OPTIMISATION OF BLUEBERRY POSTHARVEST HANDLING AND STORAGE

BY

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DECLARATION

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

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SUMMARY

By world standards South Africa's contribution to fresh blueberry production is very small, a mere 0.25 %. This seemingly insignificant and unimpressive statistic is, however, by no means indicative of the impending impressive potential the South African blueberry industry holds. South Africa is encountering a rapid growth phase off a very small base and the current 100-150 ton export crop is expected to increase to a range of 500 to 600 tons or more in the next five years. Consequently, export by sea must be considered as a logistic alternative. Seafreight success relies on controlled atmosphere storage which in turn relies on berries of exceptional quality. To achieve this, fundamental differences between blueberry cultivars must be examined and blueberry handling and storage regimes must be assessed and optimised.

This study investigates the storage potential of certain rabbiteye blueberries relative to controlled atmospheres and short pre-cooling delays. Quality assessments were based on external colour, soluble solids, titratible acidity, firmness and decay development. Furthermore, the respiration and ethylene production rates as well as moisture loss, of various cultivars were determined to characterize fundamental differences amongst cultivars and to gain insight into their postharvest behaviour. Lastly the effect of infection on pigment levels of blueberry flesh and peel was examined for the purpose of laboratory experience.

Controlled atmosphere storage had the greatest impact on berry firmness and percentage decay. No off flavours were detected during sensory analysis at the CA levels tested. CA-stored berries were firmer and incidence of decay was reduced significantly. Significant decay reduction was challenged by the market tolerance of no more than 2 %. Decay suppression and storage extension was insufficient (for the set period), particularly after the shelf life period. Cultivar and seasonal differences as well as picking maturity and inoculum pressure determine blueberry CA storability. CA may be a valuable technology, however advances must first be made in ensuring optimal blueberry quality to warrant export by sea.

Short pre-cooling delays (0, 2, 4, 6 h) at 20 ºC had no effect on colour (L, C, H), total soluble solids, titratable acidity and firmness of blueberry cultivars, 'Centurion', 'Premier' and 'Tifblue'. Results for percentage decay indicate that prompt cooling becomes more critical as the duration of storage increases, particularly when market specification of no more than 2 % decay must be met. Berries subjected to longer delays appear to be more susceptible to decay. Differences exist among cultivars and knowledge gained from this research can be used to adapt handling and cooling regimes. Information generated will benefit cultivar selection for marketing (local vs. export), storage (short vs. long term) and logistics (air vs. seafreight).

Rabbiteye blueberry cultivars differ considerably in terms of their respiration rate, ethylene production and moisture loss. These quality parameters were evaluated for various cultivars at 15 ºC. 'Climax' appears to have the highest respiration rate, ethylene production and moisture loss, whilst 'Premier' has the lowest respiration rate and ethylene production was too low to be detected by gas chromatography. The lowest moisture loss proportionality constant was found for 'Tifblue' and 'Centurion'.

A change in blueberry flesh colour from white to dark red was noted in infected berries. Artificial inoculation with *Botrytis cinerea* showed a similar result. The colour change was evaluated by comparing anthocyanin pigment levels (determined by spectrophotometry and HPLC) of the peel and flesh. Anthocyanin content of infected flesh doubled, whilst that of infected peel was less than 50 % of the control. Pigment leakage has been reported in blueberries. It would appear that cell disruption, induced by infection may facilitate migration of anthocyanin from the peel to the flesh.

The storage potential of blueberries depends on cultivar, maturity at harvest, climatic conditions as well as the inoculum pressure. Under favourable conditions storage life of blueberries can be manipulated effectively. Further cultivar specific research that targets relevant quality issues should be investigated. Reliable techniques should be developed to quantify blueberry firmness changes. Research on inoculum levels may give insight into

the reduction of decay pressure and decay suppressing alternatives such as antibacterial pads can be examined. Peel integrity relative to decay susceptibility can also be researched on a cellular level.

Optimalisering van bloubessie na-oes hantering en opberging

OPSOMMING

Suid-Afrika se bydrae tot wêreld bloubessie produksie is baie klein, ‘n skrale 0.25 %. Hierdie onindrukwekkende statistiek weerspieel egter glad nie die indrukwekkende potensiaal van die Suid-Afrikaanse bloubessie bedryf nie. Die bedryf ondervind tans ‘n dramatiese groei met ‘n klein begin vanaf die huidige 100-150 ton opbrengs tot wat volgens verwagting binne die volgende vyf jaar sal styg tot in die orde van 500-600 ton. Na aanleiding van die verwagte toename in produksie, sal seevrag verskeping oorweeg moet word as alternatief vir uitvoer eerder as lugvrag. Die sukses van seevrag lê in die gebruik van beheerde atmosfeer opberging (BA), waarvan die sukses op sy beurt bepaal word deur bessies van hoogstaande kwaliteit. Om steeds aan die verwagtings van die oorsese mark te voldoen moet grondliggende verskille tussen bloubessie kultivars ondersoek word en daaropvolgend moet hantering en opbergingstelsels aangepas en geoptimeer word.

Die navorsing bestudeer die opbergingspotensiaal van verskeie rabbiteye bloubessies relatief tot beheerde atmosfere en kort vertragings in verkoeling. Kwaliteit, gebasseer op eksterne kleur, oplosbare deeltjies, titreerbare sure, fermheid en persentasie bederf, is ondersoek. Die respirasie en etileenproduksie tempos sowel as vogverlies van verskeie kultivars was verder bepaal om die fundamentele verskille tussen kultivars te bepaal, sowel as insig te verwerf in na-oes gedrag. Die effek van infeksie op pigment vlakke van bloubessie vlees en skil was ondersoek vir die doel van laboratorium ondervinding.

Beheerde atmosfeer opberging het die grootste effek getoon op bessie fermheid en persentasie bederf. Na aanleiding van sensoriese toetse is daar geen wansmaake aangeteken ten op sigte van die BA vlakke wat getoets is nie. Bessies onderworpe aan beheerde atmosfeeriese toestande was fermer en bederfonderdrukking was beduidend. Die markverdraagsaamheid ten opsigte van bessiebederf, is egter 2 %. Hiervolgens was onderdrukking van verval asook opbergingsvermoë, onvoldoende (spesifiek na die

rakleeftyd). Kultivar en seisoenale verskille asook plukrypheid en inokulum druk, bepaal ook die sukses van BA opberging. BA word beskou as ‘n waardevolle tegnologie vir verlenging van opberging. Dit blyk egter dat daar eers ‘n vooruitgang gemaak moet word in die versekering van optimale bessie kwaliteit voordat verskeping oorweeg kan word.

Kort vertragings (0, 2, 4, 6 uur by 20 ºC) in verkoeling, het geen invloed gehad op kleur, (L, C, H), totale oplosbare vastestowwe, titreerbare suur en fermheid van bloubessie kultivars ‘Centurion’, ‘Premier’ en ‘Tifblue’ nie. Bederfresultate dui daarop dat onmiddelike verkoeling krities is, veral by verlengde opberging, om aan die 2 % bederf drempelwaarde te voldoen. Bessies onderhewig aan langer vertragings voor verkoeling, blyk meer vatbaar te wees vir bederf. Verskille bestaan tussen kultivars en kennis ingewin tydens hierdie navorsing kan toegepas word om hanteringsprosedures en verkoeling aan te pas. Ingeligte besluitneming kan dus ten opsigte van kultivarspesifieke bemarking (plaaslik en uitvoer), opberging (kort- en langtermyn) en vervoer (lug- en seevrag) gemaak word.

Rabbiteye bloubessie kultivars verskil aansienlik ten opsigte van hul respirasietempo, etileenproduksie en vogverlies. Laasgenoemde kwaliteitsbepalers is by verskillende kultivars ondersoek by 15 ºC. ‘Climax’ het die hoogste respirasietempo, etileenproduksie en vogverlies getoon, terwyl ‘Premier’ die laagste respirasietempo gehad het, en die etileenproduksie te laag was om met die gaschromatograaf te bepaal. Die laagste vogverlies proporsionaliteitskonstante is waargeneem tussen ‘Tifblue’ en ‘Centurion’.

‘n Verandering is waargeneem in bloubessie vrugvleeskleur van wit tot donkerrooi by besmette bessies. ‘n Soortgelyke resultaat is verkry met kunsmatige inokulering met *Botrytis cinerea*. Die kleurverskil is geëvalueer deur vergelyking te tref met die antosianienpigment vlakke tussen die skil en die vlees (bepaal deur spektrofotometrie en HPLC). Antosianieninhoud van besmette vrugvlees het verdubbel, terwyl die van besmette vrugskil minder as 50 % van die kontrole groep was. Pigmentlekkasie is al vermeld by bloubessies. Dit wil voorkom of selskade veroorsaak deur infeksie beweging van antosianien vanaf die skil na die vlees kan aanhelp.

Die opbergingspotensiaal van bloubessies hang af van kultivar, rypheid by oes, omgewings toestande sowel as inokulum druk. Onder gunstige toestande kan bloubessies se opbergingsvermoë effektief gemanipuleer word. Verder kultivar-spesifieke navorsing wat relevante kwaliteits kwessies aanspreek, kan gedoen word. Verder kan betroubare tegnieke in die meting van bloubessie fermheid veranderinge ondersoek word. Navorsing ten opsigte van inokulum vlakke mag insig gee in terme van bederf-druk vermindering en die gebruik van bederf-onderdrukking alternatiewe soos anti-bakteriese lappies. Skil integriteit relatief tot bederf-vatbaarheid kan ook op sellulere vlak ondersoek word.

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CHAPTER 1: Literature Review

1. Introduction

1.1 Blueberry classification

Blueberries originated in North America and belong to the family, Ericaceae, subfamily, *Vaccinioidiae*, and genus *Vaccinium* (Eck *et al*., 1990). Species of major economic importance include the highbush blueberry, *V. corymbosum* L., the lowbush blueberry, *V. angustifolium* Aiton, and the rabbiteye blueberry, *V. ashei* Reade (Hancock *et al*., 1996). Highbush blueberries are subdivided into northern and southern highbush types. Northern highbush blueberries crop late in the season and require a minimum of 800-1000 chilling hours for proper release from dormancy (Anon, 2004), whilst southern highbush cultivars ripen early (before rabbiteye cultivars) and have a low chilling requirement of 150 to 300 hours below 7.2 ºC (Lyrene, 1990 cited in Perkins-Veazie *et al*., 1994; Anon, 2004). The rabbiteye blueberry (*V. ashei* Reade) derived its name from the wild *V. ashei* berries which, while ripening, have a pink spot at their calyx end, resembling the eye of a wild rabbit (Greeff, 2003). This is a mid season blueberry, suited to South Africa due to its moderate chilling requirement of 400 to 600 hours at 7 ºC (Eck, 1988).

1.2 The South African blueberry industry

By world standards South Africa's contribution to fresh blueberry production is very small, a mere 0.25 % (D. Brazelton, personal communication, 2004). This seemingly insignificant and unimpressive statistic is, however, by no means indicative of the impending impressive potential the South African blueberry industry holds. With the explosion of positive blueberry publicity regarding potential health benefits there has been a dramatic world wide increase in the demand for fresh blueberries. In particular, new markets in the east such as Japan relying heavily on Australasia and South America for berries during the northern hemisphere off season, have opened the way for South African exports into Europe.

South African blueberry production areas are spread throughout the country, situated predominantly in the Western Cape. The vast majority of current plantings belong to rabbiteye cultivars which cater for a short harvesting season wherein fifty percent of the crop is picked in two to three weeks. This peak coincides with Christmas and New Year, a time when labour productivity is generally poor and market prices are on the decline. There is an urgent need to attenuate this peak not only through better selection of cultivars but also by exploiting the storage potential of rabbiteye blueberries.

Diversification of blueberry plantings, by the introduction of northern and southern highbush cultivars is necessary to extend the production period and in so doing, increase the blueberry export market potential during the European off season. Expansion of southern highbush plantings is taking place rapidly. Northern highbush blueberries are less suited to South Africa's climatic conditions but their potential for the South African market is being assessed in the Free State and in colder, higher altitude locations of the Western Cape (Greeff, 2003). South Africa is encountering a rapid growth phase off a very small base and the current 100-150 ton export crop is expected to increase to a range of 500 to 600 tons or more in the next five years (T.D. McKenzie, personal communications, 2004).

Blueberries are widely adaptable plants, contending with a broad spectrum of climatic circumstances, stretching from Texas to Canada and from Spain to Scandinavia as far as their particular soil requirements can be met. Their shallow adventitious root system thrives in well drained acidic (pH 4.5-5.2) sandy loams with an organic matter content ranging from 3-20 % (Eck *et al*., 1990). In South Africa most good soils have been pH adjusted to suit the needs of fruit and vine orchards, leaving only marginal soils and soils with unfavourable pH's. Furthermore the prerequisite of a minimum organic matter content of 3 % is rarely encountered in South Africa and therefore intensive soil preparation is crucial for successful blueberry production.

The first South African blueberries were planted in the early 1980's in Mpumalanga, followed by the Western Cape, however production only started taking off in the 1990's (T.D. McKenzie, personal communications, 2004). These early plantings were established, lacking sufficient knowledge of the crop, with plant material of uncertain origin in soils with too high clay contents, too high pH's and negligible organic material. It is only in recent years that enough attention has been given to specific blueberry plant needs which include aggressive soil preparation, incorporation of organic matter into the soil and appropriate nutritional programs. As a result, production is becoming more profitable, further motivating expansion and development of the industry. Advances are also being made to refine conditions during handling, cooling, storage and transport, which will optimize and maintain fruit quality so that consumer demands can be met.

2. Factors affecting postharvest potential

2.1. Biological factors

2.1.1. Respiration

Respiration is the energy-generating process fueling all metabolic activities in the plant. It is a catabolic process, involving the oxidative breakdown of stored organic materials (carbohydrates, proteins, fats) to simple end products, viz carbon dioxide and water, liberating heat and energy in the form of adenosine triphosphate (Salisbury and Ross, 1992**;** Kader, 1985). Blueberries have a moderate respiration rate, between 10-20 mg CO_2 $kg^{-1}.h^{-1}$ at 5 ºC, falling in the same category as plums (Kader, 1985).

While berries are still attached to the bush, substrates used for respiration are replenished by photosynthates from the leaves. Once berries are picked, they maintain their living status and replace substances used for respiration by consuming reserve organic materials. Consequently, this leads to depletion of reserves and rapid decline in quality. The rate of respiration is proportional to the rate of perishability after picking (Kader,

1985). Therefore, there is an inverse relationship between respiration rate and storage life of fresh commodities (Wills *et al*., 1998; Ritenour, 2003).

Respiration involves many enzymatic reactions (Wills *et al*., 1998), and the rate of enzyme activity is controlled directly by temperature. Temperature, therefore, has a dramatic effect on respiration rate. The temperature quotient or Q_{10} can be used to describe the exponential relationship of respiration for every 10 ºC change in temperature. Blueberries have a Q_{10} of 3 and therefore, every 10 ºC increase in temperature dictates a three fold increase in respiration. This emphasizes that temperature management narrates the respiration rate, deterioration and the post harvest potential of the berry.

2.1.2. Ethylene production

Storage potential is also dictated by the presence of ethylene. Ethylene is the natural aging and ripening hormone, and is physiologically active in trace amounts (less than 0.1 $\mu L.L^{-1}$) (Kader, 1985). Blueberries have a low ethylene production rate, between 0.1 and 1.0 $\mu L.kg^{-1}.h^{-1}$ at 20 °C. Ethylene is produced in small amounts throughout fruit development. However, its production rate generally increases with maturity at harvest, physical injuries, disease (incidence), temperature increases (up to 30 ºC) and water stress (Kader, 1985).

The precursors of ethylene are methionine, SAM (S-adenosylmethionine) and ACC (1-aminocyclopropane-1-carboxylic acid). Enzymes, ACC synthase and ACC oxidase, catalyse the conversion of SAM to ACC and ACC to ethylene, respectively. ACC synthase is regarded as the rate limiting step (Wills *et al*., 1998) of this pathway, characterized by Adams and Yang (as reported by Yang, 1985) and known as ethylene biosynthesis. Ethylene then binds to a receptor, forming an activated complex, which

triggers the primary reaction and subsequently initiates a sequence of physiological responses associated with fruit ripening and senescence.

Respiratory and ethylene production patterns during maturation and ripening are used to classify fruit as either climacteric or non-climacteric (Kader, 1985). Climacteric fruit are distinguished from non-climacteric fruit by their increased respiration and ethylene production rates, coincident with ripening (Kader, 1985). Watada *et al*. (1984) describe the climacteric period as an interval during the development of some plant parts, involving a series of biochemical changes associated with the natural respiration rise and autocatalytic production of ethylene. By definition, non-climacteric fruit exhibit a consistent, generally low CO_2 and C_2H_4 production rate during ripening (Kader, 1985).

The question of whether the blueberry is a climacteric fruit has been debated (Eck, 1988; Windus *et al*., 1976). Blueberries have been described as climacteric fruit, due to an association between the onset of the climacteric rise and changes in berry colour (Windus *et al*., 1976; Ismail and Kender, 1969; Lipe, 1978; Mitcham *et al*., 2000). Windus *et al*. (1976) segregated berries into colour grades, representing advancing maturity stages, namely; immature green, mature green, green pink, blue pink, and blue. In general, the respiration peak correlated with the green pink (75 % green and 25 % pink), or blue pink (75 % blue and 25 % pink) stage (Windus *et al*., 1976; Ismail and Kender, 1969). The CO_2 evolution increment at room temperature (± 23 ºC) from immature green to green pink varied from as little as 15 to 50 $mg.kg^{-1}h^{-1}$ depending on the cultivar. The CO_2 peak is followed by a decline as the berry transforms to blue.

According to Lipe (1978) and Windus *et al*. (1976), an ethylene peak arises in synchrony with the green pink stage. A 0.1 $\mu L.kg^{-1}.h^{-1}$ C_2H_4 production increase was reported by Windus *et al*. (1976) for highbush blueberries, while Lipe (1978) observed a 3.25 $mg.L^{-1}$

C_2H_4 production increase for rabbiteye blueberries (cultivar Tifblue). When one compares this rise in ethylene with that of other climacteric fruit, it becomes clear that blueberry ethylene production is exceedingly low, increasing only one or two fold, while apples and bananas may display ethylene production increases as high as 100 or 1000 fold (R. Beaudry personal communication, 2003). Even though these slight respiration and ethylene increases are regarded by some as evidence of the climacteric nature of blueberries, most references suggest that blueberries should be harvested near to full ripeness (and even be left for a few days after they have turned blue) for the best flavour and quality (Mitcham *et al*., 2000; Eck *et al*., 1990).

A lot of ambiguity exists around the relevance of the timing of the climacteric peak relative to the time of harvest. According to Kader (1985), climacteric fruit can be picked at a physiologically mature stage when they are still unripe. Physiological maturity is described by Watada *et al*. (1984), as the stage of development when a plant or plant part will continue ontogeny (process of development), even if detached from the plant. As for ripening, this begins during the later stages of maturation, and is a dramatic event in the life of a fruit. It transforms a physiologically mature but inedible plant organ into a visually attractive olfactory and taste sensation (Wills *et al*., 1998). Ripening marks the completion of development and the commencement of senescence and is usually an irreversible event (Wills *et al*., 1998).

Sommer (1985) relates that maximum postharvest life of climacteric fruits can be attained only by harvesting before the start of their respiratory climacteric rise. Climacteric fruit ripening can therefore be suppressed by certain technologies and can be induced by exposure to ethylene or favourable environmental conditions. However, deductions made from personal communication with Kader (2003) insinuate that there is no clear association between the climacteric and correct picking stage. "Classifying fruit into climacteric and non climacteric groups is interesting for post harvest physiologists, but

what is more important commercially is whether the fruit is capable of ripening and attaining good eating quality when harvested at the mature green stage and kept at 20 ºC and 90-95 % relative humidity" (A. Kader, personal communication 2003). The timing of the ethylene increase and respiration rise (both minimal) for blueberries is depicted as such that it does not coincide very well with the onset of ripening (R. Beaudry, personal communication 2003). It can, therefore, be said that even though the observed climacteric peak correlates with the green pink blueberry stage, this is not indicative of physiological maturity for the blueberry, and ripening is unlikely to follow. Harvesting at this green pink stage will deter the blueberries from colouring up completely and the specifications for commercial use will not be met.

More recently, sharing the views of Forney and Beaudry (personal communication, 2003), Ehlenfeldt (2002) describes the blueberry as a non-climacteric fruit. Supporting evidence is given by Beaudry (1993) and Beaudry *et al*. (1992), where it is noted that blueberries undergo minimal changes in respiration associated with ripening. With regard to ethylene production, Ehlenfeldt (2002) mentions that for blueberries, ethylene is not a significant factor at normal storage levels. In addition, Beaudry (personal communication, 2003) states that it has yet to be shown that ethylene perception is responsible for ripening in blueberries. In accordance, Forney (personal communication, 2003), states that blueberries appear to be unresponsive to ethylene and relates that a recent study with 1-MCP, which blocks ethylene action, had no effect on the postharvest quality or storage life of blueberries. Grapes show much the same type of behaviour and have respiration (5-10 $mg.kg^{-1}h^{-1}$ at 5 ºC) (Kader, 1985) and ethylene production rates (less than 0.1 $\mu L.kg^{-1}.h^{-1}$ at 20 ºC) (Kader, 1985) similar to that of blueberries. Grapes are, however, classified as non climacteric (Berry and Aked, 1996; Kader, 1985).

2.1.3. Transpiration and moisture loss

Fresh commodities constantly lose water to the surrounding environment by transpiration (Wills *et al.*, 1998). The driving force behind this process is the vapour pressure deficit or VPD. The VPD regulates the movement of water out of the fruit by means of a water vapour gradient (Wills *et al.*, 1998, Kader, 1985). The gradient exists between the fruit and the atmosphere and facilitates water migration from the fruit's saturated intercellular spaces, to the less saturated atmosphere. Factors affecting the transpiration rate include relative humidity, air velocity and temperature as well as barrier properties of the fruit surface, the ratio of fruit surface area to volume and fruit colour (Paull, 1999). The impact of these environmental and biological factors is accentuated after harvest when moisture lost from the berry can no longer be replaced by the flow of sap from the plant. This leads not only to quantitative losses (loss of salable weight), but also losses of aesthetic, textural and nutritional quality (Kader, 1985). The environmental contributors will be discussed in more detail later.

Considering the basic morphology of the blueberry, a small, dark and round fruit, it becomes clear that these characteristics contribute to heat build up (creating a higher vapour pressure difference) and thus moisture loss from the fruit. The average diameter of a blueberry is between 11-15 mm, which accounts for a high surface area to unit volume ratio and therefore a greater loss in weight due to transpiration (Eck *et al.,* 1990; Wills *et al*., 1998, Maguire *et al*., 2001). Its dark blue colour serves in the absorption of a high percentage of radiant energy and therefore also the build up of heat. During picking the berry is detached from the stalk, leaving a stem scar. This scar facilitates moisture loss and also acts as a site for easy access of pathogens (Cappellini and Ceponis, 1977, cited in Smittle and Miller, 1988; Cline, 1997). In contrast, Eck and Childers (1966), Anderson *et al*. (1979) and Teramura *et al*. (1979), cited in Eck *et al*. (1990) describe the blueberry plant as a very effective utiliser of water, rendering it tolerant to high temperatures and drought conditions. The berry surface is covered with a delicate waxy or powdery substance known as bloom, which is resistant to the passage of water (Wills

et al., 1998). During harvest care must be taken not to over handle the berries as this will remove the bloom (Boyette *et al.*, 1993). Taking into account all these factors and considering that temperature during harvest is usually high (Boyette *et al.*, 1993), the importance of minimizing berry stress and moisture loss are accentuated and the importance of optimizing blueberry handling and storage is emphasized.

2.1.4. Decay

Diseases are the greatest cause of postharvest losses and the principle factor determining blueberry shelf life (Ballinger *et al*, 1978; Cappellini *et al.*, 1982; Ceponis and Cappellini, 1985; Cline, 1997; Mitcham *et al.*, 2000). Susceptibility to decay seems to be area, cultivar and climatically related (Cappellini *et al.*, 1982; Ehlenfeldt, 2002). Cappellini *et al*. (1982), report that fungal decay accounts for two thirds of the total defective fruit, responsible for spoilage. The most common post harvest disease-causing organism is grey mould (*Botrytis cinerea*). Infection is characterized by a soft, watery decay followed by the development of grayish-white mycelium on the berry surface (Anon, 1995; Beattie and O'Loughlin, 1989).

For blueberries, the severity of decay in storage seems to be directly affected by the weather conditions before harvest (Beattie and O'Loughlin, 1989). Decay incidence is higher if harvest occurs during cool, rainy weather as wet berries are more susceptible and conditions are optimal for decay development (Boyette *et al.*, 1993). Seeing that blueberry stems are detached during harvest, the scar serves as a major avenue of entrance for diseases (Cappellini and Ceponis, 1977; Cline, 1997). Decay is only evident after berries are placed in cold storage (Anon, 1995), therefore attention must be given to factors that promote infection.

Disease progression is common in both highbush (*V. corymbosum* L) and rabbiteye (*Vaccinium ashei* Reade) blueberries (Cline, 1997), while lowbush berries (*V. angustifolium*) show little decay (Sanford *et al*., 1991). For rabbiteye, 'Premier' and 'Tifblue' are considered particularly susceptible to certain pathogens (Cline, 1997), and 'Climax' is reported to be more resistant to decay than 'Woodard' (Smittle and Miller, 1988). A relationship between decay susceptibility and cultivar differences was also reported by Smith *et al.* (1996).

Overripe blueberries rapidly lose their firmness and are more subject to disease infection (Peano *et al*., 2002). Ballinger *et al.* (1978) investigated the relationship of ripeness stage relative to decay development in terms of soluble solids (SS) / titratable acidity (TA) ratio. They concluded that blueberries destined for long distance shipment should have SS/TA no higher than 20 and locally marketed fruit, SS/TA no higher than 30. Fruit with SS/TA above 30, were recommended for processing within 24 hours and overripe fruit were categorized in the SS/TA range of 40 or higher.

Combating disease is a combination of timely harvesting (Cline, 1997), prompt cooling, storage at the lowest safe temperature, prevention of physical injury to the fruit and shipment under high carbon dioxide atmospheres (Mitcham *et al*., 2000). CO_2 enriched atmospheres reduced decay in blueberries by 50 % (Ceponis and Cappellini, 1983). In addition, care should be taken to avoid contamination originating from infested picking containers and throughout the handling chain (Cline, 1997). In the event of rain picking should be delayed (Ehlenfeldt, 2002), and diseased or wounded berries should be kept out of packages as decay can spread from diseased to nearby healthy berries (Ceponis and Cappellini, 1983; Smittle and Miller, 1988; Song, *et al*., 1992; Mitcham *et al*., 2000). Storage potential of blueberries, relative to decay, can therefore be optimized by basic management and cultural practices, most importantly the maintenance of the cold chain.

2.2. Environmental factors

2.2.1. Temperature

As previously discussed, temperature affects all facets of the post harvest life of fruit. High temperatures stimulate rapid respiration and ethylene production and increase the moisture loss from the fruit, therefore accelerating senescence (Nunes *et al*., 1995). Rapid cooling and good temperature management are, therefore, vital if ripening and deterioration processes are to be delayed (Mitchell, 1985).

2.2.2. Relative humidity

Relative humidity (RH) is defined as the ratio of water vapour present relative to the maximum amount of water vapour which can be present at the same temperature and atmospheric pressure. Relative humidity plays a fundamental role in preserving freshness of perishable products. It has a direct effect on the moisture loss of a product (Forney and Brandl, 1992) and thereby, a myriad of other subordinates regulating product quality. Moisture loss in harvested produce is, therefore, said to be one of the main causes of deterioration. It can affect anything from loss of salable weight, appearance (wilting and shriveling), textural quality, nutritional quality and uniformity of fruit ripening to susceptibility to decay. Berries are reported to have a low moisture loss rate of approximately 1 % moisture per week (m/m) when stored in uncovered containers, and 0.3 % moisture loss per week, in plastic lined containers at 1 ºC (Dekazos and Smit, 1976; Hrushka and Kushman, 1963, cited in Miller *et al*., 1984).

The vapour pressure deficit (VPD) is directly influenced by RH and is determined by the vapour pressure of the product minus the vapour pressure of the air (Wills *et al*., 1998). Relative humidity within a berry is considered to be 100 %, therefore the water movement equilibrium is never reached and air maintains its affinity for water causing movement out of the fruit to alleviate the difference.

The optimum relative humidity for perishable products is between 90-95 % and is achieved in blueberries with the lowest safe temperature of 0-2 ºC. The higher the RH at a given temperature the closer the atmosphere is to dew point temperature and greater the danger of condensation on the product due to small temperature fluctuations. The dew point temperature is the temperature at which air is saturated with water vapour (i.e. 100 % RH). If the temperature drops further the air becomes oversaturated and no longer has the capacity to hold the vapour, therefore condensation occurs.

Growers and shippers are reluctant to refrigerate blueberries to low temperatures because they believe the condensate or 'sweat' formed on the berries, when removed from the cold, erodes the bloom on the fruit and enhances excessive decay development (Ceponis and Cappellini, 1983; Hudson and Tietjen, 1981). However, preliminary studies done by Schulze *et al*. (1981) do not support the premise that berry 'sweat' will result in increased decay (Miller and Smittle, 1987; Ceponis and Cappellini, 1983). Condensation of moisture on the commodity over long periods of time is more important than is the relative humidity of the ambient air in enhancing decay (Schulze *et al*., 1981).

Modified atmosphere packaging (MAP) makes use of semi permeable films, designed to create favourable gas and RH micro environments during storage and form a barrier against moisture loss. The high humidity developing in the shrink wrapped packs however, has been reported to increase the incidence of fungal disorders (Anon, 1995). In contrast, in a study on grapes, Artés-Hernández *et al*. (2004) report that MAP treatment prevented rachis browning, controlled weight loss and reduced decay development. Raising the relative humidity in the air surrounding the fruit is therefore considered a viable practice to reduce quality degradation of fruit.

2.2.3. Rain

The occurrence of rain during the harvesting season, particularly in combination with high temperatures, is known to reduce fruit storage potential and lead to unsatisfactory fruit quality (Ehlenfeldt, 2002). Pritts and Hancock (1992), cited in Prange and DeEll (1997) report that rain during harvest can adversely affect quality of highbush berries as it causes harvest delays, washing off of fungicides, softening of berries, moistened stem scars and split berries resulting in increased disease. The most visible symptom caused by rain is berry split or cracking. This has been researched extensively in sweet cherries, grapes and tomatoes (Marshall *et al*., 2002) but has received little attention with blueberries. In studies done on cherries, the occurrence of split has been attributed to a number of factors. Belman and Keulemans (1996) define the rate and quantity of water uptake which in turn, are influenced by skin nitrogen content, number of stomata and cuticle thickness, as determinants of split. Water uptake is not only encountered through the vascular system, but also through the skin of the fruit (Marshall *et al*., 2002). The high rate of water absorption as well as irregularity in uptake, lead to irregular cell expansion and fruit growth which eventually exhausts the capacity of the fruit cuticle to accommodate the changes in turgidity and results in cracking (Sekse, 1995).

Cherries are most susceptible to rain induced cracking during the final three weeks of maturation and ripening. For blueberries, splitting was most frequent at the beginning of the harvest season and decreased throughout the season. Even when fruit are not visibly cracked following rainfall, microscopic cracks may develop that can greatly reduce fresh market shelf life due to increased susceptibility to attack by fungal pathogens (Marshall *et al*., 2002). In studies on grapes, Uys and Calitz (1997) describe the same weakening in tissue due to the continual increase and decrease in moisture stress as a result of variations in moisture supply and moisture withdrawal. They also relate susceptibility to cracking with berry size. According to Meynhardt (1956), cited in Uys and Calitz (1997), smaller berries are softer, with weaker skin, and have a rounder shape, leaving little room for expansion during water uptake. Blueberries are small and round, however these

characteristics may not be correlated to the observed consequences in grapes. In grapes, fruit integrity is maintained by stem attachment to the berry. For blueberries, removing the stem creates a stem scar, a weak point that could enhance splitting (Marshall *et al*., 2002). Components of the cell wall, such as lignin, cellulose and fibre, give rigidity and structure and their relative quantities may also contribute to berry split susceptibility (Silva, 1985 cited in Marshall *et al*., 2002).

Specialized cultural practices such as plastic row covers or calcium applications by over-tree sprinklers (Marshall *et al*., 2002) may reduce incidence of fruit cracking. However, a more important consideration for areas prone to rain during the harvesting season, is to choose cultivars less susceptible to cracking. Fruit splitting in rabbiteye blueberries seems to be cultivar specific. 'Premier' exhibits a low incidence of splitting whereas 'Tifblue' berries have a much greater propensity to split. Silva (1985), as reported in Marshall *et al*. (2002) also found 'Tifblue' to contain less lignin, cellulose and fibre than 'Premier'. Studies with regard to cultivars and split susceptibility can be useful for optimizing fruit quality and storage potential in rainy areas.

3. Handling and storage chain: from picking to purchasing

Good fruit quality is critical for good storage (Ehlenfeldt, 2002) and originates on the tree. From the chosen maturity stage at the point of picking the natural sequence of the fruit is interfered with, continuing throughout the handling and storage chain until it is eventually consumed. Preservation of fruit quality entails minimizing handling and transportation, and holding the fruit under optimal conditions from beginning to end. There is no substitute for maintaining the cold chain throughout the postharvest handling system (Kader, 2003). Proper temperature management, starting in the field, is the most effective method for extending postharvest life. Cooling must be prompt and thorough (Hayakawa and Succar, 1982; Dincer, 1995), whereafter the optimal storage temperature must be maintained until the time of purchase.

3.1. Harvesting process

Blueberries are handpicked at the 100 % blue stage (Eck *et al*., 1990) on more or less a weekly basis, depending on the rate of ripening. Blueberries, even those growing on the same bush, do not ripen at the same time. Ripening takes place over a period of three to four weeks (Greeff, 2003). The red-blue stage is the crucial time of quick improvement in quality properties. Picking at or before this stage prevents further postharvest evolution of favourable organoleptic traits (Giacalone *et al*., 2002) and may even compromise storage life (Peano *et al*., 2002). On the other hand, over mature berries are easily damaged, rapidly lose their firmness and become more susceptible to fungal decay, also reducing storage potential (Peano *et al*., 2002; Ballinger *et al*., 1978; Boyette *et al*., 1993).

Ehlenfeldt (2002) mentions an increased consciousness of harvest frequency and its effect on improving keeping quality. Cline and Milholland (1995) emphasize that since blueberries are picked at 100 % blue, growers must maintain the all-important seven-day (or less) harvest interval in a given field and pickers must thoroughly harvest all ripe fruit before moving on to the next bush. However, frequency of harvesting is always a tradeoff between labour availability, ripeness and yield. Labour availability is a relevant issue in South Africa as the current production peak coincides with Christmas and New Year, a time when labour productivity is generally poor. It is during this time that difficulty is experienced in handling berry volumes, overripe fruit are abundant and quality issues such as shrivel, soft and decayed fruit arise.

Traditionally, blueberries were picked into buckets, taken to the packhouse, pre-cooled to 10 ºC whereafter sorting and repacking took place. Increasingly, however, hand harvesting, grading and packing are being treated as a single operation in South African blueberry fields. Berries are hand picked directly into the 125 g, 150 g, or 170 g punnets in which the fruit is sold to the consumer. During peak production, berries are picked into bulk 600 g tubs in an attempt to cope with the large volumes. Sorting and packing in the field reduces the amount of handling but renders careful attention and adequate training

for pickers and supervisors essential. Criteria for grading include size (berries must be larger than 11 mm or 13 mm in diameter for Holland and UK, respectively), presence of bloom, as well as absence of blemishes and bruises. Bloom is easily removed through rough handling and by holding berries in a hand before placing them into a punnet. This not only detracts from berry appearance, but may cause bruising and physical damage which encourages moisture loss, increases respiration and accelerates senescence.

Filled punnets are packed into trays and left in the shade until transport to the pack shed is possible. Protection against heat deterioration is important as fruit temperature can rise above the air temperature in less than an hour. Trays left in the shade will have fruit temperatures 5-6 ºC lower than ambient air temperature (Boyette *et al*., 1993). Berries are generally removed from the field within 1 or 2 hours whereafter they are cooled to approximately 0-2 ºC. Transport to the refrigeration facility should be slow and smooth to avoid bruising and shaking

3.2. Cooling

Temperature management starts in the field and since deterioration is a function of time and temperature, faster cooling retains fruit quality and can significantly extend shelf life (Mitchell, 1987; Paull, 1999; Nunes *et al*., 1995; Jackson *et al*., 1999). Dincer (1995) and Brecht *et al*. (2003) associate rapid forced air pre-cooling to about 0 ºC immediately after harvest with improved preservation of natural blueberry properties during storage, distribution and export. Quick removal of field heat and reduction of delays can also retain firmness, reduce weight loss and shrivel (Nunes *et al*., 1995), decrease decay development (Hudson and Tietjen, 1981; Ceponis and Cappellini, 1985; Harvey and Harris, 1986) and maintain fruit marketability (Jackson *et al*., 1999).

The time required to bring fruit to optimum storage temperature is affected by delays between harvest and placing fruit in the cooling facility and by completeness of cooling. Incomplete cooling results in a wide range of temperatures among individual fruit and is a cause of above-optimal average fruit temperatures for undesirable lengths of time (Harvey and Harris, 1986). In some cases the designated product temperature may never be reached.

The North Carolina blueberry website (Boyette *et al*., 1993), illustrates a practical example of incomplete cooling. Reference is made to a pallet of blueberries with an initial temperature of 28 ºC, held in a 7 ºC coldroom (room cooling) for approximately an hour. Appreciable cooling was observed in berries situated on the outside of the pallet. However, readings near the centre of the pallet showed a temperature increase during the first hour of cooling. Central punnets are said to require more than 36 hours to be cooled below 10 ºC. This highlights that room cooling is not uniform enough or efficient enough to cool a pallet of blueberries within a short timespan. Taking into consideration that South African growers rely heavily on room cooling, this is a valuable point. It must, however, be noted that South African blueberries are only palletized after proper cooling has taken place. This motivates that cooling should be monitored and that the correct temperature should be reached before final packaging and transport seeing that refrigerated transport cannot be depended on to provide adequate cooling. Forced air cooling ensures rapid and thorough cooling resulting from intimate contact between the cold air and the product as it is channeled through the packaging (Kader, 1985). It facilitates cooling to occur in one-quarter to one-tenth of the time required by room cooling (Wills *et al*., 1998). For fresh produce to reach distant markets in a satisfactory condition, more uniform and faster cooling must be prioritized (Mitchell, 1985) and the cold chain must be maintained from the point of picking, packaging, handling, transporting, marketing to distribution. Kader (2003) describes the cold chain as an irreplaceable entity for extended quality and storage. According to Miller *et al*. (1984) careful cultivar selection, proper pre-cooling, packaging, and maintenance of transit

temperature, should ensure successful export of rabbiteye blueberries to distant markets via air or sea transport.

Considering the sequence of events prior to shipment and the expectations on arrival in the UK, the importance of extended storage life is highlighted. A week at 0 ºC can elapse from picking to loading followed by 24 hours to reach the UK distribution agent (airfreight). Seafreight berries spend 16 days at sea under CA storage at 0 ºC plus 24 hours to reach the distribution agent. Potentially the berries can then be stored in CA for a further two weeks, otherwise they should withstand a shelf life period of a minimum of ten days at 2 ºC and still have the capacity to spend four days on the supermarket display plus an extra day for the consumer. There are two supermarket scenarios, namely ambient (22-24 ºC) or 2 ºC in refrigerated displays (which run closer to 6-7 ºC). One advantage of exporting from South Africa is that the berries reach the UK in winter therefore temperature fluctuation related issues are less substantial. In effect, berries must last at 0 ºC for a minimum of three weeks (airfreight) or five to six weeks (seafreight) plus five days of shelf life at 15 ºC.

3.3. Storage

Storage conditions must decrease, as much as possible, the rate of physiological processes in fruit and prolong the good quality of the fresh fruit (Borecka and Pliszka, 1985). Blueberries have a short storage potential of 2-3 weeks (Boyette *et al*.,1993). Sekse (1996) associates a short storage potential with non-climacteric fruit which require administration of adequate precautions against post harvest deterioration. Berries must be stored at the lowest safe temperature (0-2 ºC) and a relative humidity close to 100 %. Storage prolonging techniques such as controlled atmosphere storage and modified atmosphere packaging have also been used.

3.4. Controlled atmosphere (CA) and modified atmosphere packaging (MAP)

After temperature control, controlled and modified atmosphere storage is the next most popular storage prolonging techniques (Borecka and Pliszka, 1985). Controlled atmosphere (CA) storage involves the manipulation of the gaseous environment around the fruit to precisely controlled, predetermined levels. This is usually accomplished by decreasing oxygen and increasing carbon dioxide content of the air (Wills *et al*., 1998). Carbon dioxide enriched atmospheres are used as a means of slowing ripening and senescence of many fresh commodities during storage, transport and marketing (Mattheis and Fellman, 2000). Carbon dioxide levels are said to have a more pronounced effect than the oxygen level (Boesveld, 2000). However, both contribute in reducing respiration rate, and influence nutritive value, flavour, aroma, moisture loss, (Clayton-Greene, 1993), textural changes and softening (Mattheis and Fellman, 2000; Beaudry, 1999, 2000; Song *et al.*, 1992). Lowering O_2 levels to 2 % can, however, cause poor flavour and apparently does not enhance CO_2 effectiveness (Ceponis and Capellini, 1985). According to Ceponis *et al*. (1983), Fan *et al.* (1993) and Smittle and Miller (1988), a further advantage of CA is that it is fairly effective in inhibiting post harvest decay. Artés-Hernández *et al*. (2004) state that CA is even seen as an alternative to replace SO_2 for keeping quality of 'Autumn Seedless' grapes. Much room for refinement of CA conditions exists as temperature regimes and cultivars differ in response to enriched atmospheres and a fine balance exists between the ratios of the gas concentrations (Kader, 2003).

In South Africa controlled atmosphere technology of blueberries has not yet been examined. However, its success will be a valuable step forward as blueberry tonnage increases, especially considering that air space is limited. CA storage must prove competent over an extended period and must also be exceptionally effective. The UK market tolerance for decay is set at 2 % at the end of the shelf life period, therefore decay must be reduced almost to zero.

Various theories exist around the recipe for the most favourable blueberry gas combinations. In general, parameters for optimal blueberry storage are given by Ehlenfeldt (2002) as temperatures between 1-2 ºC, RH of 95 %, 10-15 % CO_2 and O_2 levels no lower than 3-4 %. The recipe used is dependent on the goal to be achieved and the circumstances involved, i.e. duration of storage, handling practices, susceptibility to decay, maintenance of berry firmness, as well as cultivar differences (Beaudry *et al*., 1998; Brecht *et al*., 2003). An important consideration is that different processes of ripening and senescence have different O_2 and CO_2 optima for maximum beneficial responses (Mattheis and Fellman, 2000). Subjecting fruit to atmospheres outside their optimum level, could result in increased susceptibility to decay or off flavours, therefore certain compromises must be made.

Blueberries might have a considerable ability to be stored (Ceponis and Cappellini 1979, 1983, 1985) and CA is said only to be advantageous over regular air for longer storage periods (Clayton-Greene, 1993; Ehlenfeldt, 2002). According to Boesveld (2000) a storage period of eight weeks is categorized as long and a CO_2 level of 10 %, together with an O_2 range between 5 % and 10 %, is considered to be suitable. For shorter periods, a higher CO_2 level of 15 % is advised, however this level can be detrimental to taste and firmness for longer storage periods. Bounous *et al*. (1997) report that CA can sustain a six week storage period when CO_2 conditions fall between 3-10 % or 5 -12 % as quoted by Clayton-Green (1993) and Cameron *et al* (1994), respectively, in combination with 2 % O_2 levels. The best treatment for six weeks storage, described in the north west berry and grape information network (Anon, 1995), is 1.8 % O_2 and 12 % CO_2 at 97 % RH. An extreme example of storage life is described by Forney *et al.* (1999)**,** where comparable results were obtained after 9 weeks of storage, for both regular air (RA) and CA. The incidence of decay was 2 % for RA, while 1 % decay was observed for CO_2 levels of 10-15 %. CO_2 enriched atmospheres have been reported to reduce decay by 50 % (Ceponis and Cappellini, 1983). Greater percentages of marketable fruit, firm fruit, as well as better sensory ratings were attained for CA combinations (10 %, 15 % or 20 % CO_2 with 5 % O_2), than for storage in air (Smittle and Miller, 1988).

Temperature also has a role to play in CA atmospheres as lower temperatures justify lower CO_2 concentrations, thus reducing the chance of off flavour, and CO_2 damage. For non-precooled fresh blueberries a combination of adequate refrigeration and CO_2 concentrations of 15 % can control postharvest diseases and maintain marketable fruit quality well enough to warrant transportation to distant markets. This information appears to present CO_2 enriched atmospheres as a viable alternative to pre-cooling. However, Ceponis and Cappellini (1983) state that efficacy of disease control is enhanced by rapid pre-cooling. Best CA results are achieved when employed with good temperature and RH control (Brecht *et al*., 2003).

Possible disadvantages associated with CA storage include the development of off flavours and shortened shelf life periods. Off flavours develop when O_2 levels are too low, or CO_2 levels too high, resulting in anaerobic respiration and a characteristic fermented taste (Kader *et al*., 1985). Off flavours can be avoided by using CO_2 levels below 15 % (Ceponis and Cappellini, 1985; Fan *et al*., 1993). Blueberries stored in 15 % CO_2 were off flavour, after 76 days (Fan *et al.*, 1993).

CO_2 enriched atmospheres are effective in suppressing decay. However, when berries are removed from CA storage the inhibiting effect is alleviated and fruit deteriorate more rapidly than freshly marketed berries (Ehlenfeldt, 2002). According to Ceponis and Cappellini (1985), upon removal of berries from CA (CO_2 concentrations of 10 %, 15 %, or 20 %) after seven or 14 days at 2 ºC plus shelf life of three days at 21ºC decay was reduced significantly for only one or two days.

CA storage requires that large quantities of fruit should be stored within a few days (Bounous *et al*., 1997). Modified atmosphere packaging (MAP) has the potential to provide low O_2/high CO_2 regimes similar to those of CA storage (Beaudry *et al*., 1992;

Cameron *et al*., 1994). Smaller quantities of fruit are sealed in pallets and wrapped in plastic film so that fruit are exposed to the enriched atmospheres throughout the handling chain (Bounous *et al*., 1997). Unlike CA, MAP does not rely on expensive gas regulation equipment. A constant gas composition is maintained in the package by matching the respiration of the product with the permeation rates of the package (Song *et al*., 1992). Bounous *et al*. (1997) reported successful maintenance of fruit quality over six weeks of MAP storage at 8-10 % O_2 and 10-13 % CO_2. However MAP was described as unsuccessful for atmospheres of 15 % O_2 and 3 % - 25 % CO_2, due to the development of off flavours (Bunemann *et al*. 1957, cited in Kotecha and Madhavi, 1993). Due to inevitable temperature fluctuations occurring during handling, transit and storage, MAP should maintain the appropriate atmospheric condition over a range of temperatures (Beaudry *et al*., 1992; Cameron *et al*., 1994). Since respiration related O_2 consumption of blueberries is more sensitive to increases in temperature than the film permeability, hypoxic levels are easily reached causing off flavours (Beaudry *et al*., 1992). Song *et al.* (1992) report that MAP of blueberries is impeded by the lack of understanding of the respiration of the fruit. In the case of pore regulated MAP, optimized to 2 kPa O_2 at 0 ºC anaerobic conditions were predicted with a temperature increase of as little as 5 ºC, should steady-state conditions be reached (Cameron *et al*., 1994; Rosenfeld *et al*., 1999). Rosenfeld *et al*. (1999) describe the effect of temperature as more important than film type and initial atmosphere modification when related to berry quality. Before MAP can be considered as a dependable technology, further advances should be made with regard to film manufacture, package configuration, packing, handling and marketing (Beaudry *et al*., 1992; Cameron *et al*., 1994).

Traditionally, optimal CA and MA storage conditions were selected on the basis of stretching the post harvest life of a commodity to its limit (Brecht *et al*., 2003). This would entail choosing the least advanced maturity or ripeness and subjecting the commodity to the most extreme CO_2 and O_2 levels tolerable, without injury, while still attaining the minimum specifications (Brecht *et al*., 2003). CA can therefore contribute to the year round availability of the fresh commodity and lead to numerous other economic

benefits. The movement towards promoting greater consumption of fresh produce has shown that meeting consumer demands must be prioritized (Kader, 2003). Ehlenfeldt (2002) highlights that consumer acceptance for stored blueberries require the maintenance of good quality and that fruit availability without quality is worse than no availability at all. With this realization, there is a need for a more holistic approach in CA and MA storage, combining extended storage life with the assurance of good quality and not merely borderline acceptability. Post harvest life based on flavour and nutritional quality is shorter than post harvest life based on firmness or appearance for most fresh fruit and vegetables (Kader, 2003). Therefore, more attention must be given to the former attributes in order to optimize blueberry storage and product popularity.

4. Motivation for study

This MSc study is focused on blueberries belonging to the midseason, rabbiteye cultivars. The production period for these blueberries stretches from week 48 until week eight, with a production peak arising between week 51 and week four, coinciding with Christmas and New Year. There is an urgent need to attenuate this peak, not only through better use of cultivars, but also by exploiting the storage potential of rabbiteye cultivars.

Reports by Ceponis and Cappellini (1979, 1983, 1985) indicate that blueberries might have a considerable potential to be stored. However, there is a need to refine this knowledge and develop commercial procedures applicable to the South African blueberry export market. The storage conditions should stretch the postharvest life of the blueberries without compromising the favourable qualities associated with fresh blueberries. Techniques such as controlled atmosphere may extend storage life and warrant export by sea rather than by air during peak production (Clayton-Greene, 1993). The berries can therefore be stored, reach their destination two weeks later, and can be sold in a rising market. Considering that seafreight is cheaper than airfreight, blueberry preservation, logistics, arrival time and price issues can all be addressed simultaneously in a cost effective way.

The objective of this study is to establish norms for South African blueberries, which will justify sea shipment to Europe and successfully extend the postharvest life while maintaining the desired fruit quality. According to Miller *et al*. (1984) careful cultivar selection, proper pre-cooling, packaging and maintenance of transit temperature, should ensure successful export of rabbiteye blueberries to distant markets via air or sea transport. To develop appropriate seafreight technology, differences existing between rabbiteye cultivars will be assessed, cooling and storage regimes will be evaluated and optimal controlled atmosphere environments will be investigated.

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CHAPTER 2: Controlled atmosphere storage of 'Premier' and 'Tifblue' blueberries.

Abstract

During peak blueberry production, seafreight is considered a favourable logistic alternative for export to the UK. Controlled atmospheres (CA) have been used successfully elsewhere to prolong and optimise berry quality and storage life during shipment. To establish CA norms for South African blueberries, the effect of two atmospheres were assessed relative to RA on rabbiteye blueberries, cultivars 'Tifblue' and 'Premier'. Assessments were done at six evaluation dates up until 12 weeks of storage at 0 ºC, followed by a shelf life period of five days at 15 ºC. Colour (L, C, H), total soluble solids and titratable acidity were not affected by CA. No off flavours were detected after sensory analysis at the CA levels tested. CA-stored berries were firmer and incidence of decay was reduced significantly. Decay differed considerably between seasons and cultivars. The market tolerance for decay is 2 % and this specification was difficult to meet, particularly in 2003. CA may be a valuable technology, however its success relies on good quality fruit and low levels of decay. Once this can be achieved sea shipment may be justified.

Keywords: CA, 'Tifblue', 'Premier', storability, blueberry quality, decay

Introduction

The South African blueberry industry is relatively young, contributing a mere 0.25 % to world fresh production (D. Brazelton, personal communication, 2004). However, with the introduction of new cultivars an accelerated phase of development has been reached (T.D. McKenzie, personal communication, 2004). Until recently production has been limited to rabbiteye cultivars which cater for a short harvesting season wherein fifty percent of the crop is picked in two to three weeks. This peak coincides with Christmas and New Year, a time when labour productivity is generally poor and market prices are

on the decline. There is an urgent need to attenuate this peak, not only through better selection of cultivars but also by exploiting the storage potential of rabbiteye blueberries.

Reports by Ceponis and Cappellini (1979, 1983, and 1985) indicate that blueberries have a considerable storage potential. Smittle and Miller (1988) describe CA as a successful method of extending blueberry shelf life. Furthermore, Clayton Greene (1993) considers CA technology sufficient to warrant export by sea. Sea freighting blueberries is a favourable alternative during peak production. The labour issue is reduced through the use of bulk packaging, packaging and logistics are cheaper and berries reach their destination two weeks later on a rising market. According to Miller *et al*. (1984) careful cultivar selection, proper pre-cooling, packaging, and maintenance of transit temperature, should ensure successful export of rabbiteye blueberries to distant markets via air or sea transport.

In South Africa controlled atmosphere technology of blueberries has not yet been examined. However, its success will be a valuable step forward as blueberry tonnage increases, especially considering that air space is limited. CA must prove competent over an extended period and therefore it is important to consider the sequence of events prior to shipment and the expectations on arrival in the UK. Filling a 12 m container is a timely process. A week at 0 ºC can elapse from picking to loading during the accumulation period (berry 'age' will range between 2-7 days). Hereafter, berries spend 16 days at sea at 0 ºC and a further 24 hours to reach the UK distribution agent. One advantage of exporting from South Africa is that the berries reach the UK in winter therefore temperature fluctuation related issues are less substantial. Potentially the berries can then be stored in CA for a further two weeks, otherwise they should withstand a storage period of a minimum of ten days at 2 ºC and still have the capacity to spend four days on the supermarket display plus an extra day for the consumer. There are two supermarket scenarios, namely ambient (22-24 ºC) or '2 ºC' in refrigerated displays (which in reality run closer to 6-7 ºC).

To safely achieve the above, berries must last at 0 ºC for a minimum of five or six weeks plus five days of shelf life at 15 ºC, of which CA exposure constitutes 16 days. CA must therefore be exceptionally effective, reducing decay almost to zero and CA effects should preferably be residual. The objective of this study was to establish controlled atmosphere norms for South African blueberries, which will justify sea shipment to Europe and successfully extend the postharvest life while maintaining the desired fruit quality.

Materials and methods

Rabbiteye blueberries, cultivars 'Premier' from By-den-Weg, Stellenbosch and 'Tifblue', from Gelukstroom, Vyeboom (season 1), were hand picked at the 100 % blue stage with 30 berries to a punnet. The punnets were divided into three sub-samples in the field by means of randomly pre-labeled, colour coded lids. Transportation to the laboratory occurred within 2 hours of picking. 'Tifblue' berries for season 2 originated from Highfields, George and were obtained from an export pallet designated for sea shipment to the UK. Immediately upon arrival a zero time quality assessment was done on a representative set of 180 berries (i.e. six replicate punnets of 30 berries), per treatment.

Treatments included three atmospheres, namely a regular atmosphere (RA) control, controlled atmosphere A (CA A, 2 % O_2 and 10 % CO_2) and controlled atmosphere B (CA B, 6 % O_2 and 15 % CO_2). Both the RA and CA were supplied and humidified by means of flowboards, running at a flowrate of approximately 100 ml/min and governed by glass capillaries.

During the first season CA compositions were made up and maintained within 10 % of the required concentration by using an O_2/CO_2 analyser (PBI-Dansensor, Combi Check 9800-1, Ringsted, Denmark). In the second season CA compositions were pre-mixed in gas cylinders within a 2 % range of the required concentration by Air Products South Africa (Pty) Ltd.

For both cultivars the blueberries were stored at 0 ºC, 14 replicates (of 30 berries each) to a bucket with a total of five buckets per treatment representing each evaluation date, namely 4, 6, 8, 10 and 12 weeks. Shelf life following cold storage was simulated for five days at 15 ºC.

Quality assessment: Each punnet of blueberries was used for all quality assessments. Two additional punnets were held under the same conditions for sensory analysis.

Firmness: An automated penetrometer, in 2002 a Texture Analyzer (TA-XT2, Stable Micro Systems, UK) and in 2003, an Instron, (model 4444) was used to puncture ten berries per replicate. The berry was placed on its side and a 2 mm flat surfaced cylindrical probe was lowered 4 mm into the berry at 100 mm/min to give a firmness reading in Newton (N).

Colour: External colour of five berries per replicate was evaluated at each of six evaluation dates (in 2003, three dates for 'Tifblue'). A colorimeter (NR – 3000, Nippon Denshoku, Tokyo, Japan) was used on the cheek of the berry at a point on the fruit where colour was most uniform.

Soluble solids (SS) and titratable acidity (TA): A punnet of 30 berries was squeezed through cheese cloth and was used as a pooled juice sample for a SS reading, using a hand held refractometer (Atago PR-100 9501, Japan). The same pooled juice sample was analysed for TA by titrating it with 0.1 N NaOH to an endpoint pH of 8.2, using an automated titrator (Titrino 719S and Sample Changer 760, Metrohm Ltd., Herisau, Switzerland). Results were expressed as percent malic acid and citric acid.

Percentage Decay: The number of mouldy berries was recorded and expressed as a percentage of the total number of berries in each replicate.

Sensory evaluation: In 2003 the sensory analysis was performed by a trained panel of three members at harvest, after six and 12 weeks for 'Premier', and at harvest and after

six weeks for ‘Tifblue’. Each panelist was served three berries in nine replicates per treatment in a randomized order. Attributes were assessed by means of an unstructured line scale, anchored with low intensity on the left side and high intensity on the right side. Tested attributes were firmness, grittiness, juiciness, blueberry taste, sweetness and acidity. Characteristics such as off flavour and storage flavour were not included in the statistical analysis as incidence thereof was too low (inadequate quantities affected). Since panelists had no previous exposure to blueberries, the 2002 sensory analysis was used on a preliminary basis to familiarize the panelists with the taste of blueberries and to identify relevant characteristics associated with storage time and the CA treatment.

Statistical analysis: The experimental design was a completely randomized factorial with six replications of each treatment. The data were analysed with the General Linear Models (GLM) procedure of SAS (Enterprise Guide VI, version 1.3). An arcsin square root transformation was performed on all percentage data prior to statistical analysis. Nontransformed data are presented.

Results

Colour

In both seasons, for ‘Tifblue’ and ‘Premier’, differences for lightness, chroma and hue angle were negligible and where significant, results were inconclusive. No trends could be linked to either storage atmosphere or length of cold storage (Table 1A, B). According to the colorimeter hue sequence (Voss, 1992) blue colour is measured in the approximate range of 225 - 290 º. Between 300 and 360 º hue passes through purplish blue, violet, purple and reddish purple, reaching red at approximately 20 º. ‘Tifblue’ hue ranged between violet and purplish blue in 2002 and between purplish blue and blue in 2003. ‘Premier’ hue ranged between 257 and 282 º in both seasons and were therefore considered bluer than ‘Tifblue’.

Soluble solids and titratable acidity

In both seasons for both cultivars, the soluble solids content ranged between 12 and 15 °Brix. The storage atmosphere (RA, CA A and CA B) had no significant effect on blueberry soluble solids and results were inconclusive relative to the length of cold storage. Results for titratable acidity were very similar, and no trends could be linked to either storage atmosphere or length of cold storage (Table 2 A, B).

Firmness

'Tifblue': In 2002, an interaction was found between the storage atmosphere and evaluation dates for both storage at 0 ºC and shelf life (Table 3). After 0 ºC storage, significant interaction contrasts were found between both the linear and quadratic time relationship with regular air and controlled atmosphere berries (RA vs. CA* T_L and RA vs. CA* T_Q) (Table 3).

In 2003, firmness for the CA treatments at 0 ºC differed significantly as is also reflected by the significant contrast found. Firmness measured for CA B was 0.12 N lower than for CA A. Significant differences occurring for evaluation dates after 0 ºC storage and shelf life were inconclusive. A quadratic time contrast was found for firmness after storage at 0 ºC.

'Premier': In 2002, firmness readings for all treatments differed significantly. CA A's firmness was 0.15 N lower than RA and CA B was 0.42 N lower than RA (Table 3). Significant contrasts were found linearly over time, between RA and CA firmness as well as between CA A and CA B. In 2003, firmness for CA A and CA B differed and contrasted significantly and both a linear and quadratic time contrast was observed. After 0 ºC storage the consecutive evaluation dates differed significantly, however, for both seasons, they followed no clear trend.

Shelf life: In both seasons interactions were found between storage atmosphere and evaluation date. After shelf life in 2002, a significant interaction contrast was found between the linear time relationship with RA and CA stored berries (RA vs. CA*T_L), as

well as between the individual controlled atmospheres (CA A vs. CA B*T_L) (Table 3). In 2003, controlled atmosphere shelf life contrasts interacted both linearly and quadratically with time (CA A vs. CA B*T_L and CA A vs. CA B*T_Q).

For both cultivars, in both seasons, for 0 ºC storage and shelf life the lowest firmness readings were recorded for CA B, which had the higher O_2 and CO_2 content (6 % O_2 and 15 % CO_2). In relation to treatment and time, firmness changes were minimal. The greatest difference between treatments was 0.2 N for 'Tifblue' and 0.42 N for 'Premier'. Firmness measurements were also consistently higher after shelf life in both years for both cultivars.

Percentage decay

'Tifblue': In both seasons an interaction was found between the storage atmosphere and evaluation date (Table 4). After 0 ºC storage in both years, a significant interaction contrast was found between the linear time relationship and the RA and CA stored berries (Table 4 and 5). In 2002 CA treatments suppressed decay development completely for up to 12 weeks of cold storage while decay set in after eight weeks for RA, reaching 7.8 % after 12 weeks of storage (Table 5 A and Fig. 1). In 2003, the evaluation period was halved due to excessive decay and regardless of the storage atmosphere, decay set in after only four weeks of cold storage. Decay levels ranged between 4 % and 60 % (Fig. 2). After four and six weeks of storage, decay levels for CA were reduced by 62.5 % and 51.9 %, respectively relative to RA (Table 5 B).

In both seasons shelf life decay levels were similar and insignificant for RA, CA A and CA B as is also clear from the insignificant contrasts (Table 4). At each evaluation date in 2002 only small differences were found between storage atmospheres and in 2003 the differences between storage atmospheres on decay development were negligible (Fig.. 2). Shelf life decay increased significantly as the storage interval increased. Relative to decay a linear time contrast was found in 2002 while 2003 showed both a linear and quadratic time contrast. In 2002 shelf life decay set in after four weeks for RA, after six weeks for CA A and after eight weeks for CA B. Decay levels for 2003 ranged between

30 % and 50 % following shelf life after harvest and for all treatments reached 100 % after six weeks (Fig. 2).

'Premier': In both seasons an interaction was found between the storage atmosphere and evaluation date after storage at 0 ºC (Table 4B). In 2002 decay was suppressed completely for CA A and CA B until 12 weeks of cold storage (Fig. 3). RA decay set in at 0.6 % after eight and 10 weeks and reached 5.6 % after 12 weeks of storage (Table 6A, Fig. 4). Higher levels of decay developed in 2003 and decay set in at 10 % after six weeks at RA relative to 2.7 % for CA after eight weeks (Table 6 B). In both seasons, significant interaction contrasts were found between both the linear and quadratic time relationship and the RA and CA stored berries. CA related decay reduction during storage at 0 °C ranged between 77.2 % and 100 % relative to RA in 2003.

'Premier' decay levels after shelf life in 2002 were exceptionally low and no significant differences were found for either storage atmosphere or evaluation dates (Table 4b). However, a linear time contrast was found. RA decay set in at 1.7 % after four weeks and at 1.1 % after six and eight weeks for CA B and CA A, respectively. Decay levels never exceeded 3.3 % (Fig. 3). In 2003, however, circumstances changed and interactions existed between storage atmosphere and evaluation date. A significant interaction contrast was found between the linear time relationship and the RA and CA stored berries (RA vs. CA*T_L, Table 4). After harvest decay levels reached 2.2 % for CA A. After four weeks, decay set in at 12.2 % for RA and 6.2 % for CA (Table 6 C). CA related decay reduction ranged between 49.2 % to 70.6 % after shelf life in 2003.

Sensory analysis

An interaction was found between the storage atmosphere (RA, CA A and CA B) and evaluation date after 0 ºC storage for 'Tifblue' sensory attributes sweetness and acidity (Table 7). Interaction results were inconclusive. Controlled atmospheres had no significant effect on firmness, grittiness, juiciness and blueberry taste. Firmness and grittiness increased significantly from harvest to six weeks. No sensory analysis was done after shelf life as decay levels were exceptionally high.

After shelf life of 'Premier' berries an interaction was found between the storage atmosphere and evaluation date for sensory attributes juiciness, blueberry taste, sweetness and acidity. Interactions were inconclusive. CA storage had no significant effect on sensory attributes after 0 ºC storage. Firmness decreased and grittiness increased significantly with increased storage duration for both 0 ºC storage and shelf life.

The purpose of the sensory analysis was to determine whether off flavours would develop in response to CA. Since incidence thereof was too low to analyse statistically, it was accepted that off flavours were not a concern for 'Tifblue' and 'Premier' blueberries at these CA levels.

Discussion

CA storage had no impact on blueberry colour, soluble solids or titatable acidity. Beaudry *et al*. (1998) report that fruit held in CA storage were in no way superior to fruit held in RA storage for most quality parameters. Artés-Hernández (2004) also detected no noticeable changes in soluble solids and titratable acidity of grapes under CA conditions. However Smittle and Miller (1988) state that soluble solids decreased during storage particularly for RA berries. Titratable acidity is said to remain constant under CA conditions whilst decreasing during air storage (Smittle and Miller, 1988). Kim *et al*. (1995), cited in Rosenfeldt *et al*. (1999) noticed that the ratio of soluble solids to titratable acidity was influenced by CO_2 concentration and was lower at higher CO_2 levels. During sensory analysis no off flavours were detected and CA effects on the remaining sensory attributes were insignificant. These sensory attributes were also considered unimportant by Rosenfeld *et al*. (1999). Donahue *et al*. (2000) discussed large variation in consumer and instrumental data, which may be related to huge variation in the berries themselves, giving a possible explanation for inconclusive results.

Firmness

Firmness is a component of fruit texture (Abbott, 1999) and is considered an important parameter of fruit (De Belie *et al*., 2000, DeLong *et al*., 2000) and berry quality (Hanson, 1995). Firmness decreases with ripening due to the dissolution of pectins present in the middle lamella which act as cementing agents for the cell walls (De Belie *et al*., 2000). Fruit firmness readings can be influenced by cell structure, size and composition (De Belie *et al*., 2000), as well as by fruit size (smaller fruit are usually firmer), temperature (warmer fruit are usually softer), position (i.e. cheek vs. calyx end) and turgidity (Bramlage, 1983, Døving and Måge, 2002).

A puncture test using an automated penetrometer (Texture analyzer, 2002 and Instron 2003) was used to measure berry firmness. Penetrometers measure the elastic response of the fruit and quantify the effect of force and deformation. However, fruit, especially softer fruit, exhibit viscoelastic behavior which is a function of force deformation and time. Tests including the viscous component are more representative for softer fruit such as tomatoes and berries but there is currently no accepted standard method for these techniques (Abbott, 1999).

In this experiment a higher firmness reading was indicative of a softer berry. As blueberries became less turgid, the flesh offered less resistance and rupturing of the peel was delayed, resulting in a higher reading although the fruit was softer. Bramlage (1983) reports that apple peel distorts the pressure test and must therefore be removed from the area tested.

Berries stored in CA B (6 % O_2 and 15 % CO_2) consistently had the lowest firmness readings indicating that these berries were generally firmer. CA storage slows processes of senescence therefore retention of berry firmness is expected. However, many reports state that higher CO_2 levels, especially above 15 % can be injurious and depending on the cultivar, concentrations between 10 % and 17 % can accelerate berry softening and reduce blueberry firmness (Forney *et al*., 1999; Fan *et al*., 1993). The critical factor is not

only the CO_2 concentration but the tolerance range of fruit which is related to the duration of storage (Mattheis and Fellman, 2000; Weichman, 1986). The tolerance level of 'Premier' and 'Tifblue' blueberries is therefore accepted to be above 15 %. Relative to firmness of berries stored at 0 ºC, firmness readings were consistently higher after shelf life and berries were, therefore, softer. Forney *et al*. (1999), mention that CO2-induced softening was enhanced by increased storage time. Artés-Hernández (2004) observed minimal changes in grape firmness after CA storage at 0 ºC with more considerable changes after shelf life, attributing the result to the absence of a residual effect of CA on maintenance of firmness.

Regardless of the instrument used (Texture analyzer or Instron), the season (2002 or 2003) or cultivar ('Tifblue' or 'Premier'), firmness differences occurring between treatments and evaluation dates were minimal. Firmness seldom increased or decreased more than 0.5 N. Interactions, contrasts and significance all fell within a 1N range and the question arises whether these changes have a noticeable impact on berry quality. Harker *et al*. (2002), in their study relating sensory texture to instrumental texture report that differences in instrumental firmness though significant are often small and difficult to interpret. They also stated that puncture test firmness had to differ by 6-8 N before the sensory panel could detect a change in apple texture. Verreydt (1995) cited in De Belie *et al.* (2000) reported that, particularly for quite ripe fruit, despite the fact that softening still proceeds, almost no change in penetrometer firmness with time is detected. Blueberries are picked at 100 % blue stage and are easily harvested over-mature (Hanson, 1995), therefore changes in firmness may be less obvious.

Another factor to consider is the size of the berry and the size of the probe. A 2 mm probe was used and therefore readings will be lower than those of a bigger probe or a flat disc (Pressure (P) = Force (F)/Area (A)). However, since differences were detected with a small probe, this suggests that they are meaningful.

Firmness is also a function of the time of season, pick, weather, cultivar and berry size. Donahue *et al*. (2000) report that berries in the 6-8 mm size class are firmer than larger

berries. 'Tifblue' blueberries in 2002 were generally small while 'Premier' berries were 13 mm or bigger. Magee (*unpublished data)* cited in Smith *et al*. (1996), states that 'Premier' blueberries are considered to be softer at full ripeness than 'Tifblue'. It may be valuable to find norms to depict acceptable firmness or to correlate readings to sensory analyses. Alternately, other measures such as shrivel or weight loss may quantify changes in texture more clearly than firmness.

Percentage Decay

Postharvest decay is the principle factor determining blueberry shelf life (Cappellini *et al*., 1982; Ceponis and Cappellini, 1985; Ballinger *et al.*, 1978; Cline, 1997; Mitcham *et al*., 2000). According to Cappellini *et al*. (1982), decay accounts for two thirds of the total defective fruit, responsible for spoilage. CA inhibits postharvest decay (Fan *et al.*, 1993; Smittle and Miller., 1988) and a 50 % reduction in blueberry decay was attributed to CA storage (Ceponis and Cappellini, 1983).

Although the difference between RA and CA on decay development over time is significant, standards set by the UK market are exceptionally high and a general tolerance level for UK supermarkets for pests and diseases is five fruits per kilogram of which no more than one should show *Botrytis* (personal communications, S. Taylor 2004). The tolerance level for collapsed berries is two berries per kilogram and for progressive defects such as over maturity stands at 2 %. For this experiment, the decay threshold was set at 2 % and a storage life of six weeks plus shelf life was regarded as acceptable. Berries reaching the UK distribution agent should have a minimum shelf life of ten days at 2 ºC, thereafter they must last for four days at 6-7 ºC on the supermarket display and another day with the consumer.

For 'Tifblue' in both years decay reduction was noticeable after storage at 0 ºC (100 % in 2002, 62.5 % and 51.9 % in 2003) (Table 5A and B, Fig. 1 and 2). However CA appeared to have no residual effect after shelf life as decay levels were comparable for all

treatments. This was particularly clear in 2003 when decay levels were considerably higher. The 2 % decay specification was met in 2002 up until four weeks plus shelf life for CA A and RA and until six weeks plus shelf life for CA B (higher CO_2 level, 6 % O_2 and 15 % CO_2). In 2003 decay levels after harvest plus shelf life ranged between 30-40 % (no CA exposure) and after four weeks plus shelf life, for all treatments reached levels between 62.5 % and 71.8 % (Table 5 B, Fig. 2) therefore CA was considered to be insufficient for 'Tifblue' in 2003.

CA decay reduction on 'Premier' in both years was evident after storage at 0 ºC (100 % in 2002, between 77.2 % and 100 % for 2003), (Table 6 A and B, Fig. 3 and 4). After shelf life, decay levels were higher. However, residual CA decay reduction ranged between 49.2 % to 70.6 % for various evaluation dates (Table 6 C). Decay suppression was effective for 'Premier' up to eight weeks plus shelf life for CA A and 12 weeks plus shelf life for CA B. In 2003 the 2 % specification was only met until harvest plus shelf life (no CA exposure), with decay levels of 12.2 %, 4.3 % and 8 % for RA, CA A and CA B, respectively, after four weeks plus shelf life. Decay suppression for 'Premier' in 2003 was therefore also considered insufficient.

Shelf life decay is the restrictive component of blueberry storage life therefore a residual CA effect can be considered a prerequisite. According to Ceponis and Cappellini (1985), removal of berries from CA (CO_2 levels of 10 %, 15 % and 20 %) after seven or 14 days of storage at 2 ºC showed residual inhibitory effects on decay for two of the three days at 21 ºC. Effects were clearer for higher CO_2 levels but after the third day the effectiveness of CA was greatly diminished. Furthermore, decay levels after the second day at 21 ºC were already above 2 %. Ehlenfeldt (2002) states that decay deterioration occurs more rapidly for fruit once out of CA than for fruit held at RA. However, Beaudry *et al*. (1998) state that CA relative to RA had no superior effect on quality parameters after shelf life, except for reducing decay incidence, thus suggesting that CA has a residual inhibitory

effect. A residual effect might be more apparent when decay levels are low and may be effective, only for shorter periods (no longer than two days). However, efficacy may also be a factor of storage duration, cultivar, decay level, maturity, season and environmental conditions during picking.

CA extends blueberry shelf life and has been described to be beneficial for up to six weeks of storage (Ceponis and Capellini, 1983, 1985). Clayton Greene (1993) states that CA is only efficacious over long storage periods (greater than six weeks). Ehlenfeldt (2002) described CA to have no advantage over RA for short term storage periods but also states that shelf life is considered to decline rapidly after three weeks or more of storage in CA. Boesveld (2002) recommends a CO_2 level of 10 % and O_2 range of 5 – 10 % for eight weeks of storage, while a CO_2 level of 15 % is regarded as suitable for shorter periods. CO_2 levels between 15 % and 20 % are associated with CO_2-induced berry softening and off flavours particularly after longer storage periods (Ceponis and Cappellini, 1985; Fan *et al*., 1993). Blueberries stored in 15 % CO_2 had off flavours after 76 days (Fan *et al.*, 1993). Therefore, off flavours can be avoided by using CO_2 levels below 15 % particularly for longer storage periods. Increasing CO_2 levels from 0 % to 10 % have been associated with decreasing decay levels. However, between 10 % and 15 %, Fan *et al.* (1993) found no further significant decay reduction. Similarly, no significant difference was found between CA A and CA B after shelf life. However in 2002 for both cultivars, CA B decay inhibition was effective longer. Irrespective of statistical difference, the deciding factor for successful decay suppression being the 2 % tolerance level, CA B appeared to be more effective. In 2003 decay suppression was insufficient, regardless of the treatment. According to Ehlenfeldt (2002) the generally accepted CA composition is 10-15% CO_2 and an O_2 range no lower than 3-4 %. Many recipes exist but none specify storage duration or assure decay suppression below 2 %. After 46 days of storage under 1.8 % O_2 and 12 % CO_2, 97 % of stored berries were rated as having very good quality (Anon, 1995). In an extreme example, Forney *et al*. (1999) observed less than 1 % decay for berries stored in 10-25 % CO_2 after nine weeks of storage. However, it is important to mention that decay incidence for RA after nine weeks of storage at 0 ºC

was 2 %, indicating that decay levels were low and that besides CA other factors also play an important role in decay incidence.

Disease problems are often cultivar-specific (Cline, 1997; Eck, 1988; Smith *et al*., 1996) and can be affected by climate, fruit maturity at harvest and post harvest temperature (Eck 1988; Hruschka and Kushman 1963; Milholland and Jones, 1972). Storability is mostly determined by cultivar and disease progression is common in both highbush (*V. corymbosum* L) and rabbiteye (*Vaccinium ashei* Reade) blueberries (Cline, 1997), whilst lowbush berries (*V. angustifolium*) show little decay (Sanford *et al*., 1991, cited in Jackson *et al.,* 1999). Austin (1994), cited in Smith *et al*. (1996) regards rabbiteye blueberries to be more resistant to diseases than highbush blueberries. However as the industry expands, it is expected that fruit diseases will increase in importance. Of the rabbiteye berries, 'Premier' and 'Tifblue' have been described as particularly susceptible to certain pathogens (Cline, 1997).

In this experiment 'Premier' appears to perform better than 'Tifblue' in both seasons. It must be borne in mind that many 'Tifblue' berries were under mature (red) in 2002 and over mature in 2003. This was also reflected in the hue angle results. Red berries had a higher decay incidence and it was presumed that these berries were physiologically immature and therefore more susceptible. Beaudry *et al*. (1998) reports that post harvest shelf life and quality control can be gained with appropriate cultivar selection and optimal harvest maturity. He also states that blueberries picked at 60 % blue performed better than those picked at the 100 % blue stage after CA storage. This is confirmed by Ballinger *et al*. (1978) who ascribe enhanced transportability to the harvesting of less mature fruit. Overripe blueberries rapidly lose their firmness and are more subject to disease infection (Peano *et al*., 2002).

Since the Western Cape season came to an unexpected abrupt end in 2003, 'Tifblue' berries originated from George. Not only was there a delay between the time of picking and CA storage, berries were also picked after a rainy period. Rain during harvest implies delays in harvesting, washing off of fungicides, softening of berries, wet stem scars, and split berries, all of which encourage decay development (Pritts and Hancock, cited in Prange and DeEll, 1997). High temperatures during rainy periods exacerbate these problems. The differences observed between 'Tifblue' and 'Premier' could therefore be related to berry maturity and environmental conditions and not necessarily to cultivar. Decay for both cultivars was higher in 2003 than in 2002 and could be described as a seasonal effect such as a higher field inoculum level.

It would appear that the inoculum level plays a substantial role in the efficacy of CA decay reduction. Under low decay levels CA may be successful and prolong blueberry shelf life, moderate decay may require higher CO_2 levels and cater for medium term storage, however CA may not have the desired effect when decay pressure is high. Having established that CA can reduce decay and that CA is more effective at lower decay levels, it is now important to pay attention to factors which promote infection and to reduce these prior to CA storage.

Susceptibility to decay seems to be area, cultivar and climatically related (Cappellini *et al*., 1982; Ehlenfeldt, 2002). In an event of rain picking should be delayed (Ehlenfeldt, 2002), and diseased or wounded berries should be kept out of packages to prevent disease spreading to healthy berries (Ceponis and Cappellini, 1983; Smittle and Miller, 1988; Song, *et al*., 1992; Mitcham *et al*., 2000). According to Cline (1997) combating disease is a combination of timely harvesting (Cline, 1997), prompt cooling, storage at the lowest safe temperature, prevention of physical injury to the fruit and shipment under high CO_2 atmospheres (Mitcham *et al*., 2000). Ceponis and Cappellini (1979) emphasize that only

blueberries of top quality from an early picking should be selected for long periods in cold storage.

Conclusion

Potentially, CA storage can be used to maintain blueberry quality and extend blueberry storage life. In our research, CA effects were unimportant for quality attributes of colour, soluble solids and titratable acidity as well as for sensory analysis. CA storage had the greatest impact on blueberry firmness and decay levels. It is difficult to quantify changes in blueberry firmness, therefore further research is required to find a more effective measure. Alternately, firmness standards related to a specific technique should be established prior to investigation, and could be associated to subjective measures. Decay is the most important storage limiting factor for blueberries. CA storage caused a significant reduction in postharvest decay. However, under high decay conditions suppression was less effective and decay levels flourished after shelf life. Considering the exceptionally low tolerance level for decay (2 %), specified by the export market, the importance of a low initial inoculum/decay level is highlighted. In our research, CA was only sufficient in suppressing decay in 2002 and the CA B gas combination (6 % O_2 and 15 % CO_2) appeared to be most effective. Blueberry cultivars react differently to CA storage and experience differing rates of deterioration under various conditions. CA storage success is also affected by season, picking maturity, environmental conditions, inoculum levels, handling and cooling as well as storage duration. Therefore, successful CA storage relies on blueberries of good quality and CA storage cannot be used to rescue or compromise below optimal fruit or conditions. Considering that CA storage was targeted to attenuate peak production, coinciding with the festive season, when labour productivity is generally poor, it is of the utmost importance that correct systems are in place to assure blueberry quality. To a certain degree bulk packaging may facilitate more thorough picking and in turn reduce the problem of over-mature fruit. However, there are many other aspects which need to be addressed. At this stage no CA storage norms can be set until basic quality maintenance can be guaranteed. This requires picking at the correct interval and maturity (slightly under-ripe is preferable to over-ripe), maintaining

low field inoculum levels, optimizing blueberry handling (to prevent bruising), prompt cooling and maintenance of the cold chain. Special attention should also be given to environmental conditions such as rain and appropriate actions should be taken to reduce negative effects thereof. Once this can be achieved successfully, suitable cultivar selection can take place and CA norms can be refined. CA storage will then warrant sea shipment to the UK and Europe.

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CHAPTER 3: Rabbiteye blueberry quality changes in response to short pre-cooling delays at 20ºC

Abstract

The effects of short pre-cooling delays (0, 2, 4, 6 h) at 20 ºC and subsequent storage times at 0 ºC (three and five weeks), followed by a shelf life period of seven days at 15 ºC were assessed for rabbiteye blueberries, cultivars 'Centurion', 'Premier' and 'Tifblue'. Colour (L, C, H), total soluble solids, titratable acidity and firmness were unaffected by pre-cooling delays of up to 6 hours. Results for percentage decay indicate that prompt cooling becomes more critical as the duration of storage increases, particularly when market specification of no more than 2 % decay must be met. Berries subjected to longer delays appear to be more susceptible to decay. Differences exist among cultivars for all quality parameters, especially susceptibility to decay. Knowledge gained from this research can be used to adapt handling and cooling of different cultivars, particularly under less favourable conditions. It may also impact cultivar selection for marketing (local vs. export), storage (short vs. long term) and logistics (air vs. seafreight).

Keywords: pre-cooling delays, rabbiteye blueberries, decay

Introduction

Prompt cooling and good temperature management are imperative to delay quality deterioration and senescence in fresh produce (Brecht *et al*., 2003; Mitchell, 1985; Paull, 1999). High temperatures stimulate rapid respiration and ethylene production and increase moisture loss from the fruit (Nunes *et al*., 1995a). Blueberries have a Q_{10} of 3 and therefore every 10 ºC increase in temperature dictates a three fold increase in respiration. Being dark, berries will readily absorb heat (Boyette *et al*., 1993). Heat absorption in apples can be responsible for fruit temperatures 10 to 12 ºC higher than ambient temperature (Bergh *et al*., 1980; Kotze *et al*., 1988; Parchomchuk and Meheriuk, 1996). For this reason faster cooling and attainment of low storage temperatures are

assumed to always have a positive effect on produce quality (Nunes *et al*., 1995b; Paull, 1999).

According to Boyette (1995) immediate cooling after harvest is more critical for perishable fruit such as blackberries, strawberries and blueberries as softening and decay can occur in less than four hours at high temperatures. In studies conducted on strawberries pre-cooling delays at 30 ºC led to significant degradation after 6 to 8 hours (Nunes *et al*., 1995a, b). Berries were softer, more shriveled, had less attractive colour and the acidity, SSC, sugar and ascorbic acid levels were lower than fruit that were cooled more promptly (Nunes *et al*., 1995b). Strawberries, however, are more perishable than blueberries (Anon, 1995).

During commercial field and transport operations delays before cooling are inevitable (Nunes *et al*., 1995b). The objective of this study was to assess the effects of short pre-cooling delays at 20 ºC on the quality of 'Centurion', 'Premier' and 'Tifblue' rabitteye blueberries (*Vaccinium ashei* Reade).

Materials and methods

In 2002 and 2003 rabbiteye blueberries (*Vaccinium ashei* Reade), cultivars 'Centurion' and 'Premier' from By-den-Weg, Stellenbosch and 'Tifblue', from Gelukstroom, Vyeboom, were hand picked into punnets and divided into four sub-samples by means of randomly pre-labeled, colour coded lids. The fruit was transported to the laboratory within 2 hours of picking. Immediately upon arrival, a zero time quality assessment was done on a representative set of 180 berries (i.e. six replicate punnets of 30 berries). Sub-sample one was immediately put into cold storage at 0 ºC. Sub-samples 2-4 were spread out in trays, one or two punnets deep, and held at 20 ºC in an air conditioned room at ambient relative humidity. After being subjected to these conditions for 2, 4 and 6 hours (postharvest delay) the berries were stored at 0 ºC for three or five weeks. Shelf life following cold storage was simulated by holding fruit at 15 ºC for seven days.

Two experimental systems were used, namely a static and flow through system.

Season 1: Static system

Replicate punnets of 30 berries each, were placed into cardboard trays, stacked eight trays high and covered with cling film (two stacks per cultivar). A relative humidity of approximately 95 % was created by using a moist newspaper lining in the trays. After each storage period, 24 replicates (six punnets per treatment) were used for quality assessments and the remaining 24 punnets were kept at 15 ºC for a further seven days.

Season 2: Flow through system.

Twelve punnets were placed into a 25L bucket, subdivided into three layers of four punnets each for storage at 0 ºC. For each cultivar four buckets per treatment were used and connected to a flowboard. Flow rates of ± 400 $mL.min^{-1}$ were achieved by means of glass capillaries and a relative humidity of 95 % was maintained by means of a humidifying bottle. For the shelf life period, six punnets were packed into four, 10L buckets and connected in the same way to a flowboard at 15 ºC.

Quality Assessment: Each punnet of blueberries was used for all quality assessments.

Colour: External colour of five berries per replicate was evaluated at each of three evaluation times. A colorimeter (NR – 3000, Nippon Denshoku, Tokyo, Japan) was used on the cheek of the berry for the colour evaluation at a point on the fruit where colour was most uniform.

Percentage decay: The number of mouldy berries was recorded and expressed as a percentage of the total number of berries in each replicate.

Firmness: An automated penetrometer, in 2002 a Texture Analyzer (TA-XT2, Stable Micro Systems, UK) and in 2003, an Instron, (model 4444) was used to puncture ten

berries per replicate. The berry was placed on its side and a 2 mm flat surfaced cylindrical probe was lowered 4 mm into the berry at 100 mm/min to give a firmness reading in N.

Soluble solids (SS): A punnet of 30 berries was squeezed through cheese cloth and was used as a pooled juice sample for a SS reading, using a hand held refractometer (Atago PR-100 9501, Japan).

Titratable acidity (TA): The same pooled juice sample was analysed for TA by titrating it with 0.1 N NaOH to an endpoint pH of 8.2, using an automated titrator (Titrino 719S and Sample Changer 760, Metrohm Ltd., Herisau, Switzerland). Results were expressed as percent malic acid and citric acid.

Statistical analysis: The experimental design was a completely randomized factorial with six replications of each treatment. The data were analysed with the General Linear Models (GLM) procedure of SAS (Enterprise Guide VI, version 1.3). An arcsin square root transformation was performed on all percentage data prior to statistical analysis. Nontransformed data are presented.

Results

Colour: In both seasons, for cultivars 'Centurion', 'Premier' and 'Tifblue', delayed cooling results on berry colour were inconclusive. No significant differences were found relative to delay and significant differences occurring between evaluation dates were inconsistent. Delay time and evaluation date interactions were uncommon and, where present, showed no clear trend (Table 1, 2, 3), (data not presented).

Total soluble solids: In both seasons for all cultivars, results were inconclusive and no trends could be linked to either cooling delays or length of cold storage (Table 1, 2, 3). In 2002 soluble solids were lowest for 'Tifblue' and highest for 'Centurion', whilst in 2003 lowest soluble solids were found in 'Premier' and highest levels in 'Tifblue'.

Titratable acidity: Significant differences for titratable acidity (expressed as percentage malic acid and citric acid) relative to pre-cooling delays were found only in 2002 but followed no apparent trend. Differences occurring relative to storage duration, for both years, though mostly significant, were inconclusive (Table 1, 2, 3). The percentage of malic acid was similar to that of citric acid in both years. Titratable acidity was higher in 2003 and titratable acidity was lowest for 'Centurion' and highest for 'Tifblue' in both seasons. Malic and citric acid seem to decrease considerably during the shelf life period of seven days at 15 ºC ('Centurion' and 'Premier', 2003 and 'Tifblue' 2002). This may be indicative of the trend that could follow either an extended pre-cooling delay or delays at higher temperatures as shown in work by Nunes *et al*. (1995).

Firmness: In 2002, for all cultivars, berry firmness after storage at 0 ºC and shelf life was unaffected by pre-cooling delays of up to 6 hours at 20 ºC (Table 4 A, B, C). Relative to storage duration, firmness readings appeared to increase, albeit minimally, from harvest to five weeks in the case of 'Centurion' and 'Premier'. Firmness of 'Centurion' berries did not differ after storage at 0 ºC whilst shelf life firmness readings increased significantly after the fifth week of storage plus shelf life. 'Premier' firmness readings after three and five weeks for both storage and shelf life increased significantly relative to those at harvest and harvest plus shelf life. Firmness of 'Tifblue' berries was unaffected by storage duration. Shelf life firmness readings for all cultivars were consistently higher that those for 0 ºC storage relative to pre-cooling delay and storage duration.

In 2003, interactions existed between pre-cooling delay and storage duration for 'Centurion' and 'Premier' after 0 ºC storage and shelf life, occurring only after 0 ºC storage for 'Tifblue' (Table 4 A, B, C). Interaction results were inconclusive. The effect of pre-cooling delays and storage duration on 'Tifblue' firmness was unclear after storage and shelf life (Appendix B).

Percentage decay (2003): Pre-cooling delays at 20 ºC had no significant effect on 'Centurion' and 'Premier' decay levels after storage at 0 ºC or shelf life (Table 5 A, B, C). The duration of storage only had an impact after five weeks plus shelf life when decay levels for 'Centurion' and 'Premier' were significantly higher. An interaction was found between the pre-cooling delay and storage duration after 0 ºC and shelf life for 'Tifblue' berries. Decay levels for all cultivars were generally low. However, considering that the export threshold for decay was set at 2 % the seemingly insignificant delay effects had to be reassessed. Clear differences were evident among cultivars and shelf life decay was considered as the restrictive component on blueberry storage life. After five weeks of cold storage plus shelf life decay levels of 'Centurion' berries were below 2 % for all berries except those exposed to a 6 hour pre-cooling delay (Fig. 1). 'Premier' berries exposed to pre-cooling delays of 2 and 6 hours exceeded the 2 % tolerance level after three weeks of cold storage plus shelf life (Fig. 2). After five weeks of cold storage plus shelf life berries cooled immediately after harvest could not even meet the 2 % specification. Unacceptable decay levels were reached for 'Tifblue' berries after three weeks of cold storage plus shelf life (Fig. 3).

Discussion

Blueberries have been described as a highly perishable product, requiring prompt cooling to increase storage capacity and maintain fruit quality (Boyette *et al*., 1993). Jackson *et al*. (1999) reported that, irrespective of the delay temperature, pre-cooling is beneficial only when delays exceed 21 hours. In our research, pre-cooling delays of up to 6 hours at 20 ºC did not affect the quality attributes of colour (lightness, chroma and hue angle), titratable acidity and soluble solids. Similar results were reported by Ferraz *et al*. (2000) after 0, 2, 4, 6 and 8 hour delays at 30 ºC.

Firmness

Fruit firmness continues to be an important quality parameter of blueberries produced for fresh market consumption, as it relates to consumer appeal and to post harvest decay of

fruit (Ballinger *et al*., 1973; Mainland *et al*., 1975; NeSmith *et al*., 2002). Firmness is a component of fruit texture (Abbott, 1999) and is considered an important parameter of fruit (De Belie *et al*., 2000, DeLong *et al*., 2000) and berry quality (Hanson, 1995). Firmness decreases with ripening due to the dissolution of pectins present in the middle lamella which act as cementing agents for the cell walls (De Belie *et al*., 2000). Fruit firmness readings can be influenced by cell structure, size and composition (De Belie *et al*., 2000), as well as by fruit size (smaller fruit are usually firmer), temperature (warmer fruit are usually softer), position (i.e. cheek vs. calyx end) and turgidity (Bramlage, 1983, Døving and Måge, 2002).

A puncture test using an automated penetrometer (Texture analyzer, 2002 and Instron 2003) was used to measure berry firmness. Penetrometers measure the elastic response of the fruit and quantify the effect of force and deformation. However, fruit, especially softer fruit, exhibit viscoelastic behavior which is a function of force deformation and time. Tests including the viscous component are more representative for softer fruit such as tomatoes and berries but there is currently no accepted standard method for these techniques (Abbott, 1999).

In our research firmness results were mostly inconclusive and in 2002 firmness readings appeared to increase with storage duration, also increasing after shelf life. Strangely, this would reveal that berries become firmer with increasing storage period, which is contradictory to results from previous work. However, Nunes *et al*. (1995a) reported similar increases in firmness and ascribed it to toughening of the epidermis as a result of water loss rather than retention of flesh firmness. NeSmith *et al*. (2004) also reported an increase in firmness readings due to high water loss and formation of raisin-like blueberries. In 2002 a higher firmness reading was indicative of a softer berry. As blueberries became less turgid, the flesh offered less resistance and rupturing of the peel was delayed, resulting in a higher reading although the fruit was softer.

In 2003, firmness results were ambiguous and differed among cultivars. NeSmith *et al*. (2004) investigated the effect of handling temperatures and storage on six rabbiteye

blueberries. Firmness deterioration was closely related to cultivar. Cultivars with a higher initial firmness, for example 'Brightwell', appeared to have a lower rate of firmness loss and were less affected by longer delays. Furthermore, cultivars responded differently to increasing storage temperatures. 'Premier' had a lower initial firmness and softened rapidly at all temperatures. Results of NeSmith *et al*. (2004) research emphasize the importance of identifying cultivar differences and suggest using this information to maximize fruit quality and postharvest shelf life.

In our situation more research is required in establishing firmness differences. It may be valuable to establish firmness norms prior to experimental testing so that tendencies for visibly softer berries can be compared to penetrometer readings. Alternately, other measures such as shrivel or weight loss may quantify changes in texture more clearly than firmness loss.

Percentage Decay

Postharvest decay is the principal factor determining blueberry shelf life (Cappellini *et al*., 1982; Ceponis and Cappellini, 1985; Ballinger *et al.*, 1978; Cline, 1997; Mitcham *et al*., 2000). According to Cappellini *et al*. (1982), decay accounts for two thirds of the total defective fruit, responsible for spoilage. Sanford *et al*. (1991) found that increasing temperature resulted in accelerated senescence and higher levels of decay. Quality retention of fresh market blueberries is favoured when the berries are not exposed to temperatures exceeding 10 ºC, the optimal holding temperature being as close as possible to 1 ºC (Ballinger *et al*., 1978). Rapid pre-cooling to 2 ºC has been associated with reduced berry decay after shelf life when compared to berries experiencing a 48 hour delay to reach temperatures of 10 or 2 ºC (Hudson and Tietjen, 1981). After shelf life of 24 hours at 21 ºC, pre-cooled berries had 60-80 % less decay (Hudson and Tietjen, 1981). In this research the overall decay percentage was considered very low and therefore Jackson *et al*. (1999) regarded the differences to be inadvertently exaggerated. In their own elaborate investigation into the effect of delay times (3, 9, 21 and 45 hours) at various pre-packing temperatures (5, 12, 19 and 26 ºC) Jackson *et al*. (1999) described the decay response relative to pre-cooling delay as unimportant. Ferraz *et al*. (2000)

observed no decay for delays of up to 8 hours at 30 ºC after four weeks of storage plus shelf life (24 hours at 20 ºC). In our research, decay levels for all cultivars were generally low. However, the 2 % decay threshold was the determining factor of the delay effect and was particularly restrictive after shelf life. Although decay incidence was seemingly insignificant, decay effects may be more important than anticipated. Depending on the cultivar, unacceptable decay levels were reached after only three or five weeks of cold storage plus shelf life and there was a tendency for greater decay potential with increasing cooling delays.

The temperature and duration of shelf life periods after storage differ across studies, therefore shelf life decay levels will also vary. Jackson *et al*. (1999) did not include a shelf life period. Shelf life conditions were set at 24 hours at 20 ºC and 48 hours at 21 ºC by Ferraz *et al*. (2000) and Hudson and Tietjen (1981), respectively. In order to assess the delay effect, the shelf life period should closely resemble the post storage situation. Airfreighted South African berries reach the UK distribution agent after 24 hours. Potentially the berries can then be stored in controlled atmosphere for a further two weeks otherwise they should endure a minimum period of ten days at 2 ºC. Hereafter berries must have the capacity to spend four days on the supermarket display plus an extra day with the consumer. There are two supermarket scenarios, namely ambient (22-24 ºC) or 2 ºC in refrigerated displays (which run closer to 6-7 ºC) (S. Taylor, personal communication, 2004). A shelf life period of five days at 15 ºC would therefore adequately simulate these post storage conditions. For practical reasons a seven day shelf life at 15 ºC was used.

Low storage temperatures do not stop postharvest physiological changes (Jackson *et al*., 1999). As storage duration increases (even at 0 ºC), fruit inevitably become more susceptible to decay. Decay results after five weeks of 0 ºC storage, met specifications. However, following the simulated shelf life period, decay development increased considerably, particularly where pre-cooling delays were longer. This was probably due to increased microbial activity at 15 ºC. As reported by Jackson *et al*. (1999) pre-cooling berries to 5 ºC efficiently slows microbial activity. Increasingly higher microbial counts

after storage of up to three weeks at 0 ºC were associated with longer pre-cooling delays (Jackson *et al*., 1999). Hence, delaying cooling increases the probability of decay development after storage at 0 ºC, and shelf life conditions encourage it. It may be valuable to assess decay development after shorter intervals than seven days at 15 ºC to determine how quickly decay development increases at this temperature. Since berry contamination may fluctuate throughout the picking season, high temperatures and long delays should be avoided to minimize quality deterioration due to microbial activity (Jackson *et al*., 1999).

Decay susceptibility is species and cultivar dependent. Highbush blueberries (*Vaccinium corymbosum* L.) are considered to be more susceptible than rabbiteyes (*Vaccinium ashei* Reade), and lowbush blueberries (*Vaccinium angustifolium*) are accepted to be more disease resistant (Austin (1994) cited in Smith *et al*., 1996; Cline, 1997; Sanford *et al*., 1991). Within species, considerable differences have also been detected. Decay was less common among 'Climax', 'Bonita' and 'Bluegem' relative to 'Tifblue', 'Becky blue' and 'Premier'. Smith *et al*. (1996) described 'Premier' as more susceptible to decay than 'Tifblue'. 'Tifblue' decay deterioration was considerably lower than 'Woodard' (Miller *et al*., 1984; Smittle and Miller, 1988). In our research 'Centurion' appeared to be more disease resistant than 'Premier' and 'Tifblue'. Ehlenfeldt (2002) also discussed cultivar differences relative to decay and emphasized the importance of such information in determining storability. Knowledge gained from this research can be used as decision making parameters when choosing suitable cultivars for longer storage or sea freight. Under less favourable conditions more attention can be given to more susceptible cultivars and preventative measures can be implemented to reduce risks.

Blueberries used for the purpose of this experiment were picked between 7 and 8 am. By this time ambient temperatures were no higher than 17-24 ºC and individual berry temperature was approximately 23 ºC. Furthermore, pre-cooling delays were performed at 20 ºC. These conditions do not resemble the commercial situation closely. Field temperatures at harvest can easily be between 30 and 35 ºC and picking often takes place

late in the afternoon. Berries exposed to these conditions will have experienced much higher levels of stress and fruit temperature will also be considerably higher. Time of day when harvest is performed can influence shelf life (Paull, 1999). Therefore, results seen here may not be indicative of those expected for berries subjected to commercial harvest conditions.

Conclusion

In response to pre-cooling delays of up to 6 hours at 20 ºC, results for blueberry cultivars ‘Centurion’, ‘Premier’ and ‘Tifblue’ picked early in the morning, were mostly inconclusive. Quality attributes appeared to be unaffected by pre-cooling delays under these conditions. This supports results observed by Ehlenfeldt (2002), Ferraz *et al*. (2000) and Jackson *et al*. (1999). Despite low and statistically insignificant decay levels, the 2 % market threshold necessitates further investigation into delayed cooling effects on development of decay, particularly after a shelf life period. Delays in cooling and exposure to high temperatures should be minimized to reduce incidence of decay after storage and shelf life periods (Jackson *et al*., 1999). Further studies on microbial activity relative to delay temperatures may also be valuable. Should delays after picking be unavoidable, partial cooling to 10 ºC can be considered as a viable alternative for no longer than 21 hours before full cooling.

Cultivar differences were evident for all quality parameters, especially relative to decay. This emphasizes the importance of marketing cultivars separately and motivates careful cultivar allocation to different markets (local vs. export). Where a lower rate of quality loss relative to temperature is experienced, handling and cooling can be adapted particularly under less favourable conditions. As an example, NeSmith *et al*. (2004) reported that during a time of limited cooling space and multiple cultivar harvesting, certain cultivars can be left to endure longer delays without compromising quality. Knowledge of cultivar differences can also impact cultivar selection for storage (short vs. long) and logistics (air vs. sea shipment).

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CHAPTER 4: Respiration, ethylene production and moisture loss of rabbiteye blueberries.

Abstract

Rabbiteye blueberry cultivars differ considerably in terms of their respiration rate, ethylene production and moisture loss. To maximise fruit quality and postharvest storability differences existing between cultivars must be determined. As yet little research has been done on individual cultivar parameters. A series of trials was conducted to evaluate respiration, ethylene production and moisture loss of various rabbiteye cultivars at 15 ºC. 'Climax' appears to have the highest respiration rate, ethylene production and moisture loss, whilst 'Premier' has the lowest respiration rate and ethylene production was too low to be detected by gas chromatography. The lowest proportionality constant was found for 'Tifblue' and 'Centurion'. Information obtained from this research can be used to adjust postharvest procedures accordingly.

Keywords: Rabbiteye blueberry, respiration rate, ethylene production , moisture loss, cultivar differences

Introduction

Postharvest product characteristics such as respiration rate, ethylene production and moisture loss can be used to predict product perishability and storage potential. Accordingly, appropriate quality maintenance strategies can be put in place and advanced technologies such as controlled atmosphere storage and modified atmosphere packaging can be assessed to reduce postharvest losses. High respiration rates are associated with rapid deterioration (Raghavan and Gariepy, 1989 cited in Emond *et al*., 1993). Ethylene is said to promote senescence, accelerate deterioration and reduce postharvest life (Kader, 2003). Moisture loss is associated with increased ethylene and CO_2 production, shriveling and loss of firmness, and reduction thereof is essential for preservation of fruit quality.

All these characteristics are directly affected by temperature, therefore good temperature management plays an important role in delaying product senescence (Mitchell, 1985).

Respiration is the energy-generating process fueling all metabolic activities in the plant. It is a catabolic process, involving the oxidative breakdown of stored organic materials (carbohydrates, proteins, fats) to simple end products, viz. carbon dioxide and water, liberating heat and energy in the form of adenosine triphosphate (ATP) (Salisbury and Ross, 1992**;** Kader, 1985). Blueberries have a low to moderate respiration rate, between 10-20 mg $CO_2.kg^{-1}.h^{-1}$ at 5 ºC, falling in the same category as plums (Kader, 1985; Emond *et al*., 1993).

While berries are still attached to the bush, substrates used for respiration are replenished by photosynthates from the leaves. Once berries are picked, they maintain their living status and replace substances used for respiration by consuming reserve organic materials. Consequently, this leads to depletion of reserves and rapid decline in quality. The rate of respiration is proportional to the rate of perishability after picking (Kader, 1985). Therefore, there is an inverse relationship between respiration rate and storage life of fresh commodities (Wills *et al.*, 1998).

Storage potential is also dictated by the presence of ethylene. Ethylene is the natural aging and ripening hormone, and is physiologically active in trace amounts (less than 0.1 $\mu L.L^{-1}$) (Kader, 1985). Blueberries have a low ethylene production rate, between 0.1 and 1.0 $\mu L.kg^{-1}.h^{-1}$ at 20 ºC. Ethylene is produced in small amounts throughout fruit development. However, its production rate generally increases with maturity at harvest, physical injuries, disease incidence, temperature increases (up to 30 ºC) and water stress (Kader, 1985). Ethylene may in some cases also increase decay development due to fruit softening and the inhibition of the formation of antifungal compounds in the host tissue (Kader, 2003).

Blueberries are said to be quite susceptible to moisture loss. Fresh commodities constantly lose water to the surrounding environment by transpiration (Wills *et al.*, 1998). The driving force behind this process is the vapour pressure deficit or VPD. The VPD regulates the movement of water out of the fruit by means of a water vapour gradient (Wills *et al.*, 1998, Kader, 1985). The gradient exists between the fruit and the atmosphere and facilitates water migration from the fruit's saturated intercellular spaces, to the less saturated atmosphere. Factors affecting the transpiration rate include relative humidity, air velocity and temperature as well as barrier properties of the fruit surface, the ratio of fruit surface area to volume and fruit colour (Paull, 1999). The impact of these environmental and biological factors is accentuated after harvest when moisture lost from the berry can no longer be replaced by the flow of sap from the plant. This leads not only to quantitative losses (loss of salable weight), but also losses of aesthetic, textural and nutritional quality (Kader, 1985).

NeSmith's (2004) research emphasizes the importance of identifying cultivar differences and suggests using this information to maximize fruit quality and postharvest shelf life. General standards for rabbiteye blueberry respiration rates, ethylene production and moisture loss are accessible but little or no cultivar specific details are available. The objective of these experiments was to assess respiration rates, ethylene production and moisture loss of different rabbiteye blueberry cultivars.

Materials and methods

Rabbiteye blueberries from By-den-Weg farm, Stellenbosch, cultivars 'Brightwell' and 'Climax' were picked on the 19th of December, and Premier on the 26th of December, 2001, between 14h00 and 16h00, at a temperature of 25 ºC and 50 % relative humidity. Berries were used to determine respiration rates and ethylene production. Rabbiteye blueberries, cultivars, 'Bonita', 'Brightwell', 'Centurion', 'Climax' and 'Premier' originating from Stellenbosch and 'Tifblue', from Gelukstroom Farm, Vyeboom were

used to determine the moisture loss proportionality constant. Cultivars and picking times differed across the two seasons, as is summarized in Table 1.

Table 1: Experimental set up

	Season 1		Season 2	
Cultivar	**picking date**	**time**	**picking date**	**time**
'Bonita'	18/12/2001	15h00-16h00	21/12/2002	08h50-09h10
'Premier'	18/12/2001	15h00-16h00	21/12/2002 and 08/01/03	08h50-09h10
'Brightwell'	18/12/2001	17h00-17h30	21/12/2002 and 08/01/03	11h00-11h30
'Centurion'	08/01/2002	11h00	26/12/2002 and 08/01/03	11h00-11h30
'Climax'			26/12/2002	09h00-09h10
'Tifblue'			15/01/2003	10h00

For each cultivar, four punnets (of 150 g each) of blueberries were picked at the 100 % blue stage, placed into 5L and connected to a flowboard on air. Flow rates of approximately 100 $mL.min^{-1}$ were regulated by means of capillaries.

Respiration and ethylene production**:** Berries were kept at 15 ºC and 95 % relative humidity and air (maintained by means of a humidifying bottle) until decay developed.

Respiration: Carbon dioxide levels were measured with the use of an infra-red gas analyzer (IRGA) (Qubit Systems Inc Model S-151, Kingston, Ontario), which was connected to the out flow of each of the 5L buckets. Readings were taken daily throughout the storage period.

Ethylene production: Gas samples were taken from the out flow of each bucket on a daily basis, and analysed for ethylene by gas chromatograph (Varian Series 3000, Varian 4290 integrator, Varian Instrument Group, Palo Alto, California). After three days, with negligible ethylene readings, 5L buckets were sealed for four hours before the gas sample was drawn for analysis.

Moisture loss: Berries were subjected to five days at 15 ºC and a relative humidity of 50 %, which was achieved by means of a glycerol-water solution, described by Forney and Brandl (1992). Berry punnets were weighed on day zero and day five and the difference was used to determine J, the rate of moisture loss. The vapour pressure deficit, VPD, was determined (Vp(fruit) – Vp(atm)) and the proportionality constant (k) for each cultivar was calculated from the equation $J = k \times VPD$. This equation is a simplification of Fick's law of diffusion described in Maguire *et al.* (2001).

Statistical Analysis: A completely randomised experimental design with four replicates of 150g of blueberries per replicate was used. Proportionality constant data were pooled for each cultivar and standard deviations determined with SAS (Enterprise Guide VI, version 1.3).

Results

Respiration: Differences among cultivars were evident (Fig. 1). The respiration rate appeared to decrease over the evaluation period of 14 days at 15 ºC. 'Climax' had the highest respiration rate, ranging between 30-40 $ml.kg^{-1}.h^{-1}$, followed by 'Brightwell', ranging between 25-35 $ml.kg^{-1}.h^{-1}$ and 'Premier' had the lowest respiration rate, ranging between 15-29 $ml.kg^{-1}.h^{-1}$.

Ethylene production: Blueberry ethylene production at 15 ºC was exceptionally low and differences between cultivars were clear (Fig. 2 A and B). Ethylene levels were negligible using the flow-through system, therefore buckets were sealed for 4 hours prior to sampling. Using this static system, 'Premier' ethylene levels were still too low to be detected by gas chromatography. 'Climax' blueberries produced 4-6.5 $\mu L.kg^{-1}.h^{-1}$ of ethylene compared to 0.012-0.047 $\mu L.kg^{-1}.h^{-1}$ produced by 'Brightwell' berries.

Moisture loss: Considerable differences were found between cultivars relative to moisture loss over five days at 15 ºC and 50 % relative humidity (Table 2). Moisture loss

was below 1 % per day for all cultivars and was greatest for ‘Climax’. The cultivar ranking for the proportionality constant k from highest to lowest was ‘Climax’, ‘Bonita’, ‘Brightwell’, ‘Premier’, ‘Tifblue’ and lastly ‘Centurion’. A lower k value is associated with lower moisture loss from the berry.

Discussion

Considerable differences existed among cultivars for respiration rate, ethylene production and moisture loss. For ‘Climax’, ‘Brightwell’ and ‘Premier’, respiration, ethylene production and moisture loss appeared to correspond relative to ranking orders (i.e. highest levels were found for ‘Climax’ and lowest levels for ‘Premier’). This may give an indication of the cultivar’s individual storability. It may therefore be expected that ‘Climax’ is more perishable than ‘Brightwell’ or ‘Premier’.

Interestingly, according to Clayton-Greene (1993) postharvest decay was less common among ‘Climax’, ‘Bonita’ and ‘Bluegem’ compared to ‘Tifblue’, ‘Becky blue’ and ‘Premier’. However Smith *et al*. (1996) described ‘Premier’ as more susceptible to decay than ‘Tifblue’. Decay susceptibility may therefore not necessarily be related to these parameters of berry perishability and many other factors are known to influence disease development. In our research on delayed cooling ‘Centurion’ appeared to be more disease resistant than ‘Premier’ and ‘Tifblue’. ‘Centurion’ had a lower proportionality constant than ‘Premier’ and ‘Tifblue’. NeSmith *et al*. (2004) investigated the effect of handling temperatures and storage on six rabbiteye blueberry cultivars where firmness deterioration depended on cultivar differences. Cultivars with a higher initial firmness, for example ‘Brightwell’, appeared to have a lower rate of firmness loss and berries were less affected by longer pre-cooling delays. Furthermore, cultivars responded differently to increasing storage temperatures. ‘Premier’ had a lower initial firmness and softened rapidly at all temperatures. Interestingly, in our experiment, ‘Brightwell’ had a higher respiration rate, ethylene production and proportionality constant when compared to ‘Premier’. Magee (*unpublished data)* cited in Smith *et al*. (1996), states that ‘Premier’ blueberries are considered to be softer at full ripeness than ‘Tifblue’. In our research,

‘Tifblue’ had a lower proportionality constant than ‘Premier’. The relationship between initial firmness and moisture loss is, therefore, uncertain.

Conclusion

Respiration rate, ethylene production and moisture loss differ considerably among rabbiteye blueberry cultivars. Knowledge of these differences is essential to optimize blueberry postharvest storage life and can be used to determine storage procedures. For example, berries with a higher proportionality constant, respiration rate and ethylene production rate may be less suited to long term storage or export by sea. Emond *et al*., (1993) state that knowledge of the exact rate of consumption of O_2 and production of CO_2 by the product is rudimentary for developing modified atmosphere packaging (MAP) systems. The same can be said for controlled atmosphere storage. Respiration rates, ethylene production and moisture loss are affected by a wide range of factors, such as stage of development, growing area, physical stress, pathogen attack and the storage temperature (Kader, 1987, cited in Emond *et al*., 1993). However, by optimizing blueberry handling and storage and in particular by maintaining the cold chain, product deterioration governed by respiration rate, ethylene production and moisture loss can be reduced. Further research is required to ascertain cultivar specific norms relating to these quality determining factors.

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CHAPTER 5: Anthocyanin levels in blueberry peel and flesh as affected by *Botrytis cinerea* inoculation

Abstract

A flesh colour change from white to dark red was noted in infected blueberries. The pathogen was identified and 'Tifblue' blueberries were artificially inoculated with *Botrytis cinerea* to investigate whether this response could be reproduced. Flesh colour changes were evaluated and anthocyanin pigment levels (determined by spectrophotometry and HPLC) of the peel and flesh were compared. The infected blueberry flesh underwent a colour change from white to red. As the pigment content of the peel decreased, that of the flesh increased. Relative to the control, anthocyanin content of infected flesh doubled, whilst that of infected peel was less than 50 % of the control. Infection involves cell disruption, a characteristic associated in other reports with pigment leakage in blueberries. Therefore, a plausible explanation for the flesh colour change could be *Botrytis* induced migration of anthocyanin from the peel to the flesh.

Keywords: Anthocyanin, *Botrytis*, blueberry flesh colour

Introduction

Nutritionally, blueberries rank as one of the richest sources of antioxidants, owing to their high anthocyanin, flavonoid and phenolic content (Connor *et al*., 2002 (a) and (b); Skrede *et al*., 2000; Prior *et al*., 1998; Wang *et al*., 1996; Wang *et al*., 1997). Anthocyanins are confined principally to the blueberry peel in the epidermal and subepidermal cell layers (Connor *et al*., 2002 (b); Kalt *et al*., 2003; Sapers and Phillips, 1985).

Anthocyanin pigments belong to a group of secondary metabolites known as flavonoids. The accumulation of red or purple flavonoids has been described by Winkel-Shirley (2002), as a hallmark of plant stress. In response to infectious agents, plants undergo a shift in the metabolic pattern which includes the synthesis and accumulation of an array of secondary substances such as phenolics and flavonoids (Goodman *et al*., 1986).

Sorghum resists pathogen infection by the synthesis of the phytoalexin, 3-deoxyanthocyanidin (Snyder and Nicholson, 1990). Kong *et al*. (2003) also mention that besides being attractants, anthocyanins and 3-deoxyanthocyanidins can act as phytoalexins and antibacterial agents. In a study on the effect of storage on anthocyanin content of highbush blueberries, Kalt *et al*. (2003) acknowledge the possibility of postharvest anthocyanin synthesis, attributing increased anthocyanin concentrations to the conversion of non anthocyanin phenolic precursors into anthocyanin.

Anthocyanins have been described as colour-indicating dyes (Wrolstad, 1976, cited in Sanford *et al*., 1991). Profound changes in anthocyanin expression and stability in strawberries and litchis have been ascribed to changes in pH (Holcroft and Kader, 1999; Leung *et al*., 2003). The increased pH of the litchi pericarp is responsible for the colour change from red to dull brown as the pigment transforms into a colourless carbinol base (Zauberman *et al*., 1991). Colour loss of the internal tissue of strawberries was attributed to a controlled atmosphere (CA) related pH increase and reduced titratible acidity (Holcroft and Kader, 1999). Anthocyanins are more stable at a lower pH (Holcroft and Kader, 1999). In hydrangeas, the same anthocyanin is responsible for both blue and red petals and colour expression is dependent on the vacuolar pH (Yoshida *et al*., 2003). Sanford *et al*., (1991), observed a shift in blueberry peel colour from blue to blue-red in response to increasing storage temperature and related it to cell breakdown and concurring changes in titratable acids and pH.

Anthocyanin leakage is described as pigment diffusion from the epidermal cells within the peel to the berry interior (Sanford *et al*., 1991; Sapers and Phillips, 1985; Sapers *et al*., 1985). Leakage has been associated with berry softening (Sanford *et al*., 1991), peel ruptures or splits (Sapers and Phillips, 1985) as well as tissue damage during freezing resulting in collapsing cells during thawing (Sapers *et al*., 1985). Seeing that *Botrytis* infection involves the penetration of host cell membranes by enzymatic dissolution, membrane permeability may be altered and may facilitate the movement of pigments into the mesocarp (Goodman *et al*., 1986).

The flesh of infected blueberries has been noted to change colour, from white to dark red. Discolouration seems to start just below the peel and then spread to the centre of the fruit. The intensity of the flesh colour also increases to a deep blue-black as disease development progresses. The origin of this pigmentation is unknown and the question arises whether it is synthesized in the flesh in reaction to a stress situation, or whether infection causes pigment migration from the peel to the flesh. The aim of this experiment was to observe berry colour and compare changes in pigment level between the peel and flesh as brought about by *Botrytis* inoculation.

Materials and Methods

Plant material

Tifblue blueberries from Highfields, George, were obtained from an export pallet held at 0 ºC for ten weeks. As the plant material was old and it was uncertain whether berries with no visible symptoms were infected, an initial sample (I) of putatively healthy berries was taken. Berries were halved, those showing discolouration were put aside and twenty berries with white flesh were chosen and peeled. Peel and flesh were dipped (separately) in liquid nitrogen whereafter samples were ground in liquid nitrogen with a mortar and pestle, and stored at -80 ºC.

Pathogen identification

Berries used for pathogen identification were dipped into 70 % ethanol for 3 seconds whereafter 2 x 2 mm samples of blueberry peel were excised from the berries. Peel samples were taken from berries with discoloured (red) and healthy (white) flesh. The samples were isolated (eight samples to a petri dish) on potato-dextrose agar (PDA). Ten petri dishes for both the clean and discoloured samples were put into a plastic bag and left at 20 ºC for three days to allow fungal development.

Inoculation

Treatments included duplicate inoculations with *Botrytis cinerea* (B1 and B2) and a noninoculated control (C). *Botrytis cinerea* inoculum was provided by the Department of

Plant Pathology, University of Stellenbosch. Inoculum was obtained from a naturally infected grape berry and was prepared according to the method described by Coertze *et al*. (2001). Treatments B1 and B2 were dealt with separately as two inoculum subcultures originating from the same source were used. Four replicates (punnets containing 20 blueberries each) per treatment were sterilized (dipped into 70 % ethanol for 3 seconds) prior to inoculation. Replicates of B1 were placed at the bottom of an inoculation tower (Plexiglass, 3 by 1 by 1 m = height by depth by width) (Coertze *et al*., 2001) and showered with dry conidia of *Botrytis cinerea*. After allowing 20 minutes for the conidia to settle the replicates were placed in a moisture chamber (described by Coertze *et al*., 2001) at 20 ºC and ≥ 93 % relative humidity. An identical duplicate treatment (B2) was done on another set of four replicates and a clean petri dish containing PDA was included to verify spore viability.

Colour and pigment assessment of blueberry flesh and peel

Fruit were examined after seven days of incubation and the internal colour of each replicate (20 berries) was assessed subjectively. The blueberries were halved and categorized as discoloured (flesh completely red), partially discoloured (red flesh only visible just beneath the peel) and white (no visible discolouration). This was expressed as a percentage of the number of berries per category in each replicate. Flesh colour readings for chroma, lightness and hue angle of five berries per replicate were also measured with a colorimeter (NR – 3000, Nippon Denshoku, Tokyo, Japan). Blueberries were then peeled, peel and flesh were dipped (separately) in liquid nitrogen and ground under liquid nitrogen followed by storage at -80 ºC until anthocyanin extraction was done.

Since anthocyanin standards are expensive, and this is a preliminary study, it was decided to compare the different treatments and not to determine the precise concentration of the pigments. The anthocyanin content of the peel and flesh was determined using High Performance Liquid Chromatography (HPLC) and spectrophotometry. Preparation for spectrophotometry and HPLC was as follows.

Blueberry peel anthocyanin was extracted by weighing 0.4 g ± 0.005 g of the frozen material in small plastic centrifuge tubes and adding 2 mL of 5 % formic acid. Blueberry flesh is much paler in colour (light pink) than the peel (dark blue), therefore anthocyanin concentration was expected to be much lower and more material was required. For blueberry flesh 1 g ± 0.005 g of the material was extracted with 2 mL of the solvent. The solutions were homogenized by quick vortex pulsation and then refrigerated overnight for anthocyanin extraction. The homogenate was centrifuged for 10 min at 2900 g_n. The clear supernatant was then filtered through 0.45 µm syringe filters into vials suitable for HPLC analysis.

Spectrophotometry: Spectrophotometry was used to determine the absorbance of blueberry peel and flesh extracts. A UV visible spectrum was taken between wavelengths of 400-700 nm. The maximum absorbance was found at 517 nm (± 2 nm). Extracts of blueberry peel and flesh were diluted (1:99 and 1:2, respectively) with 5 % formic acid. This gave absorbances between 0.2 and 1.0 at 517 nm. Absorbance was measured in plastic cuvettes with a 1cm path length, using a Cary 50 Conc UV-visible spectrophotometer and 5 % formic acid was used as the reference.

High Performance Liquid Chromatography (HPLC): Anthocyanins were assessed by reverse-phase high performance liquid chromatography (HP 1100; Agilent Technologies, Palo Alto, Calif.).A C_{18} column (150 mm x 4.6 mm with 5 µm; Spherisorb; Phase Separations, Deeside, UK), with a Zorbax SB-C18 guard column (Agilent Technologies, Palo Alto, Calif.) was used. The mobile phase consisted of 5 % formic acid in water (A) and Methanol (B) with the gradient programme, summarized as follows:.

minutes	% solvent A	% solvent B	Flow rate ($mL.min^{-1}$)
0	81	19	0.7
25	78	22	1.0
31	75	25	1.0
39	63	37	1.0
40	0	100	1.0
45	0	100	1.0

The injection volume of the blueberry extracts was 8 μL and the eluted anthocyanins were monitored at 520 nm.

Data obtained from spectrophotometry and HPLC analysis were used to draw comparisons between treatments (control, C vs. inoculated berries, B1 and B2). Conclusions were based on the assumption that absorbance increases as pigment concentration increases (Beers law: Absorbance and pigment concentration are proportional (Jaffé and Orchin, 1964)).

Statistical analysis

The experimental design was completely randomized and the data were analyzed with the general linear models (GLM) and correlation (CORR) procedures of SAS (Enterprise Guide VI, version 1.3). An arcsin square root transformation was performed on all percentage data prior to statistical analysis. Non-transformed data are presented.

Results

Pathogen Identification

All peel samples used for pathogen identification, originating from discoloured blueberries which were incubated on potato dextrose agar (PDA), showed *Botrytis* development. Peel samples from white fleshed fruit were primarily free of fungi. *Botrytis* was identified from its sporulation, namely conidiophores with conidia, therefore an association could exist between this pathogen and flesh discolouration.

Inoculation

The spore viability test indicated a germination percentage of 98 %. There was no record of spore viability for B1, therefore no deductions could be made relative to the discolouration and the differences existing between inoculations (B1 and B2).

Initial peel and flesh sample

Extracts from the initial sample consisting of white fleshed berries (I), were almost colourless in the case of the flesh sample and dark and turbid in the case of the peel sample (Fig. 1). The anthocyanin profiles obtained from the HPLC for the initial peel and flesh samples are presented in Fig.s 2 and 3. Although no standards were available for this experiment, comparable profiles were obtained by Rossi *et al.* (2003) who used similar reverse-phase HPLC separation techniques. This leads us to suspect that the 14 (peel) peaks found in this experiment represent those of Rossi *et al.*, 2003. Peak assignments, made on the basis of elution order are as follows. 1, Delphinidin-3-galactoside; 2, Delphinidin-3-glucoside; 3, Cyanidin-3-galactoside; 4, Delphinidin-3-arabinoside; 5, Cyanidin-3-glucoside; 6, Petunidin-3-galactoside; 7, Cyanidin-3-arabinoside; 8, Petunidin-3-glucoside; 9, Peonidin-3-galactoside; 10, Petunidin-3-arabinoside; 11, Peonidin-3-glucoside; 12, Malvidin-3-galactoside; 13, Malvidin-3-glucoside; 14, Malvidin-3-arabinoside.

The lightness and hue angle of the initial sample resembled the Control (Table 1). Initial anthocyanin levels (indicated as absorbance and cumulative area under the HPLC chromatograms (ACY)) for blueberry flesh, was dramatically lower than the Control and for blueberry peel, noticeably higher (Table 2). The differences observed between the Initial sample and Control are verified in Fig. 4, which indicates the percentage of berries falling in each flesh colour category. Flesh discolouration was visible in the control, while the initial sample consisted only of white fleshed fruit.

Blueberry flesh

Samples inoculated with *Botrytis* differed appreciably from the initial sample and control, and the difference between peel and flesh samples became indistinguishable. Both peel and flesh samples turned a murky red (Fig.1). Control and inoculated berry flesh differed significantly for lightness and hue angle (Table 1). Inoculated berries were slightly darker and redder as indicated by the respective lightness and hue angle values, which were approximately 10-15 units and 15–20 º lower, respectively than the control (Table

1). Similar trends were observed subjectively for flesh colour categories, expressed as percentage red and percentage white flesh. In comparison to the control, twice as many inoculated berries had red flesh, and the prevalence of white fleshed berries was 56 % (C), 14 % (B1) and 6 % (B2) (Table 1). No significant difference in anthocyanin content was observed between control and inoculated berries, despite considerable increases in anthocyanin levels (Table 2). The percentage of red and white fleshed berries correlated significantly and strongly with lightness, hue angle and flesh anthocyanin level (Table 3). Lightness correlated with flesh absorbance but not with flesh cumulative area under the HPLC chromatogram (Table 4).

Blueberry peel

Blueberry peel anthocyanin level decreased significantly in *Botrytis* infected blueberries (Table 2). Absorbance of the infected peel was 57 and 71 percent lower (B1 and B2, respectively) than the control. The cumulative area under the HPLC chromatogram was 62 and 80 percent lower (B1 and B2, respectively) than the control. Blueberry peel anthocyanin level correlated significantly and strongly with flesh lightness, hue angle, percentage red and white fleshed berries as well as the anthocyanin level of the flesh (Table 5 and 6).

Discussion

The visual resemblances (Fig. 1) between the initial sample (I), and Control (C), allude to similar pigment content. It was therefore assumed that the putatively uninfected Control (C) berries underwent negligible colour change subsequent to inoculation of the B1 and B2 treatments. Notwithstanding, anthocyanin content of the initial sample (I) and Control (C) differ greatly for both peel and flesh (Table 2). Considering the differences between the subjective discolouration ratings (Fig. 4) these deviations can be explained by possible natural infection of the control, or membrane leakage due to senescence (Sanford *et al*., 1991), leading to flesh discolouration.

Results from the duplicate inoculations denoted a change in flesh colour. Initially white berry flesh turned red and underwent an increase in anthocyanin level, indicating a higher pigment content resulting from *Botrytis* infection. The strong correlation between the subjective categories (% red and % white) relative to lightness, hue angle and flesh anthocyanin level (Table 3) give supporting evidence of the flesh colour change. The reduced peel anthocyanin level of infected berries is indicative of the loss of peel pigmentation. Strong, yet divergent correlations between the anthocyanin levels of the peel relative to percentage red fleshed fruit and flesh anthocyanin level (Table 5), reinforced the observation that peel pigment content decreases as flesh pigment content increases.

Samples consisting of a higher percentage of white fleshed berries had higher peel anthocyanin contents (HPLC). Infected, red fleshed, berries with corresponding lower peel anthocyanin levels therefore give supporting evidence that *Botrytis* infection could be associated with these pigment changes, and migration of anthocyanin pigment from the peel to the flesh seems plausible. However, this analytical approach does not negate the possibility of anthocyanin degradation in the peel concomitant with *de novo* anthocyanin synthesis in the flesh.

It is also interesting that the crude measure of total anthocyanin content of the peel and flesh, obtained by adding the peak areas for both, appears to indicate a very similar total content in the control and infected berries (Fig. 5). Considering that the method of extraction for the peel and flesh differed, it is important to note that the peel anthocyanin content is actually much higher than is apparent in Fig. 5.

In blueberries, *Botrytis* infection is characterized by fruit softening and is described as a watery decay followed by the development of grayish-white mycelium on the berry surface (Anon, 1995). The stem scar of the blueberry serves as a major avenue of entrance for diseases (Cappellini and Ceponis, 1977; Cline, 1997). Fungal penetration of *Botrytis* into the host involves the chemical degradation of cell walls and membranes (Goodman *et al*., 1986). Alterations in membrane structural and metabolic integrity can

lead to decompartmentalization and then to uncontrolled mixing of enzymes and substrates that are normally isolated (Goodman *et al*., 1986). Anthocyanin leakage has been associated with soft berries (Sanford *et al*., 1991), split berries, collapsed cells and breaks in the berry epiderm (Sapers and Phillips, 1985; Sapers *et al*., 1985). These symptoms correspond with those seen in infected berries and therefore support the *Botrytis* induced pigment migration theory where anthocyanin moves from the blueberry peel to the flesh. The exact implications of these disruptions on tissue pH and metabolic processes of blueberries are unknown. However, cell breakdown has been associated with a pH and increased titratable acidity related shift in lowbush blueberry peel colour, from blue to blue-red (Sanford *et al*., 1991).

Conclusion

Blueberry flesh and peel analyses confirm the observed visual colour changes associated with *Botrytis* infection. Inoculated berry flesh was darker and redder than the Control, consisted of a higher percentage of red coloured fruit and the flesh absorbance and concentration increased as blueberry peel anthocyanin level decreased. *Botrytis* related cell disruption and softening may facilitate the migration of pigments from the peel to the flesh. Alternatively, anthocyanin degradation in the peel, concomitant with *de novo* anthocyanin synthesis in the flesh may also be possible. Cell breakdown can be associated with pH changes, which may also contribute to the reduced anthocyanin content of the peel. Loss of blue colour from the peel can therefore be attributed to leakage, chemical disruption and pigment expression (Sanford *et al*., 1991) all of which may be affected by *Botrytis* infection. Sapers *et al*. (1985) observed pigment leakage with a microscope. Such observations may also be valuable as evidence of this process in response to infection. Considering that the blueberries used in this experiment were old and that discolouration may be related to aging processes, clearer contrasts between the control and inoculated berries may become visible with fresher berries. Little or nothing has been documented on blueberry flesh anthocyanins, de novo synthesis of anthocyanin or the effect of infection, pH changes and chemical disruption on the blueberry flesh leaving many possibilities for further research.

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CHAPTER 6: GENERAL CONCLUSION

Optimisation of blueberry postharvest handling and storage is essential for the maintenance of berry quality and the exploitation of berry storage potential. Considering the rapid expansion of the South African blueberry industry the logistic alternative of export by sea must be investigated. Seafreighting blueberries will only be viable if berry storage life can be extended to a minimum of six weeks plus a shelf life period equivalent to five days at 15 ºC. Consequently controlled atmosphere storage is regarded as a valuable technology and a means to this goal. Controlled atmosphere norms for South African blueberries must be established and should ensure exceptional fruit quality. Sensory analysis is a valuable tool and a method looked at more frequently to assess fruit quality. From our CA research, incidence of off flavour (evaluated by sensory analysis) was too low for statistical analysis and it was accepted that this was not a concern for 'Tifblue' and 'Premier' blueberries at the CA levels tested. A further challenge is that of meeting the rigorous market specification for decay (no more than 2 % is tolerated). Decay suppression must be achieved throughout the handling chain until the end of the shelf life period in UK supermarkets. Controlled atmosphere storage was insufficient relative to decay reduction. It can therefore be said that the success of advanced technologies such as CA still rely on the basics and methods to reduce decay pressure must be investigated. Temperature management, maintenance of the cold chain and certainty of initial product quality are essential. Factors such as harvest maturity, seasonal conditions, inoculum pressure and cultivar traits all influence blueberry quality and storability. Further research is required before CA norms can be established.

Handling and cooling regimes are of the utmost importance in ensuring quality maintenance. Blueberry cultivars react differently and experience differing rates of deterioration under various conditions. Considering South Africa's latitude and climatic condition and that temperature narrates the rate of respiration (effectively determining perishability), the importance of prompt cooling and maintenance of the cold chain must be emphasized. In response to pre-cooling delays of up to 6 hours at 20 ºC results for blueberry cultivars 'Centurion', 'Premier' and 'Tifblue' picked early in the morning,

were mostly inconclusive. However these conditions do not closely resemble the commercial situation. Field temperatures at harvest can easily be between 30 and 35 ºC. Delays in cooling and exposure to high temperatures should be minimized to reduce incidence of decay after storage and shelf life periods. Depending on the cultivar, unacceptable decay levels were reached after only three or five weeks of cold storage plus shelf life and there was a tendency for greater decay potential with increasing pre-cooling delays. Further research is required in assessing inoculum pressure and cultivar responses relative to pre-cooling delays.

Postharvest blueberry behaviour is directly influenced by cultivar characteristics. Respiration rate, ethylene production and moisture loss differs considerably among rabbiteye cultivars. Knowledge of these differences is essential to optimize blueberry postharvest storage life and can be used to determine storage procedures. For example, berries with a higher proportionality constant, respiration rate and ethylene production rate may be less suited to long term storage or export by sea. This emphasizes the importance of marketing cultivars separately and motivates careful cultivar allocation to different markets (local vs. export), storage regimes (short vs. long) and logistics (air vs. sea shipment).

The optimisation of blueberry postharvest handling and storage requires further research that is cultivar specific and targets relevant quality issues. Difficulty was experienced in quantifying blueberry firmness changes therefore development of reliable techniques will be useful. Reduction of decay pressure is also essential and research on inoculum levels throughout the handling chain starting in the field may be of use. Peel integrity relative to decay susceptibility may also be a factor therefore research on a cellular level may also be necessary. Organic alternatives such as the use of antibacterial pads may also be a viable consideration for decay suppression.

There is no compromise for temperature management and although technologies such as CA are appealing initial berry quality must be exceptional to meet storage expectations.

Once cultivar differences are established, handling and cooling is optimized and decay control is reached CA storage success can be assured and export by sea will be warranted.

APPENDIX A: Selected data of Chapter 2

Fig.1. 2002. ‘Tifblue’ firmness (N) for RA and CA atmospheres after 0 °C storage and shelf life (5 days at 15 °C).

Fig. 2. 2002. ‘Premier’ firmness (N) for RA and CA atmospheres after 0 °C storage and shelf life (5 days at 15 °C).

Fig. 3. 2003. ‘Premier’ firmness (N) for RA and CA atmospheres after 0 °C storage and shelf life (5 days at 15 °C).

APPENDIX B: Selected data of Chapter 3

Table 1 Interactions for 2003, ‘Centurion’, ‘Premier’ and ‘Tifblue’ firmness in Newton (N) for pre-cooling delays (0, 2, 4 and 6h) and storage duration at 0 °C and shelf life (seven days at 15 °C).