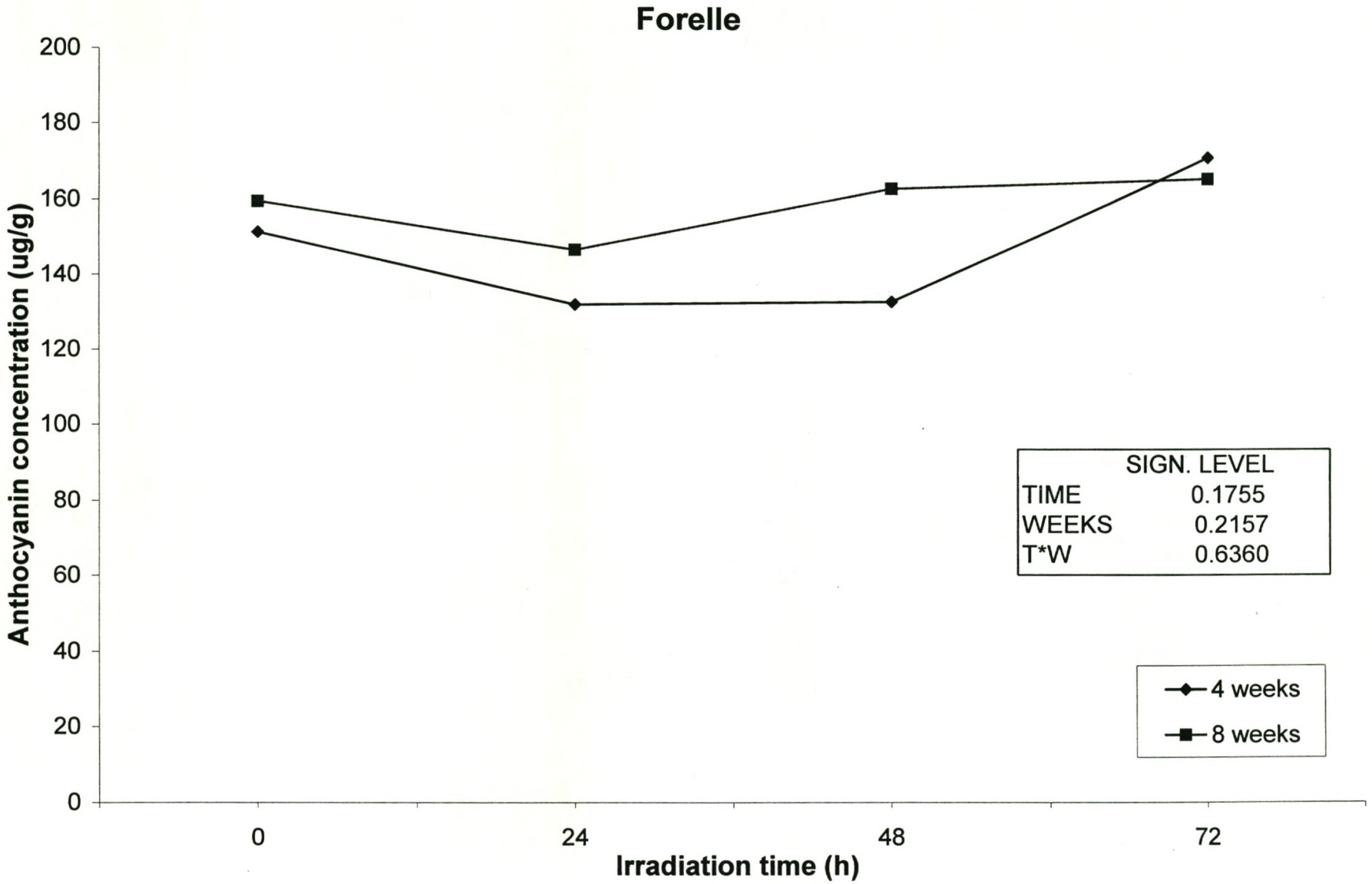

Figure 3: Effect of irradiation with HPS light on the anthocyanin concentration of 'Forelle' pears, after a storage period of 4 or 8 weeks at -0.5 °C.

PAPER 2: Fruit developmental stage affects response of 'Braeburn' and 'Cripp's Pink' apples to postharvest irradiation.

Abstract

Apples lose their colour after the initial peak of anthocyanin production, which occurs during intense cell division. The fruit appear green during the rest of the season until maturation when red of bi-coloured cultivars develop the capacity to accumulate anthocyanin. In this experiment 'Braeburn' and 'Cripp's Pink' apples were picked every week, beginning 5 weeks prior to harvest, up to the optimum harvest date. The green sides of the 'Braeburn' and 'Cripp's Pink' apples were then irradiated for 72 and 144 hours respectively, with 22 to 24 $W \cdot m^{-2}$ of a 400 W high-pressure sodium light at 20°C. 'Braeburn' apples picked 5, 4, 3 and 2 weeks prior to harvest, showed only a slight decrease in hue angle after irradiation, while the decrease for the fruit picked 1 week prior to the optimum harvest date was slightly larger. Although 'Braeburn' apples picked at the optimum harvest date had the greatest decrease in hue angle, it was only 11.6°. Consequently, the irradiation period was doubled for 'Cripp's Pink' apples. 'Cripp's Pink' apples harvested 5, 4 and 3 weeks prior to harvest showed slight decreases as a result of degreening/yellowing due to ripening and not red colour development. 'Cripp's Pink' fruit picked 2 and 1 week prior to harvest had decreases in hue angle of 25.1° and 27.6°, resulting in stripes of red colour forming. The best colour formation occurred on the fruit picked at the optimum harvest date. These 'Cripp's Pink' apples developed an overall red colour from a green fruit within only 72 hours of irradiation. It therefore seems that green coloured fruit picked 3 weeks or more prior to harvest, do not develop colour easily with postharvest artificial light. Fruit picked 2 and 1 week prior to harvest develop colour, but with longer irradiation periods than 72 hours. Fruit picked at optimum harvest needs shorter periods of postharvest irradiation for colour development to occur. Colour development occurred more readily on the 'Cripp's Pink' apples than on the 'Braeburn' apples.

Introduction

Anthocyanin production is a high-irradiance reaction and therefore needs long periods of high light intensities on each single cell in the fruit skin, for better coloration (Saure, 1990; Lancaster, 1992; Arakawa, 1991). The inferior red colour development of the inner, lower half of the tree canopy emphasises the importance of light. The second important factor involved in sufficient anthocyanin production is temperature (Creasy, 1968; Arakawa, 1988; Saure, 1990, Lancaster, 1992).

Apples and pears have a development period on the tree lasting for up to six months. During this period, two main peaks of anthocyanin production occur. Anthocyanin concentration is high in juvenile fruit during the time of intense cell division, then colour is lost during fruit growth (Saure, 1990; Lancaster, 1992; Lister *et al.*, 1994; Reay *et al.*, 1998). This is the result of anthocyanins degrading in the immature fruit skin more rapidly than they form, so that the red colour disappears. This equilibrium between synthesis and degradation is influenced by light intensity (Chalmers *et al.*, 1973). During cell division light intensities could decrease as a result of an increase in canopy size (Marais, 1995).

The second peak occurs during fruit maturation and ripening (Saure, 1990; Lancaster, 1992; Ju *et al.,* 1995; Reay *et al.*, 1998). Chalmers *et al.* (1973) proved that the second peak of anthocyanin production is a direct result of maturity. He found that while the level of anthocyanin at a particular stage of maturity may be related to environmental factors, the capacity to accumulate anthocyanin is a function of maturity and independent of environmental influences. During fruit ripening of some cultivars, the concentration of anthocyanin increased more than 3-fold, when receiving sufficient light (Gorski & Creasy, 1977; Lancaster, 1992). The transition from the immature to mature stages, regarding anthocyanin accumulation, occurs rapidly and precedes the harvest date by about two to three weeks (Chalmers *et al.,* 1973). Another reason for better colour formation is the increased PAL levels in

mature fruit as a result of ripening and lower temperatures at the end of the season, compared to lower levels of PAL in immature fruit (Faragher, 1983).

When postharvest colour development with artificial light is induced, the stage of fruit development influences the effectiveness of different wavelengths of light used (Saure, 1990), light intensities (Arthur, 1932; Chalmers *et al.*, 1973), as well as the temperatures during irradiation (Faragher, 1983).

The aim of this experiment was to determine the development stage of 'Braeburn' and 'Cripp's Pink' apples, where the fruit attains the ability to form colour postharvest with artificial light.

Material and methods

Plant material: 'Braeburn' and 'Cripp's Pink' apples were obtained from Lourensford farm in the Somerset West area in the Western Cape, South Africa (latitude: 33°10'S; longitude: 19°20'E).

Irradiation treatments: Thirty fruit were harvested weekly, until optimum harvest date, which resulted in six harvests. Fruit were divided in six replicates comprising five fruit per replicate. The green sides of the fruit were subjected to a 400 W high-pressure sodium light (HPS) (Philips SON-T, 253 mW/W). The light intensity was 22 to 24 $W \cdot m^{-2}$, measured with a Decagon light meter (AccuPAR version 4.1, Decagon devices, Pullman, Washington, U.S.A) approximately 1 m from the light source. Fruit were placed randomly in the growth chamber with a set temperature of 20°C, and the fruit were irradiated immediately after harvest.

With 'Braeburn' apples, which were harvested first, colour measurements were taken prior to irradiation and again every 24 hours for 72 hours. Up to the optimum harvest date, very little red colour developed on 'Braeburn' apples, so when 'Cripp's Pink'

apples were harvested a few weeks later, fruit were irradiated for 144 hours. Again colour measurements were taken before the irradiation treatment started and again every 24 hours.

Colour measurements: Circles were marked on the fruit and external colour was measured in the marked area. A colorimeter (model NR-3000, Nippon Denshoku; Kogyo Co. Ltd.) was used and although colour was expressed as chroma, hue angle and lightness value, only the hue angle data is presented. Hue angle (h° = arctangent [b*/a*]) refers to the angle formed by a line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).

Data analysis: The data were analysed with the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990). Analysis were conducted on the differences in hue value from the initial hue value to the final hue value.

Results and Discussion

'Braeburn' apples harvested 5 weeks before optimum harvest date had a decrease in hue angle of approximately 4.5° after 72 hours of exposure to HPS light (Table 1). The decrease in hue angle of the fruit picked 4, 3 and 2 weeks prior to harvest was very slight after exposure to 72 hours of irradiation. There was little change in fruit picked 5, 4, 3 and 2 weeks prior to harvest, despite different initial values. The fruit picked 1 and 0 weeks before optimum harvest, showed decreases in hue angle of 6 and 11°, respectively, after 72 hours of irradiation (Table 1). However, these decreases were as a result of a change from green to yellow as indicated by hue angle values (Figure 1).

In response to the poor results from the irradiation of 'Braeburn' apples, 'Cripp's Pink' apples were irradiated for 144 hours. There were only slight changes in hue angle on fruit harvested 5, 4 and 3 weeks preceding the optimum harvest date (Table 2). In all three cases, there was a decrease in hue angle, again as a result of the colour changing from green to a lighter green/yellow, and not as a result of red colour development. On the other hand, as the optimum harvest date approached, red colour development with irradiation increased. After a period of irradiation of 144 hours, the fruit picked 2 and 1 week prior to harvest showed a decrease in hue angle of 25.1° and 27.6° respectively (Table 2). At the optimum harvest date, colour development was very rapid and a decrease of 62.5° in hue angle was achieved after only 72 hours of irradiation (Figure 2). A well-coloured 'Cripp's Pink' apple will have a hue angle of 40 to 50°, whereas a good red colour for a cultivar like 'Topred' would be a hue angle of 30 to 40°. It is therefore clear that the colour development achieved at the optimum harvest date satisfied the requirements for class one pack-out.

It seems that the relationship between anthocyanin formation in young fruit and in fully developed apples is not clear, although Lancaster (1992) stated that the anthocyanins produced during early fruit development are identical in structure to those in ripe fruit. Young fruit exhibit intense coloration even in cultivars not inclined to anthocyanin formation during maturation, e.g. 'Golden Delicious' and 'Matsu' (Saure, 1990). Results shown in Table 1 and 2 indicate that there is a definite stage of maturity during which apples colour satisfactorily. Both the 'Braeburn' and 'Cripp's Pink' apples indicated an increase in capability to produce anthocyanin as the optimum harvest date approached. The results of Arakawa (1991) and Chalmers *et al.* (1973) confirm the findings that anthocyanin production with artificial light increased as the fruit ripens. Procter and Lougheed (1976) proved that the onset of rapid red colour formation in 'McIntosh' apples was about 20 days before harvest. Kubo *et al.* (1988) also reported an increase of PAL activity coinciding with the onset

of fruit coloration and the increase of ethylene production in 'Starking Delicious' and 'Golden Delicious' apples. These results indicated that fruit picked ≥ 3 weeks prior to optimum harvest do not colour easily. Irradiation of 'Braeburn' apples for 72 hours and 'Cripp's Pink' apples for 144 hours, without red colour development proved this point. Apples picked 2 weeks and 1 week prior to harvest did form colour (Cripp's Pink'), but needed a longer period than 72 hours ('Braeburn'). 'Cripp's Pink' fruit picked at the optimum harvest date developed colour, even with shorter irradiation periods, although 'Braeburn' apples did not form significant colour after 72 hours, and probably needed a longer irradiation period.

The light intensities used affected pre- and postharvest development of colour. Chalmers *et al.* (1973) found that the anthocyanin level drops in immature apple fruit skin when light intensity was reduced by shading, but in contrast to this, it continues to rise in mature apple fruit skin when kept at reduced light intensities. Thus, mature fruit requires lower light intensities than immature fruit. On the other hand, Arthur (1932) found that late harvested 'McIntosh' apples required longer exposures to light to induce a certain amount of colour development than apples harvested earlier. This is in contrast to the results in Table 1 and 2.

The increase in anthocyanin production in the field coincides with decreasing temperatures and with increasing ethylene production by the fruit. One of the reasons may be that the PAL levels are higher in ripe than in unripe fruit (Faragher, 1983). It seems therefore, that maturity plays a significant role in anthocyanin production. This may be true, but Arakawa *et al.* (1986) suggested that the ability of the fruit to produce anthocyanin differs among cultivars. For example, in poorly colouring cultivars (e.g. 'Fuji' and 'Jonagold') anthocyanin reached a peak at the same time as the rise in ethylene in the cortical tissue and decreased shortly after. However, in 'Starking Delicious', 'Jonathan' and 'McIntosh' anthocyanin accumulation continued to increase after the onset of ripening and the rise in ethylene. These

results confirm this statement, because it was clear that 'Cripp's Pink' apples developed far more colour in 72 hours than did the 'Braeburn' apples also harvested at the optimum harvest date.

The temperature at which apples are irradiated postharvest also has a substantial effect on colour development. In detached apples held under continuous white light, the temperatures for anthocyanin accumulation were 12°C in unripe apples and 16 to 24°C in ripe apples. The levels of PAL activity induced by light were inversely proportional to temperature and increased with ripening. Arakawa (1991) also indicated an increase in anthocyanin as the fruit ripens, and the optimum temperature for maximum accumulation increases continuously as fruit ripens. Again Faragher (1983) concluded that ripening has a greater effect in increasing anthocyanin levels postharvest, than does temperatures.

Chalmers *et al.* (1973) proposed that the increasing capability of mature fruit to accumulate anthocyanin when compared with immature fruit is not based on an increased endogenous rate of synthesis with maturation, but that the fundamental difference lies in the potential to degrade anthocyanin. In immature fruit anthocyanin is degraded faster than it is synthesised, whereas is mature fruit the rate of synthesis exceeds the rate of degradation. It appears that an equilibrium exists between degradation and synthesis in immature fruit when the anthocyanin level fluctuates under the control of light and other environmental conditions. However, when the fruit matures, accumulation of anthocyanin begins regardless of the light regimes, as long as the requirement for high light in the photosynthetic wavelengths is met. At this stage the rate of anthocyanin synthesis exceeds the rate of degradation. A decrease in light intensity would cause a decrease in anthocyanin in immature fruit, whereas accumulation would continue in mature fruit at the same low level of light. Faragher (1983) also proposed that the low anthocyanin production in immature fruit could be the result of the effects of inhibitory temperatures during this period.

It is concluded that maturation, together with temperature and light plays a substantial role in postharvest colour development of apples. It seems that apple fruit are required to reach a certain threshold in their development for colour to develop postharvest. After this point the more immature the fruit, the longer the required irradiation period and the more mature the fruit, the more rapid the colour develops.

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Table 1. Changes in hue angle of 'Braeburn' apples picked every week from 5 weeks prior to harvest up to the optimum harvest date, and then irradiated with HPS light for 72 hours.

'Braeburn'			
Hue angle			
Weeks prior to harvest date	Initial hue value	Final hue value	Δ Hue value
5	114.2	109.6	-4.6 b
4	113.5	113.0	-0.5 c
3	101.8	101.1	-0.7 c
2	98.3	99.3	1.0 c
1	114.5	108.3	-6.2 b
0	113.6	102.0	-11.6 a
Significance (P<0.05)			
Treatment			0.0001

Statistics were done on the differences of the final and initial hue value. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 2. Changes in hue angle of 'Cripp's Pink' apples picked every week from 5 weeks prior to harvest up to the optimum harvest date, and then irradiated with HPS light for 144 hours.

'Cripp's Pink'			
	Hue angle		
Weeks prior to harvest date	Initial hue value	Final hue value	Δ Hue value
5	116.9	107.9	-9.0 c
4	113.8	108.5	-5.2 c
3	116.4	106.1	-10.3 c
2	116.8	91.7	-25.1 b
1	114.7	87.2	-27.6 b
0	111.8	49.3*	-62.5 a*
Significance (P<0.05)			
Treatment			0.0001

Statistics were done on the differences of the final and initial hue value. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test. *In the case of 'Cripp's Pink' apples harvested on the optimum harvest date, the fruit obtained a well-developed all-over red colour and were removed after only 72 hours of irradiation.

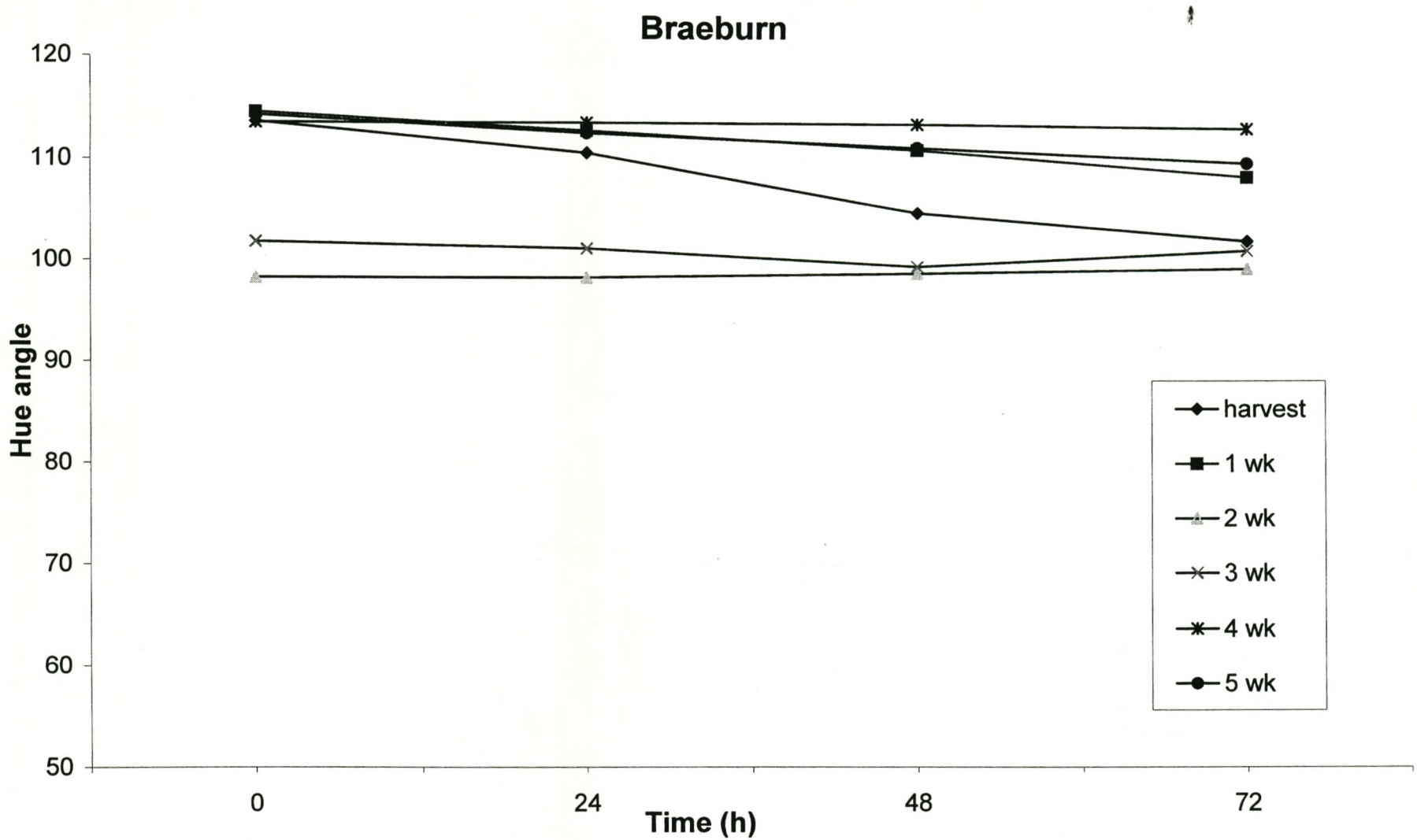

Figure. 1. Change in hue angle of 'Braeburn' apples picked every week from 5 weeks prior to harvest up to the optimum harvest date, and then irradiated with high pressure sodium (HPS) light for 72 hours.

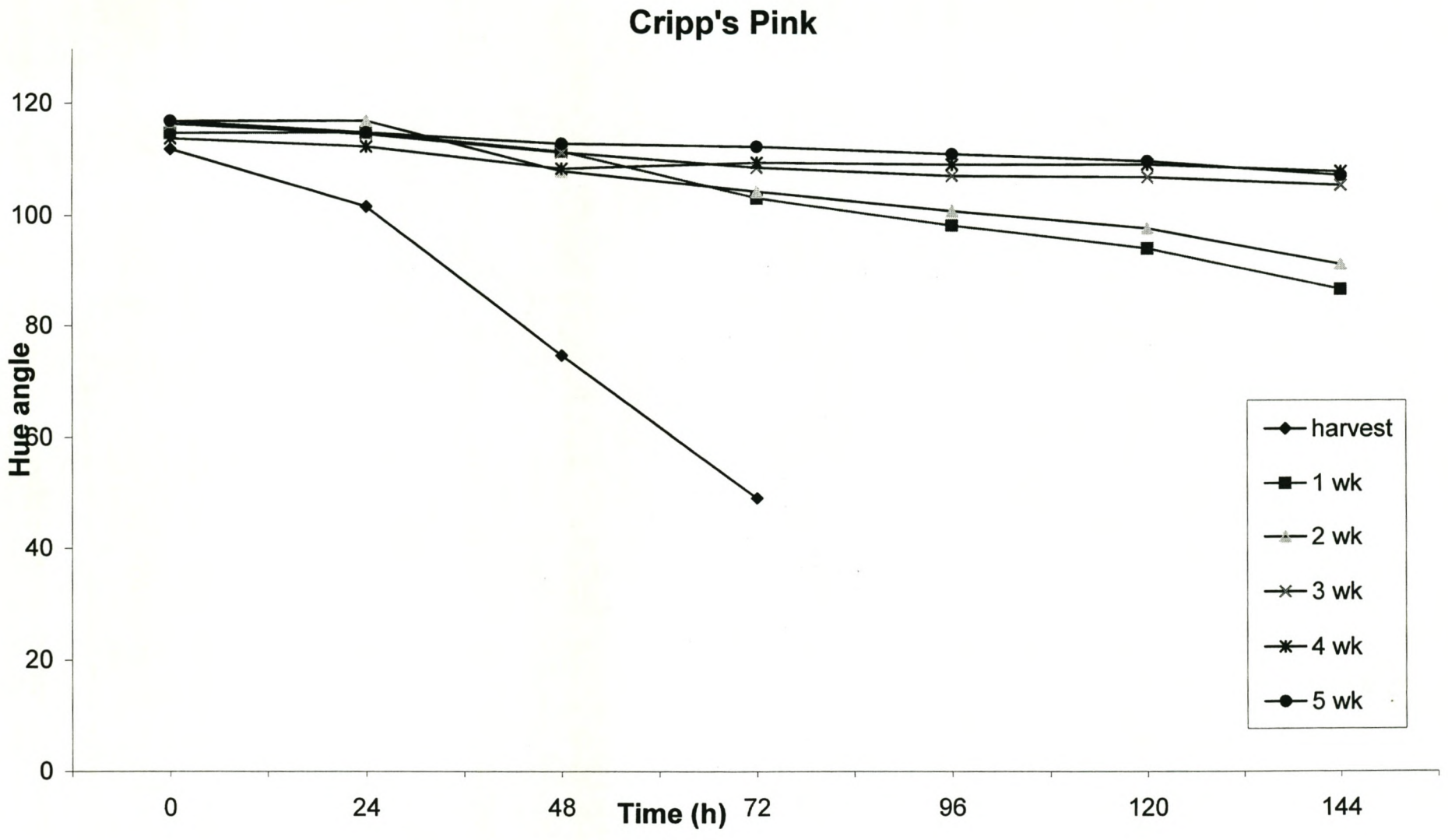

Figure. 2. Changes in hue angle of 'Cripp's Pink' apples picked every week from 5 weeks prior to harvest up to the optimum harvest date, and then irradiated with high pressure sodium (HPS) light for 144 hours.

Paper 3: Effect of storage at –0.5°C before irradiation on postharvest colour development at different temperature regimes.

Abstract

'Cripp's Pink' fruit were picked from two different areas, namely the colder Ceres area and the warmer Grabouw area. In the first experiment, colour measurements were taken at harvest and then fruit were divided into three groups. The fruit was either irradiated immediately after harvest, or stored for 2 days or 5 days at –0.5°C before irradiation. The fruit were irradiated for 120 hours with 22 to 24 $W \cdot m^{-2}$ high-pressure sodium (HPS) light, at either 6°C or at 20°C. In the case of the fruit from the Grabouw area, the best colour development occurred at a temperature of 6°C regardless of the storage period. Hue angle values decreased slightly when irradiated at 20°C, with the largest decrease (14°) after 5 days at –0.5°C before irradiation. Anthocyanin concentrations increased in relation to the decreases in hue angle values. The fruit from Ceres also produced the best colour at 6°C, but the decreases in hue angle (±40°) were greater than the Grabouw fruit. Even at a temperature of 20°C, the fruit from Ceres irradiated immediately or after 2 days at –0.5°C, showed greater decreases in hue angle values (22 and 25°, respectively), and significant increases in anthocyanin concentrations. After 5 days of storage, the hue angle was the same as fruit irradiated at 6°C. The second experiment tested 120 hours of irradiation on 'Cripp's Pink' apples with 22 to 24 $W \cdot m^{-2}$ HPS light either at a temperature of 6°C or at 6/20°C. The fluctuating temperature regime resulted in decreases in hue angle value of 70° for the fruit from Grabouw and 65° for the fruit from Ceres. The decreases were smaller (±20°) when fruit were irradiated at 6°C. Anthocyanin concentrations were significantly higher after fluctuating temperatures (6/20°C) than at 6°C for Ceres (676.9 $\mu g \cdot g^{-1}$ vs. 201.9 $\mu g \cdot g^{-1}$) and Grabouw (654.1 $\mu g \cdot g^{-1}$ vs. 206.0 $\mu g \cdot g^{-1}$).

Introduction

Red skin colour formation in apples is a result of anthocyanin production (Mancinelli, 1983; Saure, 1990; Lancaster, 1992). The level of red anthocyanin pigments in apple skin at harvest is determined mainly by the cultivar, stage of maturation (ethylene production), and the two major environmental factors responsible for anthocyanin production, namely light and temperature (Chalmers *et al.*, 1973, Faragher, 1983; Saure, 1990; Arakawa, 1991; Lancaster, 1992; Ju *et al.,* 1995).

Apples kept in the dark or at low light, do not redden (Macheix *et al.*, 1990; Lancaster, 1992). Therefore, light is required for anthocyanin synthesis (Chalmers & Faragher, 1977; Tan, 1979; Arakawa *et al.*, 1985; Saure, 1990). Light can cause *de novo* synthesis of certain enzymes involved in the biosynthesis of flavonoids (Beggs *et al.,* 1986, Bruns *et al.,* 1986; Schmelzer *et al.,* 1988; Saure, 1990; Lancaster, 1992; Ju *et al.*, 1995). PAL is a critical enzyme in controlling the flavonoid synthesis in many plant systems (Saure, 1990; Ju *et al.* 1997; Blankenship and Unrath, 1988; Chalmers and Faragher, 1977; Arakawa *et al.,* 1986; Lister *et al.,* 1994). With irradiation, PAL activity is induced after a lag phase of 10 hours, it reaches a maximum after 30 to 40 hours and then declines again (Beggs *et al.*, 1986; Macheix *et al.*, 1990; Lancaster, 1992). Irradiation also causes 10- to 20-fold increases in the enzymatic activity of PAL (Dong *et al.*, 1995).

Temperature affects both the rate of anthocyanin production, and the length of the induction period before anthocyanin synthesis (Siegelman & Hendriks, 1958b). Low temperatures enhance, and high temperatures inhibit anthocyanin synthesis. The effect of temperature varies for fruit on the tree and harvested fruit (Saure, 1990). Low temperatures (<6°C) stimulate production of PAL (Tan, 1979; Macheix *et al.*, 1990) by increasing amounts of mRNA's synthesised (Christie *et al.*, 1995; Leyva *et al.*, 1995), and as mentioned above, an increase in PAL activity is followed by an

increase in anthocyanin level (Tan, 1980). Thus low temperature and light contribute to an increased production of PAL genes and also improve the PAL activity.

The period during which *de novo* synthesis of the enzymes occurs, explains partly the existence of the lag phase prior to anthocyanin appearance, during which necessary substances are produced for anthocyanin synthesis (Siegelman and Hendriks, 1958a). Various methods have been evaluated to shorten the lag phase in an attempt to produce colour quicker, but with little success. Siegelman and Hendriks (1958a) were able to shorten the lag phase, but always ended up with the same amount of anthocyanin produced after the irradiation period. No information could be found on the use of cold storage treatment before irradiation on improvement of colour formatiion.

In view of the fact that low temperature enhances the activity of the PAL enzyme, the aim of the first experiment was to determine whether a short storage period at –0.5°C prior to irradiation would enhance postharvest colour development after irradiation at two different temperature regimes. The second experiment aimed to optimise temperature conditions for maximum colour development with artificial light.

Materials and methods

Plant material: 'Cripp's Pink' apples were obtained from two farms in two different areas in the Western Cape, South Africa. The first farm, 'De Eike' is in the Witzenberg Valley area near Ceres (latitude: 33°14'S; longitude: 19°14'E; altitude 890 m) and the second farm, 'De Rust' is situated near Grabouw (latitude: 34°10'S; longitude: 19°07'E; altitude: 330 m). The climate in the Western Cape is Mediterranean with cool, wet conditions in winter, and hot dry conditions in summer. Mean maximum and minimum temperatures from September 1998 to mid-April 1999 are presented in Figure 1. The differences in microclimate resulted in slightly different harvest dates, but fruit were harvested at commercial maturity in both cases.

Irradiation treatments: In experiment 1 'Cripp's Pink' fruit were harvested at the optimum harvest date and divided into three groups. The first group was irradiated immediately after harvest, without any cold storage period, while the second and third groups were stored for 2 or 5 days, respectively, at -0.5°C before irradiation started. The third group was stored for 5 days at -0.5°C before irradiation was applied.

After the different storage periods, fruit were placed randomly in two growth chambers, the first set at a temperature of 6°C and the second set on a temperature of 20°C. The fruit was irradiated using a 400 W high-pressure sodium light (HPS; Philips SON-T, 253 mW/W). Light intensities were measured with a Decagon light meter (AccuPAR version 4.1, Decagon devices, Pullman, Washington, U.S.A) approximately 1 m from the light source. The light intensity that reached the fruit from the HPS light was 22 to 24 $W \cdot m^{-2}$.

In each group, six irradiation periods (0, 24, 48, 72, 96 and 120 hours) were used for each area. Each period consisted of six single-fruit replicates. Colour measurements were taken every 24 hours. Fruit were peeled after either 120 hours of irradiation at 6°C or 20°C, or after 120 hours at 20°C in the dark. Only the exposed half of the fruit was peeled.

In experiment 2, mature 'Cripp's Pink' apples were stored for 20 days at –0.5°C due to space constraints in the growth chambers. When fruit were taken out of storage, colour was measured prior to irradiation with a calorimeter. Fruit were placed randomly in two growth chambers, with temperatures set at either 6°C or an alternating temperature of 6/20°C for 12 hours each. Fruit were irradiated with HPS light (Philips SON-T, 253 mW/W) at light intensities of 22 to 24 $W \cdot m^{-2}$. Six irradiation periods (0, 24, 48, 72, 96 and 120 hours) were used for each area. Each period consisted of six single-fruit replicates. Colour measurements were taken after every irradiation treatment and fruit were peeled either after 120 hours at ± 20°C in the dark

or after 120 hours irradiation at 6°C or 6/20°C. Only the exposed side of the fruit was peeled.

Colour measurements: Circles were marked on the fruit and external colour was measured in the marked area. A colorimeter (model NR-3000, Nippon Denshoku; Kogyo Co. Ltd.) was used and although colour was expressed as chroma, hue angle and lightness value, only the hue angle data is presented. Hue angle (h° = arctangent [b*/a*]) refers to the angle formed by a line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).

Anthocyanin analyses: After irradiation, the exposed side of each fruit was peeled with a potato peeler. Replicates were pooled so that there would be sufficient tissue for analysis. The fresh peel was ground to a fine powder in liquid nitrogen using a pestle and mortar. The powder was lyophilised.

Anthocyanin extraction was based on the procedure of Siegelman & Hendriks (1958a). Fifteen ml of a 1% HCl in methanol solution was added to 500 mg of sample and left for 1 hour at room temperature in the dark. Following the extraction period, the samples were centrifuged for 20 minutes at 12 000 g_n. Absorbance of the supernatant was measured at 530 nm on a spectrophotometer (Beckman Instruments Operation, DU® Series 64, 2500 Harbor Boulevard, Fullerton, Ca. 92631-3100), with 1% HCl in methanol as the blank. A standard curve was obtained with idaein chloride (cy 3-gal) (Carl Roth GmbH and Company, Germany). Anthocyanin concentration was expressed as $\mu g \cdot g^{-1}$ dry peel.

Data analysis: The data were analysed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990). The analyses of experiments 1 and 2 were performed on the differences between initial

and final hue values after 120 hours of irradiation. Anthocyanin concentrations were expressed as a percentage of the concentration in fruit that were not irradiated.

Results and Discussion

Experiment 1

Grabouw area: The storage period prior to irradiation, played no significant role in postharvest colour development for the apples from the Grabouw area (P = 0.0804) (Table 1). Fruit held at room temperature (±20°C) in the dark showed very little change in colour after 120 hours, regardless of the storage treatments. The fruit irradiated at a temperature of 6°C showed the greatest decreases in hue angle, and these decreases increased as the low temperature storage period prior to irradiation increased. This is shown in the decrease of 29° for the fruit irradiated immediately after harvest and the greater decrease of 33° for the fruit stored for 5 days before irradiation started.

When irradiated at a temperature of 20°C, both the fruit irradiated immediately after harvest and those stored for 2 days exhibited only slight decreases in hue angle. As the pre-irradiation storage period increased to 5 days, colour development improved (Table 1). There was a significant difference in the change in hue angle between fruit irradiated at 6°C and fruit irradiated at 20°C (P = 0.0001) (Table1; Figure 2).

The decreases in hue angle were clearly a result of increases in anthocyanin concentration (Table 1). The increases in anthocyanin concentration were greater when fruit were irradiated at 6°C. Although the increase in anthocyanin percentage for fruit stored for 5 days appeared lower (179.9%), the actual anthocyanin concentration clearly showed that prior to irradiation the concentration was higher (138.9 $\mu g \cdot g^{-1}$) than for the fruit stored for 0 and 2 days. This explains the interaction between storage period and temperature regimes (P = 0.0001). The interaction reflects that the differences between the anthocyanin concentrations at different

temperatures were small for fruit irradiated immediately, increased for fruit pre-stored at -0.5°C for 2 days and then increased again for fruit pre-stored at -0.5°C for 5 days.

Ceres area: For the fruit from the Ceres area, results were different (Table 2). There were no significant differences in postharvest colour development between the different periods of storage (P = 0.1806) (Table 2). Similar to Grabouw, the fruit stored at room temperature in the dark displayed little change in hue angle values. The greatest decreases in hue angle were the fruit irradiated at 6°C, regardless of treatment prior to irradiation (±40°). The greatest decrease in hue angle was obtained from the 'Cripp's Pink' apples that were stored for 2 days at -0.5°C and then irradiated for 120 hours (45°) at 6°C (Figure 3).

The fruit from the Ceres area which were irradiated immediately or stored for 2 days before irradiation at 20°C, showed decreases of 21.9 and 25.1° respectively, compared to the more or less 4.5° decreases for the fruit from Grabouw. The fruit stored for 5 days had the same decrease in hue angle whether irradiated at 6°C or at 20°C. There was thus a significant difference in colour development at the different temperatures (6 vs. 20°C) (P = 0.0180).

The increases in anthocyanin concentrations again reflect the decreases in hue angle. The highest anthocyanin concentrations were obtained when fruit were irradiated at a temperature of 6°C. The highest anthocyanin concentration was 285.3 $\mu g \cdot g^{-1}$ and this reflects the decrease of 45° obtained from the fruit stored for 2 days at -0.5°C prior to irradiation. When irradiated at 20°C, anthocyanin concentration increased with prior storage at -0.5°C. When stored for 5 days before irradiation, the % increases in anthocyanin concentrations were again relatively small due to higher anthocyanin concentrations in control fruit held in dark. The differences in anthocyanin concentration between the two temperature regimes increased with time

pre-stored, and therefore resulted in the quadratic interaction between storage period and temperature (P = 0.0001) (Table 2).

In fruit from the Grabouw area, little colour developed at a temperature of 20°C, whereas the hue angle values of the fruit from Ceres showed significant decreases at the same temperature. Fruit from the warmer Grabouw area may have a greater need for a period of cold storage prior to irradiation at 20°C. In both cases the decreases in hue angle values increased as the storage period increased, but these were much greater for the fruit from the colder Ceres area, than those from the warmer Grabouw area. This can be seen in the fruit from Ceres, because 5 days at -0.5°C prior to irradiation stimulated postharvest colour development at 20°C as much as for the fruit irradiated at 6°C with or without prior cold storage. The cold storage period is needed to increase the synthesis of PAL mRNA (Christie *et al.*, 1995; Leyva *et al.*, 1995). According to Figure 1, the fruit from the Ceres area, received lower minimum and maximum temperatures throughout the whole growing season, than the fruit from Grabouw. It is possible, therefore, that preharvest temperatures may influence the postharvest colour development. However, the influence of preharvest temperatures was not as marked at lower irradiating temperatures (6°C), possibly due to continued synthesis of PAL mRNA at this low temperature (Christie *et al.*, 1995; Leyva *et al.*, 1995). There is no literature on fruit stored at –0.5°C before being irradiation.

The fact that the better results were obtained when fruit from both Ceres and Grabouw were irradiated at a temperature of 6°C, with or without the pre storage period, confirms the results of Tan, (1979). He found improved colour development at 6°C compared to 18°C. On the other hand, Saks *et al.* (1990) and Dong *et al.* (1995) found that the best colour development was obtained when fruit were irradiated at 20°C, and that the least colour development occurred at a temperature of 2°C. Arthur (1932) also proved that colour development was better at a higher

temperature of 15°C, and that the rate of colour development was less than half this rate when the temperature was held at 5°C. Therefore twice the period of exposure was needed to colour fruit at 5°C than at 15°C. This is contrary to these results (Table 1 & 2), where colour development at 20°C was lower than at 6°C after 120 hours of irradiation. Arthur (1932) also stated that apples will not colour more rapidly when held at a temperature higher than 15°C, and since they ripen and soften much more rapidly at a higher temperature, 15°C seems to be best for practical purposes. Arakawa (1991) found that as fruit ripened the optimum temperature for anthocyanin synthesis shifted from 15°C to 25°C, but temperatures <15°C resulted in lower anthocyanin concentrations.

Siegelman and Hendriks (1958b) concluded that temperature influenced the length of the induction period, as well as the rate of anthocyanin production. Saks *et al.* (1990) confirmed this by proving slower induction of anthocyanin synthesis at a temperature of 2°C than at a temperature of 20°C. He stated that although induction was slower at a temperature of 13°C than at 20°C, the same amount of anthocyanin was produced in the end. Our results are contrary to these, because according to Figures 1 and 2, induction was not slower at 6°C than at 20°C. A temperature of 6°C resulted in faster and better colour development than fruit irradiated at a temperature of 20°C, for both the fruit from Grabouw and Ceres.

Experiment 2

Grabouw area: Fluctuating temperatures of 6/20°C had resulted in the greatest decrease in hue angle (70°), with the corresponding increase of 499.4% in anthocyanin concentration (Table 3). The treatment of irradiation at 6°C resulted in a decrease of 22.4° in hue angle value, with an increase of 158.2% in anthocyanin concentration (207.2 $\mu g \cdot g^{-2}$). The hue angle value of the fruit stored at room temperature in the dark did not change. The differences between 6/20°C and 6°C

were significant for both the hue angle changes (P = 0.0001) and anthocyanin concentration (P = 0.0001) (Figure 4).

Ceres area: The results from the Ceres area were similar to those from Grabouw (Table 4). The best decrease in hue angle was obtained by irradiation at a fluctuating temperature of 6/20°C (65.3°), compared to the decrease on only 18.3° at a constant temperature of 6°C. The anthocyanin concentration increased accordingly. The fluctuating temperature resulted in an increase of 408.5% (676.9 $\mu g \cdot g^{-2}$), compared to the 121.9% (201.9 $\mu g \cdot g^{-2}$) increase at a constant temperature. Once again, there were significant differences in hue value and anthocyanin concentrations between the two temperature regimes (P = 0.0001) (Figure 5).

Our results clearly prove that alternating temperatures of 6/20°C was more effective in colour development than a constant temperature of 6°C, on fruit from both Ceres and Grabouw. As mentioned above, the PAL enzyme affect anthocyanin synthesis, and low temperature stimulates PAL synthesis (Creasy, 1968; Chalmers and Faragher, 1977; Faragher, 1983, Arakawa *et al.*, 1986, Kubo *et al.*, 1988). Tan (1979) founnd that PAL activities in whole 'Red Spy' apples that received alternating 6/18°C and 6/25°C in light was 20 % higher than that in apples at constant 18°C and 25°C, respectively (Tan, 1979). In apple skin, PAL is regulated by a PAL-inactivating system (PAL-IS) (Macheix *et al.,* 1990; Mazza & Miniati, 1993). In constant light, the activity of PAL-IS was lower in the apples that received alternating 6/20°C treatment than the apples that received constant 25°C (Tan, 1979). However, Blankenship and Unrath (1988) reported that PAL-IS was not closely involved in regulating anthocyanin synthesis because it increased during anthocyanin accumulation and its levels during maturation were comparable between 'Starkrimson Delicious' and 'Golden Delicious'.

Although experiment 1 and 2 cannot be compared statistically, it was interesting to observe that the response at 6°C differed between the two experiments. The decreases in hue angle were greater in experiment 1 (± 31° for Grabouw, and ± 41° for Ceres) than in experiment 2 (± 22° for Grabouw, and ± 18° for Ceres). The only difference between these fruit was the duration of the cold storage period. Bishop and Klein (1975) found that the ability of apples, previously stored in air, to synthesise anthocyanin under light decreased sygmoidally with storage time, but apples may maintain considerable capacity for anthocyanin formation after 6 months of storage or even longer. Our results in experiment 1 indicated that a storage period of up to 5 days did not play any significant role in colour development at a temperature of 6°C. On the other hand, the fruit of experiment 2 showed a decreased ability to produce colour at 6°C, after a storage period of 20 days at -0.5°C. Therefore it seems that the ability to produce colour at a temperature of 6°C, possibly decreased as a result of the extended storage period.

Conclusion

Best colour development for the fruit from Ceres and Grabouw occurred at a temperature of 6°C, regardless of the number of days that fruit were held at -0.5°C prior to treatment. At a temperature of 20°C, colour development was slower, but it improved with time stored at -0.5°C prior to irradiation. Fluctuating temperatures of 6/20°C resulted in greater decreases in hue angle values and increased in anthocyanin concentration, than at a constant temperature of 6°C.

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Table 1. The effect of irradiation with HPS light at different temperatures on 'Cripp's Pink' apples grown in Grabouw and stored for different periods at -0.5°C prior to irradiation. Control fruit were stored in the dark at room temperature (± 20°C).

Grabouw area							
Storage (days)	**Time of irradiation (h)**	**Temperature (°C)**	**Initial hue value**	**Final hue value**	**Δ Hue angle**	**Anth. concent. ($\mu g \cdot g^{-1}$)**	**% Increase in Anth. concent.**
0	Dark	±20	106.4	104.7	-1.6 a	91.1 f	100.0
	120	6	107.6	78.4	-29.2 c	212.4 b	233.3
	120	20	111.0	106.4	-4.6 ab	125.8 e	138.4
2	Dark	±20	105.1	106.4	1.3 a	85.1 f	100.0
	120	6	102.1	70.5	-31.6 c	212.0 b	249.3
	120	20	103.8	99.2	-4.7 ab	124.7 e	146.6
5	Dark	±20	100.5	102.6	2.1 a	138.9 d	100.0
	120	6	102.0	68.9	-33.1c	249.7 a	179.9
	120	20	106.2	92.0	-14.2 b	160.1 c	115.3
CONTRASTS:							
Storage LIN					0.0804	0.0001	0.0001
Storage QUAD					0.6739	0.0001	0.0001
Temp. (6°C vs. 20°C) LIN					0.0001	0.0001	0.0002
INTER LIN*LIN					0.4175	0.0001	0.0001
INTER QUAD*LIN					0.5039	0.0001	0.0001

*Differences in anthocyanin concentrations were calculated comparing the percentage differences in anthocyanin concentration after the irradiation treatment. Values followed by different letters indicate significant differences ($P<0.05$) according to the LSD test.

Table 2. The effect of irradiation with HPS light at different temperatures on 'Cripp's Pink' apples grown in Ceres and stored for different periods at -0.5°C prior to irradiation. Control fruit were stored in the dark at room temperature (± 20°C).

Ceres area							
Storage (days)	**Time of irradiation (h)**	**Temperature (°C)**	**Initial hue value**	**Final hue value**	**Δ Hue angle**	**Anth. concent. ($\mu g \cdot g^{-1}$)**	**% Increase in Anth. concent.**
0	Dark	±20	105.8	107.0	1.2 a	115.0 f	100.0
	120	6	106.2	67.3	-38.9 bc	235.1 b	204.6
	120	20	108.0	86.1	-21.9 b	202.8 c	176.5
2	Dark	±20	107.5	108.7	1.2 a	81.1 g	100.0
	120	6	109.9	64.9	-45.0 c	285.3 a	318.9
	120	20	107.6	82.6	-25.1 b	193.0 d	238.3
5	Dark	±20	108.6	107.6	-1.1 a	128.1 e	100.0
	120	6	105.0	66.3	-38.7 bc	255.3 a	199.6
	120	20	106.4	64.5	-38.9 bc	239.6 b	187.2
CONTRASTS:							
Storage LIN					0.1806	0.0001	0.1305
Storage QUAD					0.8142	0.0429	0.0001
Temp. (6°C vs. 20°C) LIN					0.0180	0.0001	0.0001
INTER LIN*LIN					0.1341	0.0010	0.0493
INTER QUAD*LIN					0.3583	0.0001	0.0001

*Differences in anthocyanin concentrations were calculated comparing the percentage differences in anthocyanin concentration after the irradiation treatment. Values followed by different letters indicate significant differences ($P<0.05$) according to the LSD test.

Table 3. The effect of irradiation with HPS light at different temperatures regimes on 'Cripp's Pink' apples, grown in Grabouw and stored for 20 days at -0.5°C prior to irradiation.

Grabouw area						
Time of rradiation (h)	**Temperature (°C)**	**Initial hue value**	**Final hue value**	**Δ Hue angle**	**Anth. concent. ($\mu g \cdot g^{-1}$)**	**% Increase in Anth. concent.**
Dark	±20	106.0	106.5	0.5 a	131.0 c	100.0 c
120	6/20	109.9	39.8	-70.0 b	654.1 a	499.4 a
120	6	98.4	76.0	-22.4 c	207.2 b	158.2 b
Significance (P<0.05)						
Temp. 6/20°C vs. 6°C				0.0001	0.0001	0.0001

Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 4. The effect of irradiation with HPS light at two different temperature regimes on 'Cripp's Pink' apples, grown in Ceres and stored for 20 days at -0.5°C prior to irradiation.

Ceres area						
Time of irradiation (h)	**Temperature (°C)**	**Initial hue value**	**Final hue value**	**Δ Hue angle**	**Anth. concent. ($\mu g \cdot g^{-1}$)**	**% Increase in Anth. concent.**
Dark	±20	105.5	107.3	1.8 a	165.8 c	100.0 c
120	6/20	106.8	41.5	-65.3 c	676.9 a	408.5 a
120	6	111.3	93.0	-18.3 b	201.9 b	121.9 b
Significance (P<0.05)						
Temp. 6/20°C vs. 6°C				0.0001	0.0001	0.0001

Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

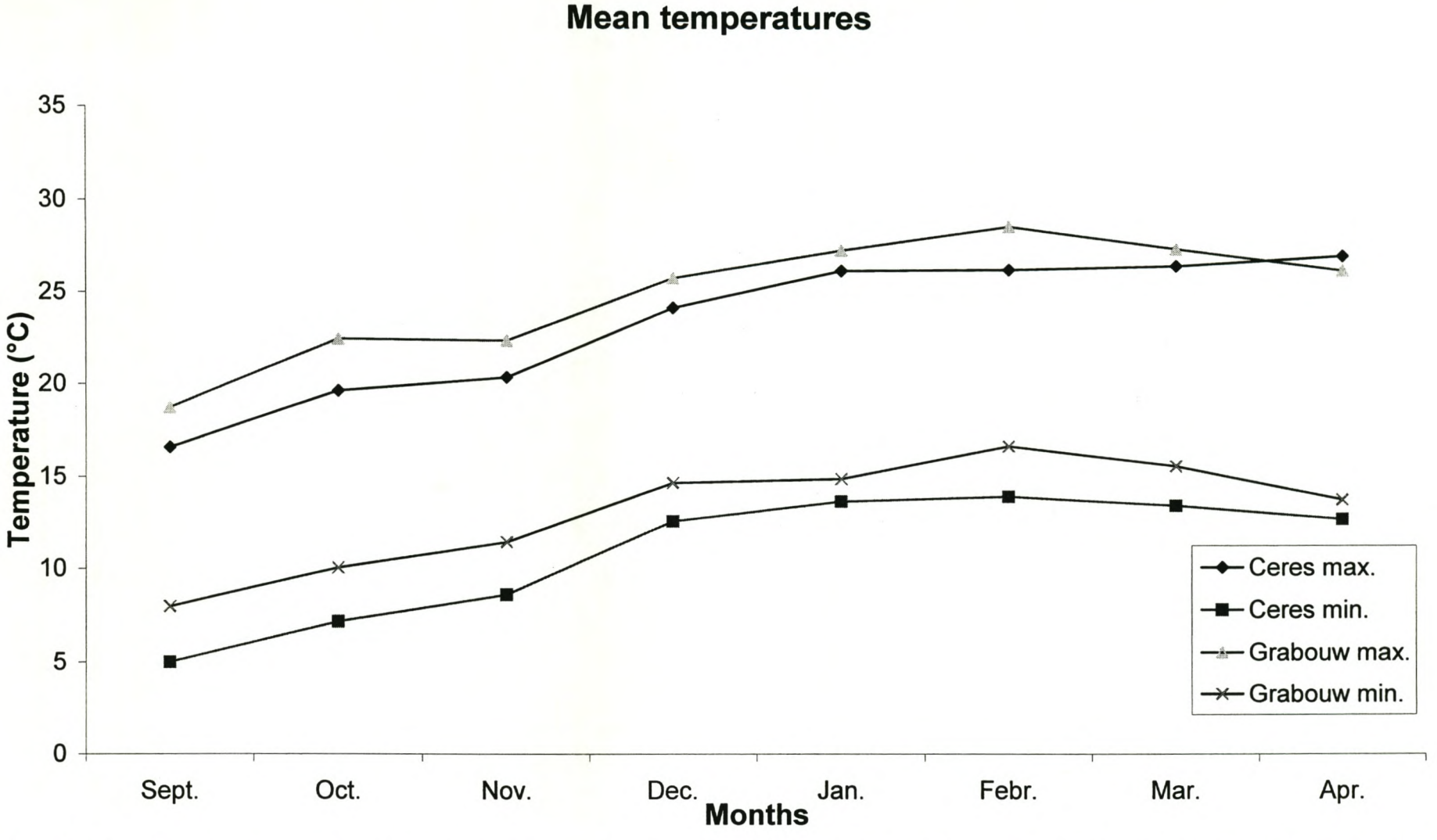

Figure 1. The mean maximum and minimum temperatures from September 1998 to April 1999 measured at the two farms in Ceres and Grabou that supplied the fruit for experiments 1 and 2. Values used for April 1999 were only until mid-April (harvest date).

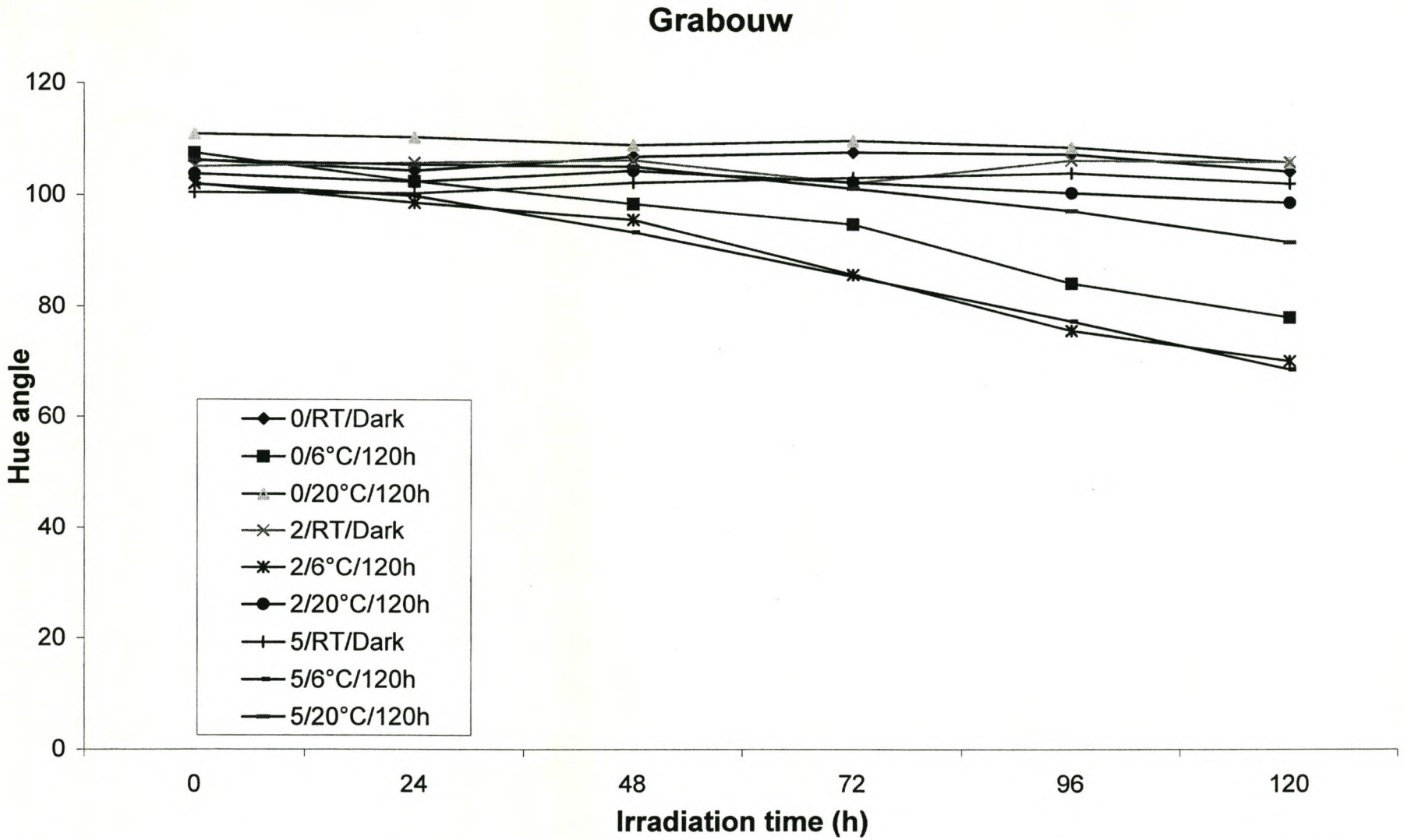

Figure 2: Effect of irradiation with HPS light on the hue angle of 'Cripp's Pink' apples stored at -0.5 °C for 0, 2, or 5 days prior to irradiation at 6 °C or 20 °C. Control fruit were stored in the dark at room temperature (20 °).

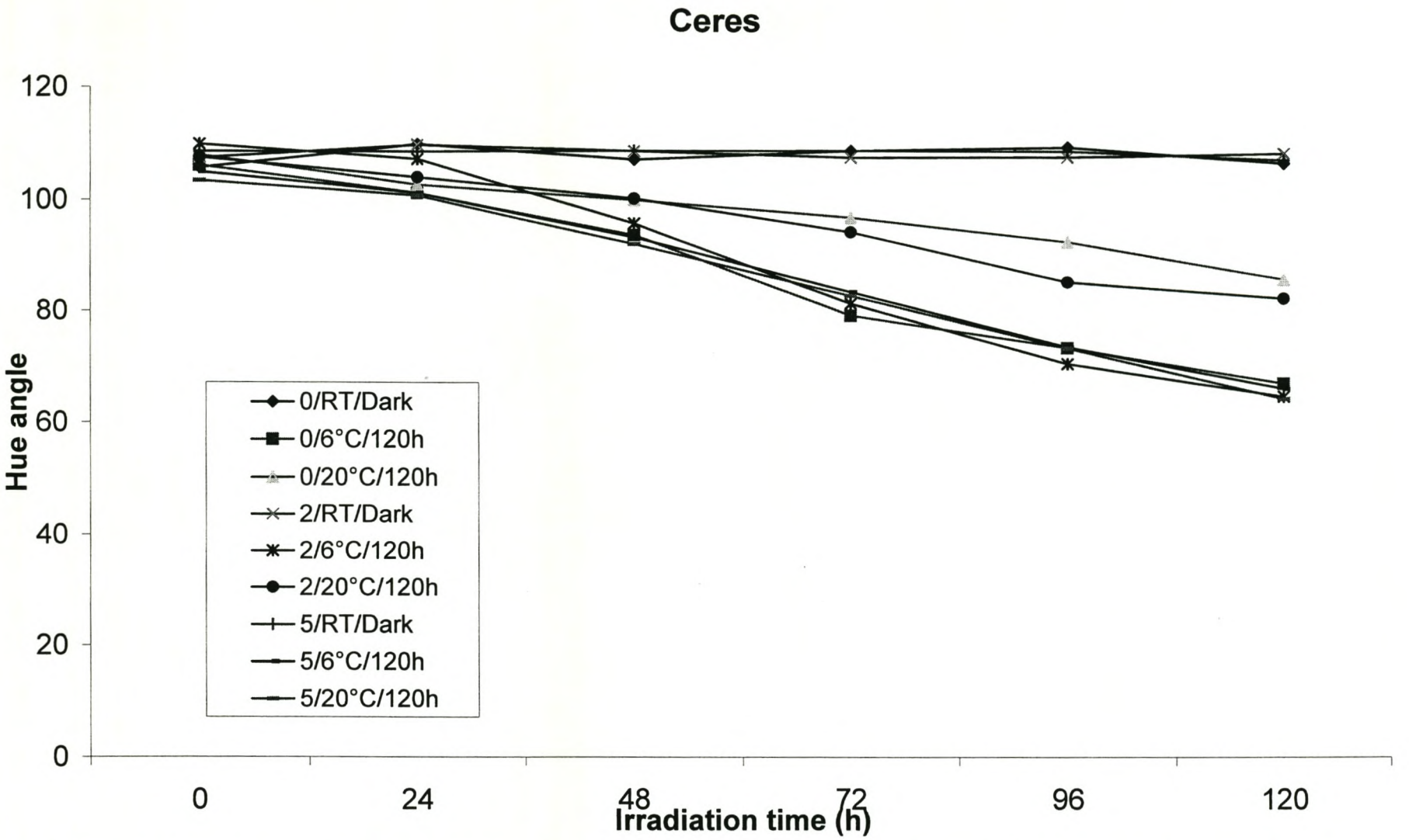

Figure 3: Effect of irradiation with HPS light on the hue angle of 'Cripp's Pink' apples stored at -0.5 °C for 0, 2, or 5 days prior to irradiation at 6 °C or 20 °C. Control fruit were stored in the dark at room temperature (20 °).

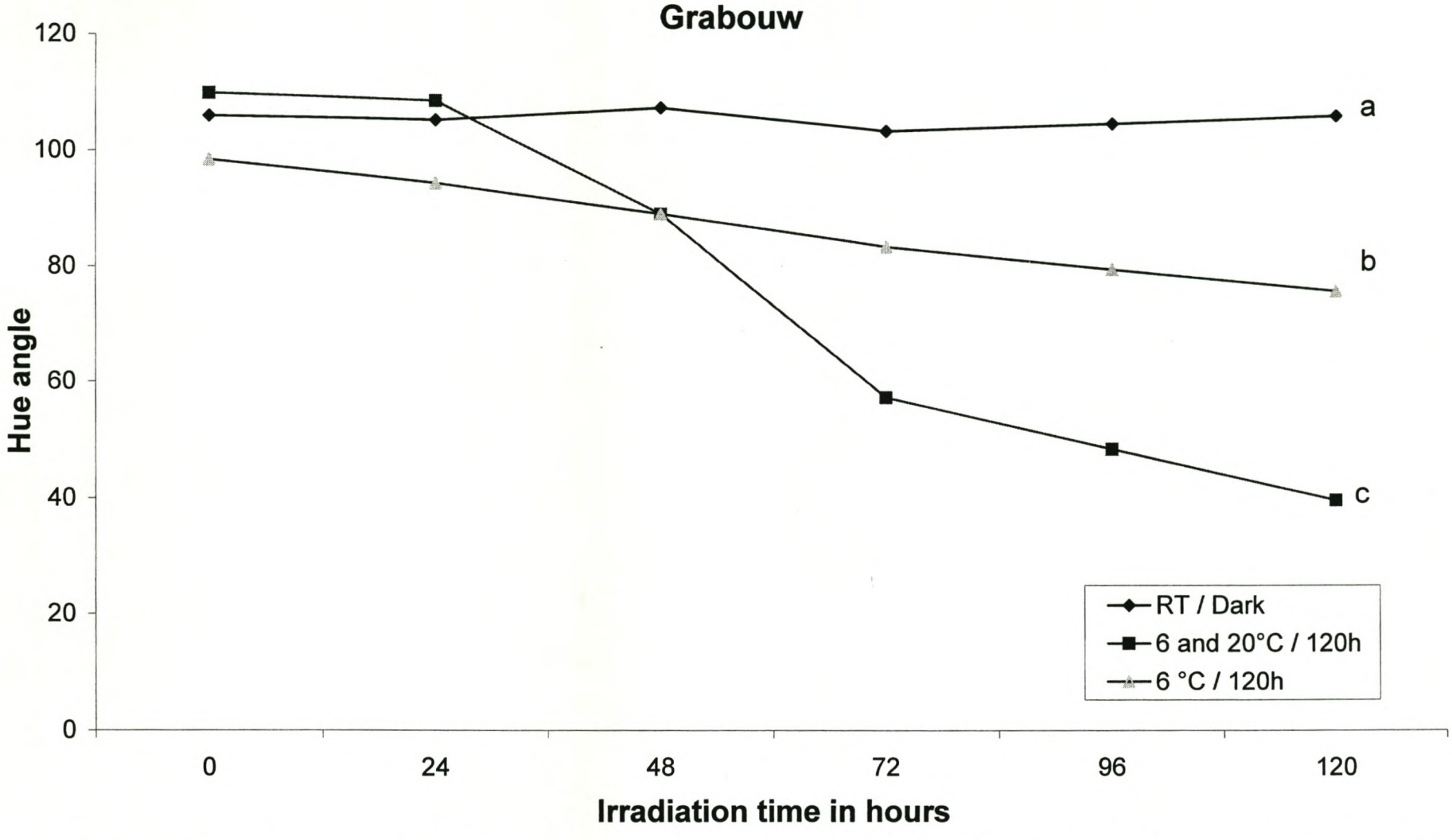

Figure 4: The effect of irradiation with HPS light at a fluctuating temperature of 6/20 °C or 6 °C on the hue angle of 'Cripp's Pink' apples from the Grabouw area. Control fruit were stored in the dark at room temperature (20 °C). The statistics were performed on the differences of the final hue values between the treatments.

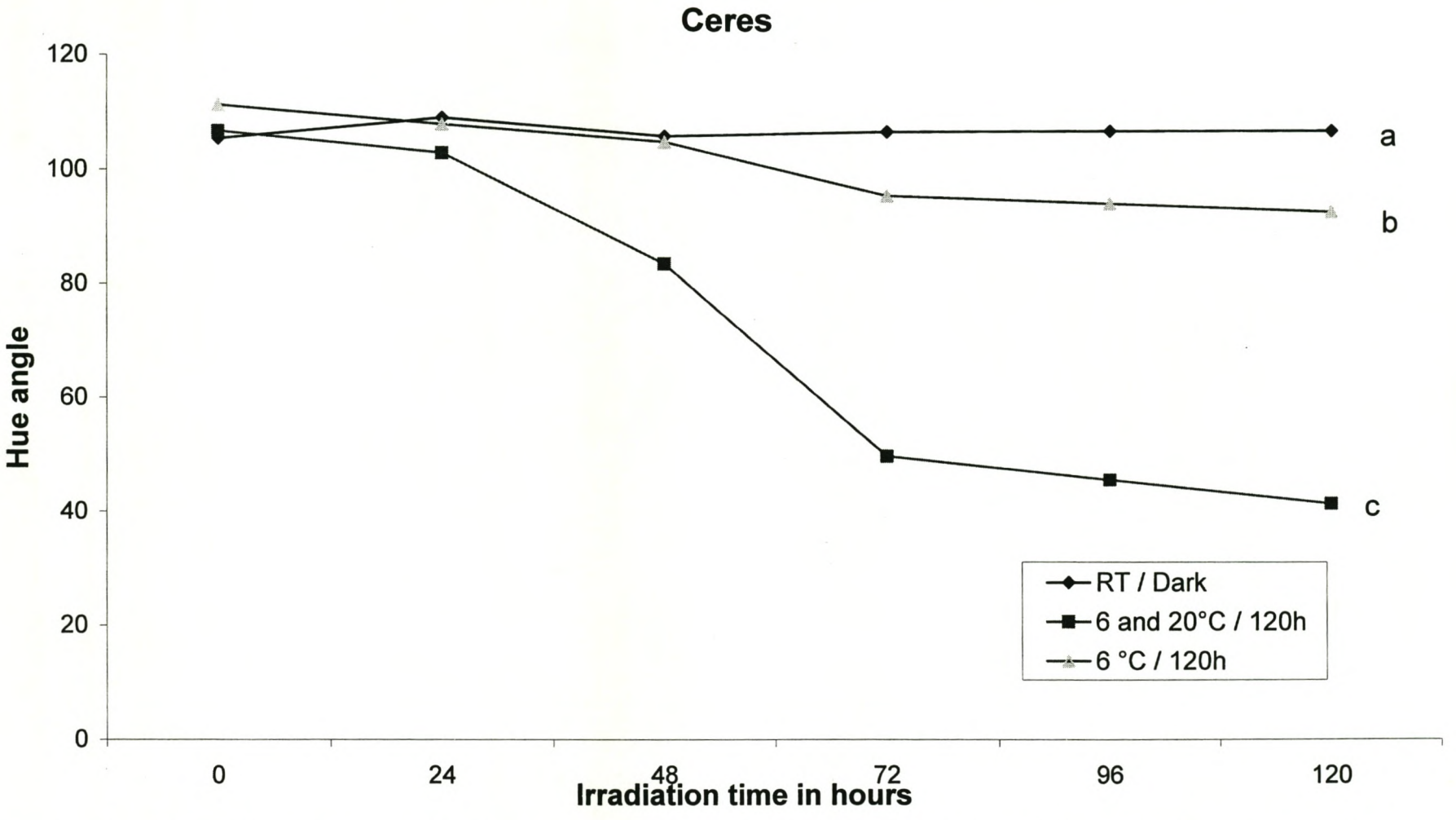

Figure 5: The effect of irradiation with HPS light at a fluctuating temperature of 6/20 °C or 6 °C on the hue angle of 'Cripp's Pink' apples from the Ceres area. Control fruit were stored in the dark at room temperature (20 °C). The statistics were performed on the differences of the final hue values between the treatments.

Paper 4: Disappearance of colour in 'Cripp's Pink' apples under high temperatures and artificial light.

Abstract

'Cripp's Pink' fruit with an initial hue angle value of 24° and an anthocyanin concentration of 739.9 μg·g^{-1} were placed in a growth chamber at 37°C. Half of the fruit were covered with polystyrene and the other half were exposed to 22 to 24W·m^{-2} high-pressure sodium light. Over a period of 144 hours of exposure to the light the hue angle value increased by 25° and the anthocyanin concentration decreased to 283.6 μg·g^{-1}. The hue angle of the covered fruit did not change significantly. Although the anthocyanin concentration in covered fruit were significantly different from each other, there was no consistent trend, and the differences were ascribed to natural variation in fruit colour. It can therefore be concluded that high temperature alone did not degrade anthocyanin, but the synergistic effect of high temperature with exposure to light affects hue angle and actual anthocyanin concentration.

Introduction

The breakdown or metabolic 'turnover' of anthocyanin is an area where there is little information. Phytochrome acts as a photoreceptor in the light-dependant anthocyanin synthesis, and anthocyanin synthesis is stimulated by the energy absorbed by phytochrome. Young meristematic tissue contains high levels of phytochrome, which result in the synthesis of anthocyanin. Young fruit do not have the ability to break the anthocyanin down, therefore anthocyanin accumulates in the young fruit which displays red colour (Mancinelli, 1985). The phytochrome level in mature fruit is low, but with the initiation of maturity of the fruit, the breakdown of chlorophyll takes places and it improves the efficiency of phytochrome (Downs *et al.*, 1965; Mancinelli and Rabino, 1984).

As the fruit grows the colour disappears, either as a result of a dilution effect from the increase in size, or as a result of degradation, either with no anthocyanin synthesis or synthesis at a slower rate than degradation. The factor responsible for the predominance of degradation over synthesis causing a depression in anthocyanin content has not yet been identified (Viljoen, 1996). The fruit again develop colour two to three weeks prior to harvest (Chalmers *et al.*, 1973). Chalmers *et al.* (1973) concluded that the increasing capability of mature fruit to form anthocyanin compared to immature fruit is based on anthocyanin degradation. The rate of degradation of anthocyanin in immature fruit is as rapid as it is formed. In mature fruit the rate of degradation is less than the rate of synthesis. Very little is known on the mechanism of preharvest degradation, or the factors and variables involved in the action of degradation.

Light and temperature are the major environmental variables involved in production of anthocyanin (Chalmers & Faragher, 1977; Tan, 1979; Arakawa *et al.*, 1985; Saure, 1990), and postharvest improvement of colour with artificial light has been accomplished

(Arthur, 1932; Siegelman and Hendriks, 1958; Downs *et al.*, 1965; Chalmers *et al.*, 1973; Arakawa *et al.*, 1985, 1988; Saks *et al.*, 1990). Irradiation at temperatures from 2°C (Saks *et al.*, 1990; Dong *et al.*, 1995) to 25°C (Faragher, 1983; Arakawa, 1991) induce anthocyanin production and therefore red colour formation. There is no recorded literature that artificial irradiation at higher temperatures adversely affect colour. Enzymatically, degradation of anthocyanin can be accomplished by polyphenol oxidase, ß-glycosidase and peroxidase (Calderon *et al.*, 1992). Yet, there is little data to support any of the hypotheses in the action of anthocyanin degradation in intact cells.

The hypothesis of this research is that irradiation at high temperatures adversely affects colour and anthocyanin concentration of 'Cripp's Pink' apples.

Material and methods

Plant material: 'Cripp's Pink' apples were obtained from the farm 'De Eike' (latitude 33°14'S, longitude 19°14'E, altitude 890 m) in the Witzenberg Valley near Ceres in the Western Cape, South Africa. The Western Cape has a Mediterranean climate with cool, wet winters and hot, dry summers.

Irradiation treatments: Well coloured 'Cripp's Pink' apples with an initial hue angle of ± 24° were picked randomly from the trees, and divided into two groups. Both groups of fruit were placed in the growth chamber set at a temperature of 37°C, after initial colour measurements were taken. Half the fruit were covered with white polystyrene and the other half were exposed to high-pressure sodium light at 22 to 24 $W \cdot m^{-1}$ (400 W HPS, Philips SON-T, 253 mW/W). The light intensities were measured with a Decagon light meter (AccuPAR version 4.1, Decagon devices, Pullman, Washington, U.S.A) approximately 1 m from the light source. Seven single-fruit replicates were used for each of the exposure periods (0, 24, 48, 72, 96, 120, 144 hours).

Colour measurements: Circles were marked on the fruit and external colour was measured in the marked area. Measurements were taken initially and again after every exposure period. A colorimeter (model NR-3000, Nippon Denshoku; Kogyo Co. Ltd.) was used and although colour was expressed as chroma, hue angle and lightness value, only hue angle data is presented. Hue angle (h° = arctangent [b*/a*]) refers to the angle formed by a line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992).

Anthocyanin analyses: After irradiation, each fruit was peeled with a potato peeler, and replicates were pooled so that there would be sufficient tissue for analysis. Only the exposed half of the fruit was peeled. The fresh peel was ground to a fine powder in liquid nitrogen using a pestle and mortar. The powder was lyophilised.

Anthocyanin extraction was based on the procedure of Siegelman & Hendriks (1958). Fifteen ml of a 1% HCl in methanol solution was added to 500 mg of sample and left for 1 hour at room temperature in the dark. Following the extraction period, the samples were centrifuged for 20 minutes at 12 000 g_n. Absorbance of the supernatant was measured at 530 nm on a spectrophotometer (Beckman Instruments Operation, DU® Series 64, 2500 Harbor Boulevard, Fullerton, Ca. 92631-3100), with 1% HCl in methanol as the blank. A standard curve was obtained with idaein chloride (cy 3-gal) (Carl Roth GmbH and Company, Germany). Anthocyanin concentration was expressed as $\mu g \cdot g^{-1}$ dry peel.

Data analysis: The data were analysed with the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

Results and Discussion

When fruit were irradiated with HPS light at a temperature of 37°C there was a significant linear increase in hue angle with time of irradiation (P = 0.0006) (Table 1; Figure 1). The increase was the greatest after 144 hours of irradiation (24.9°). The increase in hue angle is as a result of a significant decrease in red colour corresponding to a drop in anthocyanin concentration (P = 0.0001). The anthocyanin concentration decreased from 739.9 $\mu g \cdot g^{-1}$ to 283.6 $\mu g \cdot g^{-1}$, a decrease of 62% (Table 1; Figure 2).

When the fruit were stored at 37°C without any irradiation, there was no significant change in hue value after a period of 144 hours (P = 0.4881) (Figure 1). There were significant differences in relative anthocyanin concentrations between the different irradiation treatments (P = 0.0003). This is attributed to natural variation between the fruit, and a significant decrease in anthocyanin concentration (Table 1; Figure 2).

There was a significant difference in hue angle between fruit exposed to 37°C with or without light for 144 hours (P = 0.0006). The significant difference between the two treatments was also reflected in the anthocyanin concentration (P = 0.0002) and the relative anthocyanin concentration (P = 0.0002). The fruit held at 37°C in the light for 144 hours had an anthocyanin concentration of 283.6 $\mu g \cdot g^{-1}$, compared to 742.2 $\mu g \cdot g^{-1}$ in fruit held at 37°C in the dark.

This response to high temperatures has been observed previously. The red colour of apples (Saks *et al.*, 1990; Saure, 1990), grapes (Kliewer, 1977; Flora 1978) and roses (Biran and Halevy, 1973) decreases when day temperatures in the field exceed 30°C. In this study no anthocyanin degradation or decrease in hue angle values occurred at a temperature of 37°C in the dark (without any light), but the degradation occurred when the high temperatures were applied together with exposure to light (Table 1). It therefore

seems that the degradation of the anthocyanins depends on the synergistic effect of high temperature plus exposure to light.

Creasy (1968) and Saure (1990) observed that excessive high day temperatures inhibits the synthesis of anthocyanin, and that it is not only the high day temperatures that is destructive, but red skin colour was also decreased with high night temperatures (Blankenship, 1987). The synthesis of anthocyanin is inhibited at high temperatures, possibly as a result of increased inactivation of PAL at high temperatures (Faragher, 1983). The PAL-IS levels are also higher at temperatures of 18°C than at lower temperatures (6°C) (Tan, 1979). Marais (1995) observed a decrease in colour of 'Forelle' pears during the period just prior to harvest, when day temperatures often exceed 35°C. The 'Forelle' fruit acquired a brownish colour that decreased the attractiveness of the fruit.

Anthocyanins can be unstable and are decolourised by enzymatic systems during processing. They can be converted from their characteristic natural red or blue colours to undesirable brown coloured compounds. The major factors affecting degradation of anthocyanin during processing and storage include temperature, pH, phenolic compounds, sugar and sugar degradation products, oxygen and ascorbic acid (Starr and Francis, 1968; Mazza and Brouillard, 1990).

The mechanism of the degradation of anthocyanins in intact apple cells is unknown. Although anthocyanins can be degraded by polyphenol oxidase (PPO), anthocyanins are present in the vacuole and PPO is located in the chloroplast. Nothing is known about vacuolar efflux of anthocyanins to chloroplasts and it is unlikely that PPO plays a role in anthocyanin degradation in intact tissue (Calderon *et al.*, 1992; Piffaut *et al.*, 1994). Yet, anthocyanin degradation as a result of PPO does occur if fruit are processed, due to

mechanical maceration of membranes which enables reaction between enzyme and substrate.

Both ß-glycosidases and peroxidases are located in vacuoles, and therefore could participate in the degradation of anthocyanins in the vacuole. Calderon *et al.* (1992) suggested that degradation of anthocyanins in grapes is initiated by a ß-glycosidase-catalysed removal of glycoside, releasing the corresponding aglycones. The aglycone could then undergo an enzymatic oxidation catalysed by peroxidases, since while glycosides are not substrates of peroxidases, the corresponding aglycones are (Calderon *et al.*, 1992).

Another possibility for the degradation of anthocyanin is the changes in the structure, which occurs with pH fluctuations. In very acidic environments the anthocyanin molecule exists in the red flavylium cation form. As the pH increases, more of the blue quinonoidal bases are formed. At the same time slower pseudo-base reactions also forms the colourless carbinol/pseudobase. With acidification the flavylium form can be restored. The colourless carbinol can, in turn, form a pale yellow chalcone. This chalcone form is susceptible to further degradation, and this reaction is no longer reversible (Brouillard *et al.*, 1997). The above-mentioned discussions are only speculations on the possible mechanism of anthocyanin degradation, but there is no data supporting the possibilities.

In processed products, heat causes a change in the concentration of certain anthocyanins in red grape juice (Flora, 1978), and as a result, the juice changes colour. Robinson *et al.* (1966) ascribed this change in colour to the heat sensitivity of anthocyanins, like malvidin. They also proved that the concentration of delphinidin, peonidin and cyanidin in grape juice decreased when exposed to high temperatures.

Cemeroglu *et al.* (1994) tested anthocyanin degradation in sour cherry juice and found that higher temperatures and longer times promoted higher anthocyanin degradation.

In conclusion, irradiation with HPS light at a temperature of 37°C resulted in degradation of the anthocyanins, hence, supporting the hypothesis that irradiation at high temperature adversely effects colour of 'Cripp's Pink' apples, since there is very little published information on this degradation concept, learning great opportunities for further research in this field.

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Table 1. The effect of the combination of high temperature plus irradiation on the hue value and anthocyanin concentration of 'Cripp's Pink' apples.

	'Cripp's Pink'					
Treatment	**Time of irradiation (h)**	**Initial hue value**	**Final hue value**	**Δ Hue value**	**Anth. concent. ($\mu g \cdot g^{-1}$)**	**Relative Anth. concent. (%)**
37°C + Light	0	24.3			739.9	100.0
	24	25.9	29.3	3.4	681.4	92.1
	48	23.8	32.0	8.2	470.5	63.6
	72	23.7	36.6	12.0	451.9	61.1
	96	24.7	44.5	18.4	371.7	50.1
	120	24.6	43.3	19.8	340.7	46.0
	144	25.3	48.3	24.9 a	283.6 b	38.4 a
				0.0006	0.0001	0.0001
37°C – Light	0	24.3			743.3	100.0
	24	24.8	25.6	0.8	757.1	101.9
	48	22.9	26.0	2.5	737.7	99.3
	72	23.2	26.3	2.9	809.3	108.9
	96	23.8	25.9	2.2	683.6	92.0
	120	20.7	23.1	2.5	774.3	104.2
	144	24.8	25.5	-0.7 b	742.2 a	99.8 b
				0.4881	0.0002	0.0003
Significance (P<0.05)						
37°C + Light vs. 37°C – Light				0.0006	0.0002	0.0002

*Differences in hue angle and anthocyanin concentrations were calculated comparing the differences in final anthocyanin concentration and hue values after the irradiation treatment. Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

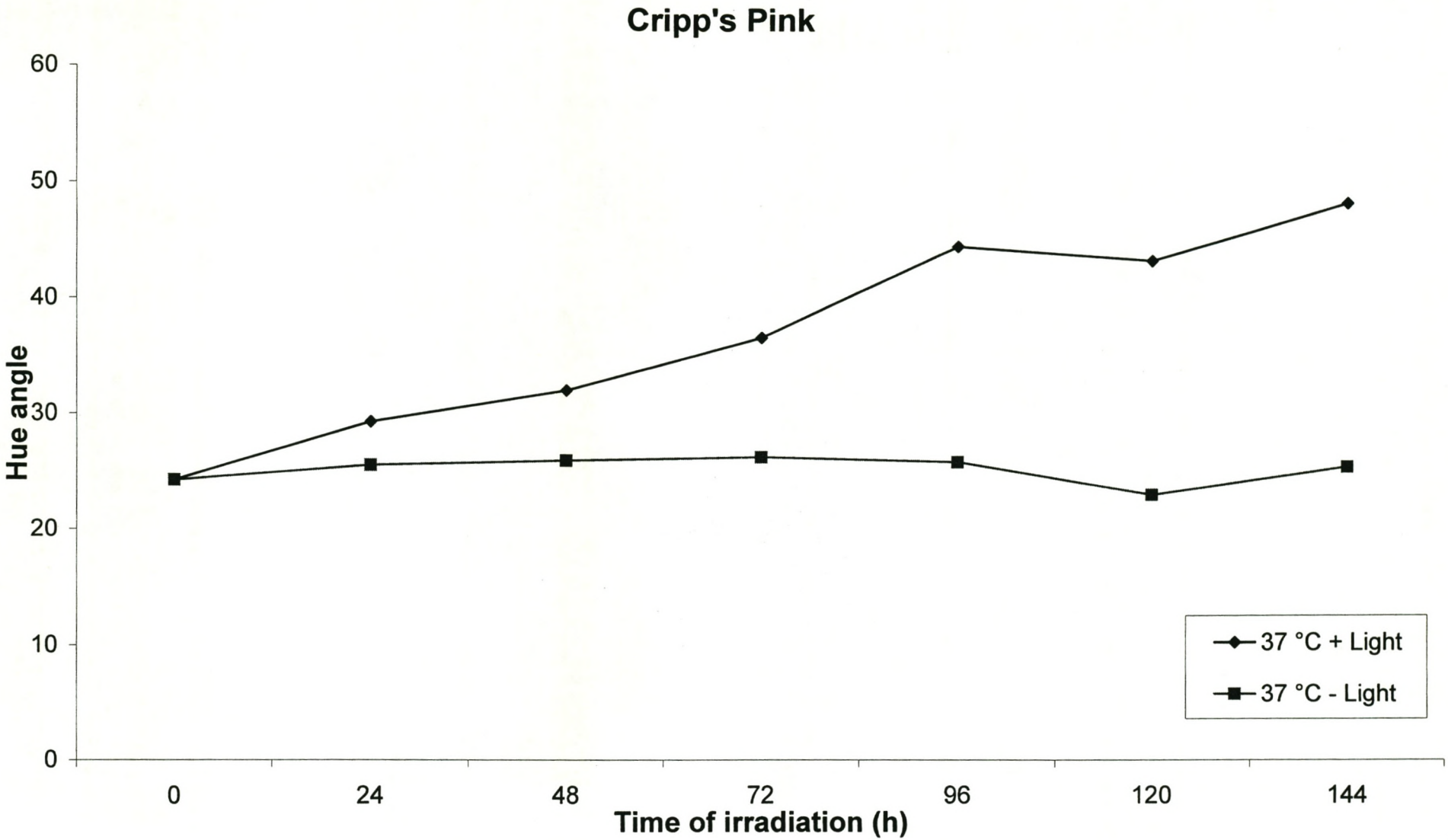

Figure 1. The effect of irradiation at a temperature of 37 °C on the hue angle of 'Cripp's Pink' apples.

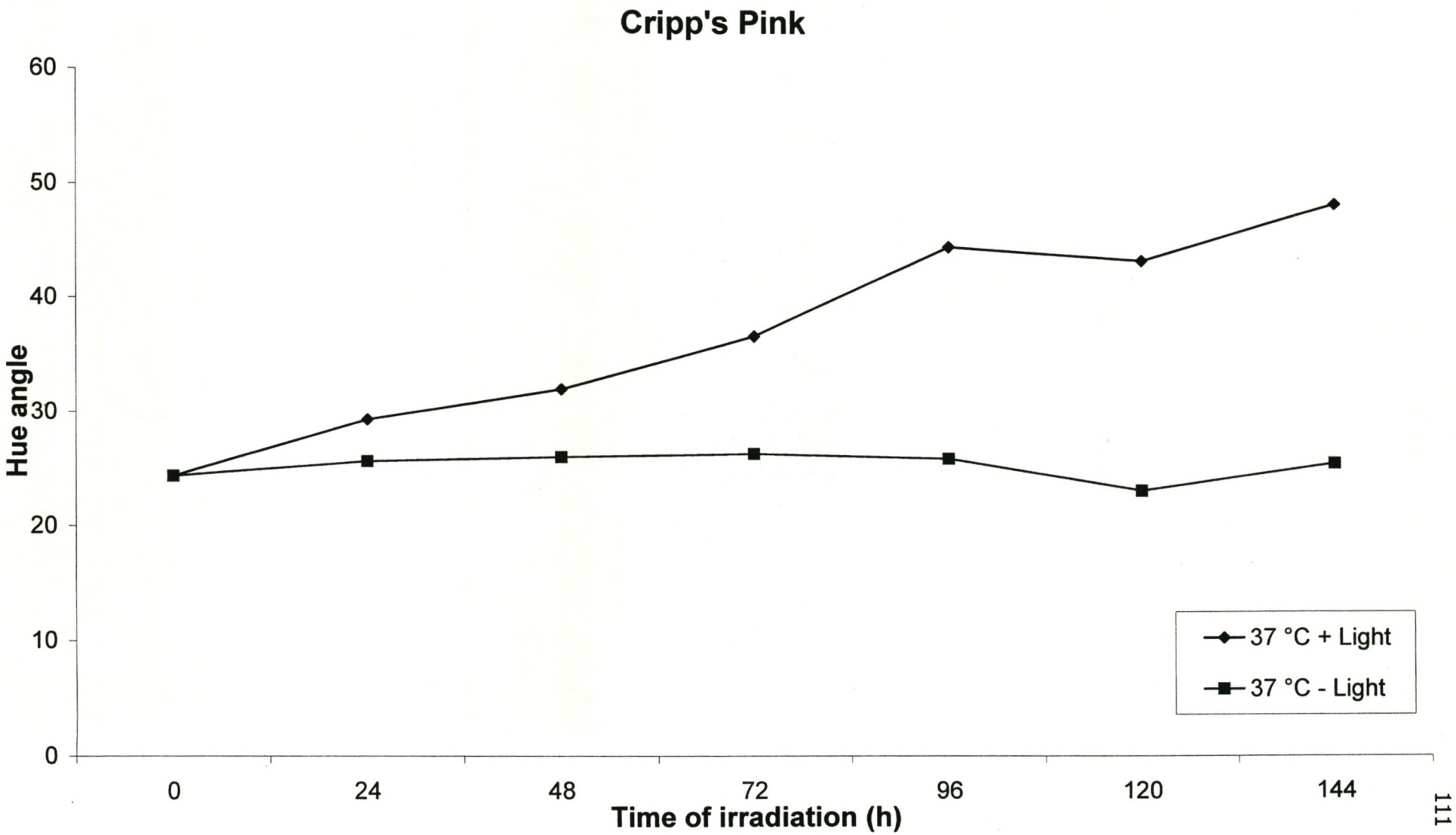

Figure 1. The effect of irradiation at a temperature of 37 °C on the hue angle of 'Cripp's Pink' apples.

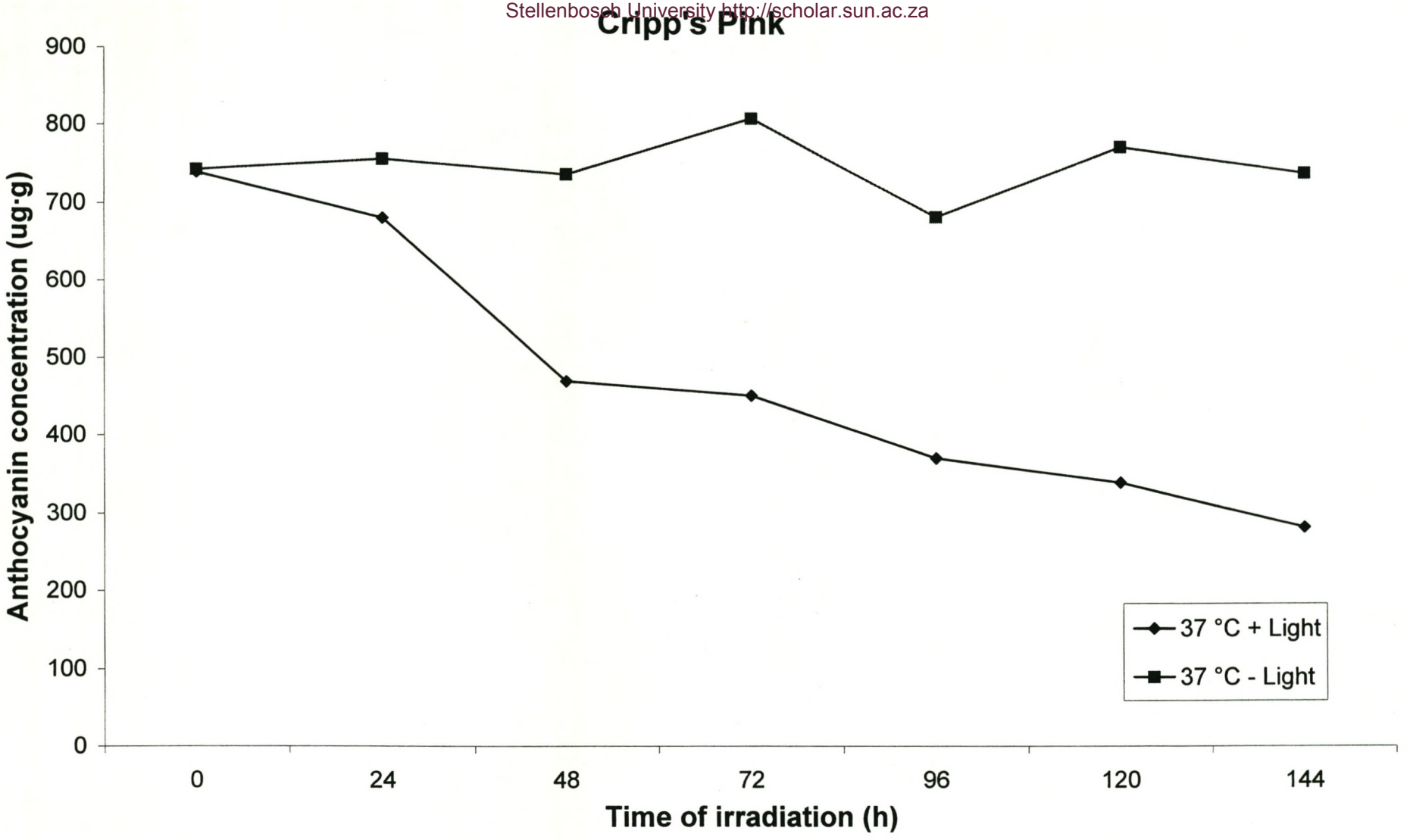

Figure 2. The effect of irradiation at a temperature of 37 °C on the anthocyanin concentration of 'Cripp's Pink' apples.

PAPER 5: Postharvest improvement of pear colour.

Abstract

In an attempt to understand red colour formation in pears, two red cultivars were irradiated immediately after harvest. Poorly coloured 'Bon Rouge' and 'Red d' Anjou' were subjected to 22 to 24 W·m^{-2} of a 400 W high-pressure sodium light. After 72 hours of irradiation there was no significant change in hue angle of 'Red d' Anjou' or 'Bon Rouge' pears. 'Red d' Anjou' pears displayed no significant difference in lightness or chroma, whereas 'Bon Rouge' pears displayed a significant increase in lightness and chroma after 72 hours of irradiation. Due to the masking effect of chlorophyll just prior to harvest resulting in an unattractive brownish colour of 'Forelle' pears the fruit were stored at 10°C for one month, to promote chlorophyll breakdown. This storage period resulted in a significant decrease in hue angle from 88 to 70°, corresponding to a change from a green to a yellow background. There was also a significant increase in lightness and chroma of 8.4 and 5.0, respectively. In a third experiment, 'Forelle' fruit still attached to its shoot segment was irradiated at two different temperature regimes (20°C and 6/20°C, respectively), to determine whether precursor availability is a problem in postharvest colour development of pears. Also present in the growth chamber were fruit without the shoot segment and fruit covered in aluminium foil. Fruit were irradiated for 166 hours with 22 to 24 W·m^{-2} of high-pressure sodium light. There was no significant difference in colour development between the two temperature regimes used. The pears without the aluminium foil displayed a greater decrease in hue angle than the pears attached to the shoot segment and the pears covered in foil, but this was as a result of yellowing and not of a visible increase in red colour development. Therefore, the 'Forelle' pears showed no increase in red colour with artificial light.

Introduction

Most research on red colour formation in pome fruit has been done on apples, with very little reference to pears. Cyanidin 3-galactoside, an anthocyanin, is the major red pigment in both apples and pears (Francis, 1970; Macheix *et al.*, 1990, Dussi *et al.*, 1995). Generally, red blushed pear varieties have a non-pigmented epidermis, with 1 to 2 additional non-pigmented cell layers below. Beneath these layers are 2 to 5 layers of cells containing anthocyanin. In certain red pear cultivars, such as 'Starkrimson', 95% of the colour is due to anthocyanin in the epidermal layer (Dayton *et al.*, 1966).

'Forelle' fruit is well coloured during the meristematic phase and loses red colour as the fruit grows. Viljoen (1996) found that the anthocyanin concentration in pear peel decreassed had a relatively sharply throughout the season, in contrast to apple peel that shows a definite increase in anthocyanin concentration throughout the season (Knee, 1972; Arakawa, 1991). The loss of visible colour may be as a result of a dilutional effect due to fruit growth (Viljoen, 1996), anthocyanin degradation (Chalmers *et al.*, 1973), or as a function of poor synthesis because of inadequate irradiation due to increased canopy size (Marais, 1995). Anthocyanin synthesis in 'Forelle' fruit is very sensitive to irradiation 30 days or more prior to harvest. Even after light exclusion for 90 days after 75% petal drop, the fruit still developed as much red colour as fruit exposed for longer periods (Marais, 1995).

Little information exists on the distribution of anthocyanin as the fruit develops. It is, however known that there is little change in the distribution of anthocyanins between 50 to 60 days after full bloom and afteer attainment of physiological maturity (Dussi *et al.*, 1995). The colour development of 'Forelle' pears is usually unsatisfactory prior to harvest since the fruit tends to change from an attractive red colour to a red-brown colour nearer to their harvest date (Marais, 1995). Viljoen (1996) found an increase in anthocyanin and

a decrease of chlorophyll and carotenoids in 'Forelle' pears with ripening. The appearance and appeal of the bi-coloured pears depend on the absolute quantities and the ratios between the various pigments present in the peel tissue. The background colour is due to the plastid pigments, chlorophyll and carotenoids, which may have a masking effect on the anthocyanins which occur in the vacuoles (Brouillard, 1982; 1983; Lancaster *et al.*, 1994; Viljoen, 1996). The enzyme, chlorophyllase, is responsible for the breakdown of chlorophyll during ripening (Knee, 1972; Gorski & Creasy, 1977).

In contrast to apples, there is no literature on postharvest colour improvement of pears with irradiation. No success was achieved with postharvest irradiation using high-pressure sodium (HPS) light on 'Forelle' pears (Paper 1). Ju *et al.* (1995) suggested that the availability of precursors for anthocyanin synthesis may play an important role in affecting fruit coloration in apples.

The objectives of this research were, firstly, to establish if postharvest improvement of colour in red pears could be achieved using artificial lights. Secondly, to improve the visibility of anthocyanins by reducing the masking effect of chlorophyll. Thirdly, to irradiate bi-coloured pears with attached stem segment, in an attempt to provide the missing precursors. Finally, cross-sectional slides of a bi-coloured pear were prepared to track pigment location over the season.

Material and methods

Plant material: 'Forelle' pears (a bi-coloured variety) were picked randomly from the inside of trees at Lourensford farm in Somerset-West. 'Red d' Anjou' and 'Bon Rouge' pears (red cultivars) were picked from orchards situated in the Koue Bokkeveld area near Ceres, South Africa (latitude: 33°10'S; longitude: 19°20'E; altitude: 800 to 900 m). The Western Cape climate is Mediterranean with cool, wet winters and dry, hot summers.

Experiment 1: Poorly coloured 'Red d' Anjou' and 'Bon Rouge' pears were picked at optimum harvest date and colour measurements were taken. Twenty pears (5 replicates comprising 4 fruit each) were placed randomly in the growth chamber set at 20°C, and irradiated for 72 hours with 22 to 24 $W \cdot m^{-2}$ provided by a 400 W high-pressure sodium light (HPS; Philips SON-T, 253 mW/W). The light intensity was measured with a Decagon light meter (AccuPAR version 4.1, Decagon devices, Pullman, Washington, U.S.A) approximately 1 m from the light source. Colour measurements were taken every 24 hours for 72 hours. A further twenty fruit of each cultivar were used as a control and placed at 20°C in the dark.

Experiment 2: Forty 'Forelle' pears were picked at optimum harvest date and colour measurements were initially taken on the blushed side of the fruit on 17 February 1999. Twenty fruit were stored at a temperature of 10°C, for optimum breakdown of chlorophyll, and the other twenty fruit were placed at room temperature (±20°) as a control. Colour measurements were taken again one month later on 18 March 1999.

Experiment 3: The third experiment was performed on 'Forelle' pears. Three treatments were applied to the fruit inside the growth chamber. Fruit with a stem segment and 5 to 6 leaves were placed in a water source and covered with plastic. Fruit without leaves were either covered with foil to exclude light, or were without foil. Fruit were placed randomly in growth chambers either set at 20°C or at fluctuating temperatures of 6/20°C (12 hours). The pears were irradiated immediately after harvest. The fruit were subjected to 22 to 24 $W \cdot m^{-2}$ HPS light (Philips SON-T, 253 mW/W). Colour measurements were taken the day of harvest and then again after 168 hours (7 days) of irradiation. Twelve fruit were used per treatment (with 3 replicates comprising 4 fruit each).

Colour measurements: Circles were marked on the fruit and external colour was measured in the marked area. A colorimeter (model NR-3000, Nippon Denshoku; Kogyo Co. Ltd.) was used and colour was expressed as chroma, hue and lightness. The chroma value ($C^* = [a^{*2} + b^{*2}]^{1/2}$) indicates the intensity or colour saturation. Hue angle (h° = arctangent [b*/a*]) refers to the angle formed by a line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue. L* is a measurement of the lightness of the fruit and its value may range from 0 = black to 100 = white (McGuire, 1992). Colour measurements were done as described in previous papers.

Data analysis: The data were analysed with the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

Results and Discussion

Experiment 1:

'Red d' Anjou' pears subjected to HPS light showed little change in the hue value after being irradiated for 72 hours. There was no significant difference between the irradiation treatment (72 h) and the control (Table 1) (P = 2820). The lightness value did not increase significantly (P = 0.2158) when irradiated. The lightness values of irradiated and non-irradiated fruit after 72 hours were significantly different (P = 0.0069), possibly because the lightness of non-irradiated fruit was lower initially. The chroma did not change significantly with (P = 0.1819) or without irradiation (P = 0.1558). There is however, a significant difference in chroma between the irradiated fruit and the non-irradiated fruit (P = 0.0228).

Results from 'Bon Rouge' pears subjected to irradiation with HPS light differed slightly from 'Red d' Anjou'. There was a significant decrease in hue value when fruit were

irradiated for 72 hours (P = 0.0548) compared to the control (P = 0.1968) (Table 2). However, this decrease was not significant between irradiated and non-irradiated fruit (P = 0.0618). The irradiation treatment resulted in a significant increase in lightness (P = 0.0200), and chroma (P = 0.0492). There was also a significant difference in lightness (P = 0.0219) when fruit were irradiated compared to non-irradiation. However this was not the case with the chroma value (P = 0.3134).

As a result of the observations made in Paper 1, where no visible improvement in colour occurred when 'Forelle', a bi-coloured pear, was irradiated, two red pear cultivars were irradiated in an attempt to improve colour postharvest. In red cultivars such as 'Starkrimson', 95% of the colour is due to anthocyanin in the epidermal layer (Dayton *et al.*, 1966). According to Viljoen (1996), two phases of anthocyanin production occurs in 'Forelle'. The first occurs in young fruit during cell division, which continues for approximately six weeks after anthesis. During this period 'Forelle' fruit is predominantly red, but with no direct sunlight (PAR), the red colour is reduced to a green colour. After this first phase there is a decrease in anthocyanin throughout the season. Viljoen (1996) proposed several possible reasons for this phenomena: (1) constant anthocyanin content per fruit, but fruit size increase, having a dilution effect on the pigment concentration, (2) active degradation, but no synthesis of anthocyanin pigments, and/or (c) active degradation with anthocyanin synthesis at a slower rate. Marais (1995) thought that red colour was lost as a result of inadequate PAR reaching fruit due to increased canopy size. The second peak in anthocyanin production takes place during maturation of apples, but there is uncertainty of the exact seasonal anthocyanin production trends in pears (Lancaster, 1992; Saure, 1990; Viljoen, 1996). In spite of the fact that the fruit did not receive any light for the first 60 days, preventing the first phase of anthocyanin synthesis, 'Forelle' fruit regained red colour when it was exposed for longer than 30 days during the period prior to harvest (Marais, 1995). According to Marais (1995), 'Forelle'

pears exposed for the first 90 days and then bagged, lost all red colour and was as green as the fruit that had been bagged for the whole season. Thus, fruit regained red colour depending on the time and length of the period of exposure before harvest.

The poor results obtained from irradiating red pears is understandable when considering that 'Forelle' pears needed 30 days of exposure before a significant difference in anthocyanin production occured. The irradiation period used in this experiment was only 72 hours. Long irradiation periods at high intensities are needed for appreciable anthocyanin synthesis (Saure, 1990). In pears, the intensity of anthocyanin in the outermost layer of pigmented cells is only slightly greater than in the one or two layers directly below. This is quite different from pigmentation in apples, where the colour intensity in the outermost pigmented layer is usually much greater than in any of the underlying layers (Dayton *et al.*, 1966). However, irradiation increased the lightness and the vividness of the existing colour, which is a positive contribution for very dark red pears such as 'Red d' Anjou' and 'Bon Rouge'.

Experiment 2:

In an attempt to improve the visibility of anthocyanins by maximising chlorophyll breakdown, fruit were subjected to 1 month of storage at 10°C. This resulted in a significant decrease of 17.6° in hue angle (P = 0.0001), compared to fruit stored at room temperature (P = 0.0001) (Table 3). There were also significant differences in lightness (P =0.0001) and chroma (P = 0.0002) between fruit stored at 10°C and fruit stored at room temperature.

As already mentioned, chlorophyll and carotenoids may mask the vacuolar anthocyanins in fruit (Creasy, 1968; Brouillard, 1982; 1983; Lancaster *et al.*, 1994; Viljoen, 1996). Since in pears, anthocyanins are in the cell layers below those containing chlorophyll,

visible light (600 to 700 nm) that is reflected by anthocyanin and carotenoids, could be absorbed by the chlorophyll (Weier *et al.*, 1982). Anthocyanin concentrations increase rapidly during the ripening process, and this coincides with a decrease in chlorophyll concentration (Lancaster, 1992; Faragher and Brohier, 1984, Ju *et al.*, 1995; Viljoen, 1996). The skin colour of the bi-coloured pears at harvest depends on the concentration of anthocyanin versus chlorophyll and carotenoids present in the peel. Due to the catabolic action of the enzyme, chlorophyllase, a loss of chlorophyll has been observed during ripening (Knee, 1972; Gorski and Creasy, 1977), and the degradation of chlorophyll peaks just before the climacteric of apples begins (Wertheim, 1990).

Low temperatures enhance and high temperatures inhibit anthocyanin synthesis (Saure, 1990). In addition to light there are three factors contributing to colour development. Firstly, colour development will vary depending on the stage of fruit maturity. Anthocyanin is produced at a much lower rate after the climacteric phase is entered. Secondly, the temperature must be high enough and of sufficient duration in the daylight hours to catalyse anthocyanin biosynthesis. Thirdly, a period of cold temperature stimulates anthocyanin production. A few nights of temperatures in the range of 2 to 5°C followed by warm, sunny days promotes red colour development (Curry, 1997).

The challenge in the second experiment was to enhance chlorophyll breakdown by storing the fruit at a temperature of 10°C, without a rapid decrease in firmness (ripening). The breakdown of chlorophyll (yellowing) redused the masking effect and improved the visibility of the anthocyanins present in apples and pears (Gorski and Creasy, 1977; Viljoen, 1996). Dixon and Herwett (1998) proved that temperature plays an important role in yellowing and that yellowing is determined by a rate constant (k) of total chlorophyll and changes in hue angle, as a function of temperature. Yellowing increases slowly from 0°C to 5°C, increases exponentially from 5°C to 20 °C, reaches a maximum

from 20°C to 24°C, and then declines at higher temperatures (Dixon and Herwett, 1998). The 'Forelle' pears stored at room temperature (± 25°C), showed no significant difference difference (P = 0.6446) in colour and support the findings of Dixon and Herwett (1998). Therefore, chlorophyll breakdown increases with temperatures up to 24°C and then declines. However, is not practical to use high temperatures for chlorophyll degradation, as a result of rapid fruit softening and ripening at these temperatures. Therefore, low temperature can be used to enhance anthocyanin production and chlorophyll breakdown (and thus a decrease in masking), and in conjunction with light optimise colour development. Although it seems that the anthocyanins are more visible with a breakdown of chlorophyll, the initial production of anthocyanins with light and low temperatures are still essential for a good appearance.

Experiment 3:

In this experiment, the fruit with leaves attached showed no significant difference whether irradiated at 6/20°C or 20°C (P = 0.0707) (Table 4). The two temperature regimes resulted in no significant difference between the pears kept in the dark (aluminium foil) (P = 0.1278) or in the light (no foil) (P = 0.0699). When irradiated at the alternating temperature, the fruit without the foil had the largest decrease in hue (12.3°), followed by the pears with the stem segment (5.6°) and then the control fruit with the foil (1.5°) (P = 0.0001). This sequence was repeated at the constant temperature (P = 0.0133), but it is clear from Table 4 that the fruit irradiated at the alternating temperature showed greater decreases than the fruit irradiated at the constant temperature. When lightness and chroma were considered there was no significant difference between the two different temperature regimes for any of the treatments (Table 4).

There is a significant difference in lightness between the treatments at 6/20°C (P = 0.0227), whereas at a temperature of 20°C there were no significant differences in lightness between the treatments (P = 0.0834). For both the temperature regimes there were significant differences in chroma between the treatments (P = 0.0083 and P = 0.0024, respectively. Thus, within a temperature regime it seems that lightness and chroma increased slightly for the pears with stem segment, while the pears without the foil showed the greatest decreases in lightness as well as chroma. However, the differences were very small.

The importance of temperature for anthocyanin production, motivated the use of the reason behind the two different temperature regimes used in experiment 3. The fluctuating temperatures were based on the results of Tan (1980), and it was compared with irradiation at a constant temperature of 20°C. The fluctuating temperature seemed to enhance colour development more than the constant temperature (Table 4). As a result of the findings in the previous paper, the proposition made by Ju *et al.*, (1995) when he suggested that the availability of precursors for anthocyanin synthesis might play an important role in affecting fruit coloration in apples was proved wrong in this experiment. It seems that either availability of the precursors in leaves had no effect, or the precursors were not in these leaves. It is however unlikely that precursors would not be found in the fruit or leaves.

In 'Rosemarie' pears, anthocyanin was located in the cell layers just underneath the epidermis soon after anthesis, but as the season progressed the anthocyanin seemed to be located a few cell layers deeper (personal observation). This could be due to either cell division, or degradation of anthocyanins in the upper cell layers. According to Wertheim (1990), the epidermis and hypodermis of pears can divide up to a 100 days

after full bloom. Therefore cell division of 'Rosemarie' could occur up to a late stage in its development.

Conclusion

It is clear from this paper that postharvest irradiation of red pear cultivars, such as 'Red d' Anjou' and 'Bon Rouge', did not produce any visible improvement of red colour. Storage at a temperature of 10°C for a month improved the visibility of the anthocyanins by reducing the masking effect of the chlorophyll on the anthocyanins, resulting in colour that was also brighter and more vivid. Irradiation of pears still attached to leaves did not improve red colour formation. None of the treatments visibly produced red colour formation on pears.

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Table 1. The effect of 72 hours of irradiation at 20°C with HPS light on hue, lightness and chroma of the peel of 'Red d' Anjou' pears.

'Red d' Anjou'				
Treatment	Irradiation time (h)	Hue angle	Lightness	Chroma
Irradiation at 20°C with HPS light				
	0	76.2	25.6	52.1
	24	75.6	27.6	52.6
	48	74.4	27.0	48.6
	72	77.7 a	30.4 a	53.4 a
Sign. Level		0.9598	0.2158	0.1819
No irradiation at 20°C				
	0	63.2	23.6	50.6
	24	60.5	23.0	48.0
	48	61.9	23.4	49.9
	72	63.3 a	23.3 b	49.6 b
Sign. Level		0.6516	0.8906	0.1558
Significance (P<0.05)				
Irradiation vs. No irradiation		0.2820	0.0069	0.0228

*Values followed by different letters indicate significant differences (P<0.05) according to the LSD test. Significant differences were calculated within a treatment, as well as between the different treatments. Differences between irradiation vs. non-irradiation were calculated comparing the final values after the specific treatment.

Table 2. The effect of 72 hours of irradiation at 20°C with HPS light on hue, lightness and chroma of the peel of 'Bon Rouge' pears.

		'Bon Rouge'		
Treatment	Irradiation time (h)	Hue value	Lightness	Chroma
Irradiation at 20°C with HPS light				
	0	96.3	33.6	55.9
	24	94.3	34.9	55.4
	48	85.5	36.3	56.6
	72	85.8 a	40.9 a	62.1 a
Sign. level		0.0548	0.0200	0.0492
No irradiation at 20°C				
	0	97.7	32.7	53.2
	24	95.5	33.1	52.8
	48	96.9	35.1	58.3
	72	93.7 a	35.3 b	57.6 a
Sign. level		0.1968	0.5180	0.1058
Significance (P<0.05)				
Irradiation vs. Non-irradiation		0.0618	0.0219	0.3134

*Values followed by different letters indicate significant differences (P<0.05) according to the LSD test. Significant differences were calculated within a treatment, as well as between the different treatments. Differences between irradiation vs. non-irradiation were calculated comparing the final values after the specific treatment.

Table 3. The effect of one month storage at 10°C on colour of 'Forelle' pears, measured as hue, lightness and chroma.

'Forelle'

	Hue value				Lightness				Chroma			
Temp. (°C)	Initial	Final	Δ	Sign. level	Initial	Final	Δ	Sign. level	Initial	Final	Δ	Sign. level
10	88.1	70.5	-17.6 a	0.0001	52.1	60.5	8.4 a	0.0001	38.2	43.1	5.0 a	0.0026
Room temp.	94.2	95.4	1.2 b	0.6446	52.5	53.1	0.6 b	0.6427	40.5	37.2	-3.4 b	0.0311
Significance (P<0.05)												
10°C vs. Room temp.			0.0001				0.0001				0.0002	

*Values followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 4. The effect of irradiation with HPS light for 168 hours at 6/20°C or 20°C on colour development of 'Forelle' pears either still attached to a stem segment with leaves, or without the stem segment but with or without foil.

'Forelle'							
Temp (°C)	6/20°C			20°C			
Treatment	Initial value	Final value	Δ Value	Initial value	Final value	Δ Value	Sign. level
Hue							
Pears + stem	115.6	110	-5.6 b	111.5	108.5	-3.0 a	0.0707
Pears – foil	114.9	102.6	-12.3 c	116.3	107.7	-8.6 b	0.0699
Pears + foil	115.4	113.9	-1.5 a	116.4	113.1	-3.3 a	0.1278
Sign. Level			0.0001			0.0133	
Lightness							
Pears + stem	62.4	65	2.6 a	63.0	65.2	2.3 ab	0.7557
Pears – foil	63.8	61.1	-2.6 b	62.9	62.9	0.0 b	0.1624
Pears + foil	63.2	64.8	1.6 a	63.2	66.7	3.5 a	0.2488
Sign. Level			0.0227			0.0834	
Chroma							
Pears + stem	44.8	48.4	3.6 a	44.6	46.2	1.6 a	0.2274
Pears – foil	48.0	42.8	-5.2 b	47.5	44.2	-3.3 c	0.3761
Pears + foil	47.4	47.3	0.1 a	47.6	47.0	-0.7 b	0.3199
Sign. Level			0.0083			0.0024	

*Values followed by different letters indicate significant differences ($P<0.05$) according to the LSD test