# Effects of dynamic controlled atmosphere and initial low oxygen stress on superficial scald of 'Granny Smith' apples and 'Packham's Triumph' pears

By

Tlou Melrose Ramokonyane

*Masters of Science in Agriculture in the Faculty of AgriSciences, Department of Horticultural Sciences, at Stellenbosch University*

Supervisor: Professor Umezuruike Linus Opara

Co-supervisors: Dr E.M. Crouch

Mr J.A. van der Merwe

Dr F.A. Vries

MARCH 2016

## DECLARATION

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Tlou Melrose Ramokonyane March 2016

# SUMMARY

'Granny Smith' apples and 'Packham's Triumph' pears are the main export pome fruit in South Africa. The fruit are stored for extended period to take advantage of main export markets such as the European Union (EU), and are susceptible to the storage physiological disorder superficial scald. Superficial scald is the main storage disorder in pome fruit, appearing as light brown to black blemishes on fruit peel, rendering affected fruit unmarketable.

Current commercial practice to control superficial scald has been to drench susceptible fruit in diphenylamine (DPA), a synthetic antioxidant. The EU recently reduced the maximum allowable residue limit (MRL) of DPA from 5 ppm for apples and 10 ppm for pears to only 0.1 ppm as effective from April 2014. This has made it necessary to urgently investigate alternative non-chemical storage protocols to control the incidence of the disorder. DPA was not used in this study to avoid cross contamination in the treatment rooms. The aim of this study was to investigate the efficacy of controlled atmosphere and low oxygen stress technologies in inhibiting superficial scald in 'Packham's Triumph' pears and 'Granny Smith' apples.

Dynamic controlled atmosphere (DCA) protocol was effective as a scald control treatment on 'Packham's Triumph' pears stored up to 7 months at -0.5 °C. Controlled atmosphere (CA) preceded by initial low oxygen stress (ILOS) was effective as a scald treatment on 'Packham's Triumph' pears when fruit were cold stored for up to 5 months. Similarly, DCA storage was effective as a scald control treatment for up to 7 months at 0 °C on 'Granny Smith' apples harvested at pre-optimal and optimal maturity stages. Storing fruit under CA preceded by ILOS was not effective in controlling scald on 'Granny Smith' apples.

Metabolomic studies showed that storing fruit under DCA and/or ILOS suppressed superficial scald development on 'Packham's Triumph' pears by probably inhibiting the auto-oxidation of α-farnesene to its by-product, 6-methyl-5-hepten-2-one (MHO), in the fruit peel. There was a strong negative correlation between the α-farnesene concentration in the fruit peel and the superficial scald severity index of 'Packham's Triumph' pears ($R^2$ = -0.90). In 'Granny Smith' apples harvested at optimal maturity there was a strong negative correlation between the α-farnesene concentration and superficial scald severity index ($R^2$ = -0.85). The correlations between MHO and the superficial scald severity index were 0.90, 0.85 and 0.57

for ‘Packham’s Triumph’ pears, for pre-optimally and optimally harvested ‘Granny Smith’ apples, respectively.

Storing fruit in ILOS for 10 days followed by CA effectively controlled superficial scald on ‘Packham’s Triumph’ pears for up to 5 months, but for only 3 months on ‘Granny Smith’ apples. Storing fruit in ILOS for 10 days followed by long term CA storage inhibited superficial scald by suppressing the auto-oxidation of α-farnesene to MHO. In summary, this study showed that CA storage technology (DCA and ILOS followed by CA) are alternative options to control ripening, delay senescence and maintain quality of pome fruit for much longer than regular air (RA) in addition to controlling superficial scald and shrivelling in ‘Packham’s Triumph’ pears. Given the possibility of shrivelling due to weight loss and decay incidence on ‘Packham’s Triumph’ pears during long term storage, correct fruit handling and sanitation practices should be adhered to at optimal storage temperatures and relative humidity. Results obtained in this study suggest that DCA is the non-chemical alternative treatment for control of superficial scald for long term storage of ‘Granny Smith’ apples and ‘Packham’s Triumph’ pears.

# OPSOMMING


‘Granny Smith’ appels en ‘Packham’s Triumph’ pere is die hoof uitvoer kernvrugte in Suid-Afrika. Die vrugte word vir lang periodes opgeberg om die voordeel te kry van die belangrikste uitvoermarkte soos die Europese Unie (EU). ‘Granny Smith’ appels en ‘Packham’s Triumph’ pere is gevoelig vir die fisiologiese defek, oppervlakkige brandvlek. Oppervlakkige brandvlek is die vernaamste opbergingsdefek in kernvrugte. Dit verskyn in die vorm van ligbruin tot swart letsels op die skil van die vrug en maak die vrugte onbemarkbaar vir die varsproduktemark.

Standaard kommersiële praktyke om oppervlakkige brandvlek te beheer was om sensitiewe vrugte met difenielamien (DFA), 'n sintetiese antioksidant, te behandel. Die EU het die maksimum toelaatbare residu limiet van DFA vanaf 5 dpm vir appels en 10 dpm vir pere na 0.1 dpm verlaag, geldig vanaf April 2014. Dit is dus nodig om dringend alternatiewe nie-chemiese opbergingsprotokolle te ondersoek om die voorkoms van die defek te beheer. DFA is nie in hierdie studie gebruik nie, om besmetting van die spesifieke behandelings kamers te voorkom. Die doel van hierdie studie was dus om die doeltreffendheid van beheerde atmosfeer en lae suurstof stres tegnologie te ondersoek wat ‘n inhiberende effek op oppervlakkige brandvlek van 'Packham's Triumph' pere en 'Granny Smith' appels kan hê.

Resultate het getoon dat die dinamies beheerde atmosfeer (DBA) protokol effektief was om oppervlakkige brandvlek op 'Packham's Triumph' pere, gestoor vir solank as 7 maande by -0.5 °C te beheer. Die opberging van vrugte onder beheerde atmosfeer (BA), voorafgegaan deur aanvanklike lae suurstof stres (ALSS), was effektief as 'n oppervlakkige brandvlek behandeling op 'Packham's Triumph' pere tot en met 5 maande in koelopberging. Soortgelyk was die DBA opberging protokolle effektief as oppervlakkige brandvlek beheer behandeling vir pre-optimum en optimum geoeste 'Granny Smith' appels gestoor tot en met 7 maande by 0 °C. Die opberging van vrugte onder beheerde atmosfeer, voorafgegaan deur aanvanklike lae suurstof, was nie effektief om oppervlakkige brandvlek op 'Granny Smith' appels te beheer nie.

Metabolomiese studies het getoon dat die opberging van vrugte onder DBA en / of ALSS, oppervlakkige brandvlek op 'Packham's Triumph' pere kon onderdruk deur waarskynlik die outo-oksidasie van α-farneseen na sy byproduk 6-metiel-5-hepten-2-een (MHE) in die skil van 'Packham's Triumph' pere te beperk. Daar was 'n sterk negatiewe

korrelasie tussen α-farneseen vlakke in die skil en die graad van oppervlakkige brandvlek indeks ($R^2$ = -0.90) in 'Packham's Triumph' pere. Daar was 'n sterk negatiewe korrelasie tussen α-farneseen vlakke en die graad van oppervlakkige brandvlek indeks ($R^2$ = -0.85) in optimum geoeste ‘Granny Smith’ appels. Die korrelasies tussen MHE en die graad van oppervlakkige brandvlek indeks was $R^2$ = 0.90, $R^2$ = 0.85 en $R^2$ = 0.57 vir 'Packham's Triumph' pere, optimum en pre-optimum geoeste ‘Granny Smith' appels, onderskeidelik.

Die resultate in hierdie studie het getoon dat DBA effektief oppervlakkige brandvlek vir tot 7 maande op ‘Packham's Triumph' pere en ‘Granny Smith’ appels beheer, deur die outo-oksidasie van α-farneseen na MHE te onderdruk. BA voorafgegaan deur ALSS vir 10 dae het oppervlakkige brandvlek effektief beheer op ‘Packham's Triumph’ pere vir tot 5 maande, maar dit onderdruk oppervlakkige brandvlek vir slegs 3 maande op 'Granny Smith' appels. BA voorafgegaan deur ALLS vir 10 dae verhoed die ontwikkeling van oppervlakkige brandvlek deur die outo-oksidasie van α-farnesene na MHE te onderdruk. Opsommend het die studie getoon dat BA stoor protokolle, DBA en ALLS alternatiewe opsies is om rypwording te beheer, veroudering te vertraag en kwaliteit van kernvrugte te behou vir baie langer as onder gewone atmosfeer, bykomend beheer dit ook oppervlakkige brandvlek, bederf en verrimpeling op 'Packham's Triumph' pere. Gegewe die moontlikheid van verrimpeling weens gewigsverlies en die voorkoms van bederf op 'Packham's Triumph' pere gedurende lang termyn opberging, moet korrekte vrug hantering en sanitasie praktyke nagekom word teen optimum temperature en relatiewe humiditeit. Resultate van hierdie studie blyk dat DBA is tans die enigste nie-chemiese alternatiewe behandeling vir die beheer van oppervlakkige brandvlek vir langtermyn opberging van 'Granny Smith' appels en ‘Packham’s Triumph’ pere.

## ACKNOWLEDGEMENTS

This work was based upon research supported by the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation.

I would like to express my sincerest gratitude to my supervisor Professor Umezuruike Linus Opara for his mentorship, patience, critique, and support.

I also thank my co-supervisors, Drs. Elke Monika Crouch and Filicity Ann Vries for their support and patience.

My appreciation also goes to Mr. Jakobus Arnoldus van der Merwe who was my co-supervisor and a team leader for the technical staff (Mr. Howard Ruiters, Mrs. Viole Combrinck and Ms. Vanessa Fortuin) in the postharvest laboratory of ARC Infruitec-Nietvoorbij for their assistance and dedication to work.

I also want to express my great appreciation to Mr. Lucky Mokwena, for his patience and readiness to share his knowledge on GC/MS on the volatiles.

My appreciation goes to Professor Martin Kidd (Stellenbosch University), and Marieta van der Rijst and Nombasa Sheroline Ntutshelo (Biometricians at the Agricultural Research Council in Stellenbosch ARC Infruitec/Nietvoorbij) for their assistance with data analysis.

I am greatly grateful to ARC Infruitec-Nietvoorbij, THRIP and Postharvest Innovation Programme (PHI) for funding my studies and providing research funding support.

I am grateful to Limpopo Department of Agriculture and Rural Development for allowing me the opportunity and the support to further my studies.

I also want to appreciate the contribution of fellow students from the SARChI Postharvest Technology Laboratory and postdocs Drs. Olaniyi Amos Fawole and Oluafemi Caleb for their assistance during the writing up of this thesis.

My thanks goes to Mr. Maxwell Canaan Mapako of the CSIR (Senior Energy Specialist) for his assistance with editing my work and for his friendship, support and encouragement.

My great appreciation also goes to my friends Mr. Luke Mugode, Mr. Mankonane Mordicae Thipe, Ms. Kedibone Blantina Chueu and Ms. Tubake Refiloe Thobejane for their patience and continued support and encouragement.

My deep gratitude goes to my mother Mrs. Mosima Maria Ramokonyane and my family (Maphuti Juliet Ramokonyane, Matlou Shielda Ramokonyane, Mrs. Phuti Phillistus Kgasago and my brother Thabang Noko Ramokonyane) for looking after my children and for her patience and support.

Many thanks to my children Kamogelo Lesiba Ramokonyane and Kopano Ramokonyane for their love, support and patience.

Overly, I thank GOD, the Almighty, for providing the gift of life, good health and the provision of loving, caring and supporting people.

# NOTE

This thesis represents a compilation of manuscripts where each chapter is an individual entity and repetition between chapters has been unavoidable.

## TABLE OF CONTENTS

# GENERAL INTRODUCTION

Superficial scald is a physiological disorder of apples and pears, causing browning of the fruit peel during or after low-temperature storage in air or after controlled atmosphere (CA) storage, reducing the appearance quality and fresh-market value of fruit. Apples and pears are major South African horticultural crops and are consumed fresh, minimally processed, sliced, and after processing as canned, bakery and juice items. The domestic and export market for these fresh fruit are significant. For fresh fruit export, the crops need to be stored for up to 9 months or more at low temperature to allow orderly marketing. The superficial scald disorder is induced by storage at low temperature (0 °C and below) which is required to delay ripening. 'Granny Smith' apples and 'Packham`s Triumph' pears are the most susceptible pome fruit cultivars to superficial scald (Ingle and D`Souza, 1989).

In the global fresh fruit industry superficial scald has been controlled by a postharvest drench treatment with diphenylamine (DPA, 1000-2000 $mg.kg^{-1}$ water) (No Scald DPA EC-283 (Decco, Cerexagri, Inc., Monrovia, CA) (Hall et al., 1961; Jung and Watkins, 2008; Zanella and Strüz, 2013) and it has been used on susceptible apples and pears for more than five decades (Hardenburg and Anderson, 1962). The use of DPA on apples and pears has been under review in the European Union (EU) for some time due to its toxicity to the environment and potentially to human health and other organisms such as aquatic life (Dzyrga, 2003). The reduction and ban of DPA in April 2014 for controlling apple and pear scald poses considerable risk to South African pome fruit industry and has prompted renewed interest among researchers in developing alternative control strategies.

Several technologies have been introduced to the market in recent years in order to aid growers, exporters and researchers in the control of superficial scald in apples and pears including 1-Methylcyclopropene (1-MCP) (SmartFresh$^{TM}$, Agro Fresh, Pennsylvania, USA), and lower oxygen (controlled atmosphere and initial low oxygen stress) storage technologies. 1-Methylcyclopropene - an ethylene inhibitor, CA, dynamic controlled atmosphere (DCA) and initial low oxygen stress (ILOS) are all technologies being applied and have the potential to inhibit superficial scald and increase the storage life of pome fruits (Robatscher et al., 2012). There is pressure from consumers and the general public on scientists to find non-chemical storage technologies such as CA and DCA as alternatives for the control of superficial scald on apples and pears.

DCA technology has shown positive results in quality maintenance and inhibition of superficial scald on several cultivars of apples grown in South Tyrol, Italy (Zanella, 2003; Zanella et al., 2008; Zanella and Strüz, 2013). It is, however, imperative that optimum storage conditions for locally grown fruit is determined through effective research (Steynor, 1996). Preliminary research trials conducted at the Agricultural Research Council (ARC) in South Africa on the use of low oxygen storage technologies such as CA, DCA and ILOS to control scald on apples and pears has shown positive results (Van der Merwe et al., 2003).

Accumulation of the volatile sesquiterpene, α-farnesene and its subsequent oxidation to conjugated trienes and 6-methyl-5-hepten-2-one has been associated with scald development (Meigh, 1969; Anet, 1972a; Anet, 1972b; Anet and Coggiola, 1974). Anet (1972b) reported that immature apples generally do not produce more α-farnesene than more mature apples, but develop more scald. It was thus suggested that the inability of immature fruit to prevent the oxidation of α-farnesene, possibly due to a less efficient antioxidant system, may be responsible for their higher susceptibility. Due to the recent restriction on the use of DPA on pome fruit to control superficial scald it became imperative to test alternative technologies. This study aims to investigate alternative technologies to control superficial scald on stored pome fruit to the use of DPA and to understand how these technologies control this disorder in pome fruit.

The specific objectives were to:

1. Determine the effects of controlled atmosphere technologies and initial low oxygen technologies to reduce incidence of physiological disorders and maintain quality of ‘Packham’s Triumph’ pear and ‘Granny Smith’ apples (paper 1, 2 and 4).
2. Investigate the mode of action of controlled atmosphere technologies and initial low oxygen stress technologies by investigating the development of implicated volatiles namely α-farnesene and MHO during long term storage of ‘Packham’s Triumph’ pears and ‘Granny Smith’ apples (paper 3 and 5).

## References

Anet, E.F.J.L. (1972a). Superficial scald, a functional disorder of stored apples. VIII. Volatile products from the autoxidation of α-farnesene. *Journal of the Science of Food and Agriculture*, 23, 605-608.

Anet, E.F.J.L. (1972b). Superficial scald, a functional disorder of stored apples. IX. Effect of maturity and ventilation. *Journal of the Science of Food and Agriculture*, 23, 763-769.

Anet, E.F.J.L. & Coggiola, I.M. (1974). Superficial scald, a functional disorder of stored apples X. Control of α-Farnesene Autoxidation. *Journal of the Science of Food and Agriculture*, 25, 293-298.

Dzyrga, O. (2003). Diphenylamine and derivatives in the environment. *Chemosphere,* 53, 809-818.

Hall, E.G., Scott, K.J. & Coote, G.G. (1961). Control of superficial scald of 'Granny Smith' apples with diphenylamine. *Australian Journal of Agricultural Research*, 12 (5), 835-853.

Hardenburg, R.E. & Anderson, R. (1962). Chemical control of scald on apples grown in the eastern United States. *Agricultural Marketing Services Marketing Quality Research Division US Department of Agriculture*, 1-47.

Ingle, M. & D' Souza, M. (1989). Physiology and control of superficial scald of apples: A review. *Journal of Food Science,* 24, 28-31.

Jung, S. & Watkins, C.B. (2008). Superficial scald control after delayed treatment of apple fruit with diphenylamine (DPA) and 1-methylcyclopropene (1-MCP). *Postharvest Biology and Technology*, 50, 45-52.

Meigh, D.F. (1969). Production of farnesene and incidence of scald in stored apples. *Quality Plant Material Vegetables*, 19, 243-254.

Robatscher, P., Eisenstecken, D., Sacco, F., Pohl, H., Berger, J., Zanella, A. & Oberhuber, M. (2012). Diphenylamine residues in apples caused by contamination in fruit storage facilities. *Journal of Agricultural and Food Chemistry*, 60 (9), 2205-2211.

Steynor, P. (1996). An introduction to cold storage design and construction. In Combrink J.C. (Ed.), *Integrated management of postharvest quality*, (p. 74). ARC Infruitec-Nietvoorbij: Paarl Printing.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae,* 600, 261-265.

Zanella, A. (2003). Control of apple superficial scald and ripening- a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra-low oxygen storage. *Postharvest Biology and Technology*, 27, 69-78.

Zanella, A., Cazzanelli, P. & Rossi, O. (2008). Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll response to firmness retention in apple. *Acta Horticulturae*, 796, 77-80.

Zanella, A. & Stürz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10$^{th}$ of EU's apples producing area. *Acta Horticulturae*, 1012, 419-426.

# LITERATURE REVIEW: THE POTENTIAL USE OF LOW OXYGEN STORAGE TECHNOLOGIES IN THE MANAGEMENT OF SUPERFICIAL SCALD ON APPLES AND PEARS

## 1. Introduction

### South African production of pome fruits

In 2014, South African agricultural production was valued at R215 135 million, which is 13.2% higher than the previous year (DAFF, 2014c). The contribution of agriculture, forestry and fisheries to the value added products for 2014 is estimated at R84 662 million representing 2.5% of the total value added product of the economy (DAFF, 2014c). Income from horticultural products increased from R50 822 million to R57 926 million, amounting to a 14% increase over the previous year (DAFF, 2014c). The income generated from deciduous fruit (including apples and pears) increased by 11.7%, from 2013 with an amount of R14 689 million (DAFF, 2014c). Apples and pears are therefore very important products in the South African agricultural industry, making important contributions to the national economy.

### Export market and quantities of pome fruit produced in South Africa

Pome fruits are the fourth most exported agricultural products from South Africa with an estimated annual value of R5 677 million following citrus fruit (R11 582 million), wine (R8 105 million) and grapes (R 6 274 million), respectively (DAFF, 2014d). The South African deciduous fruit industry is an export orientated industry. Approximately 48% of the South African pome fruit is exported while a smallest percentage is consumed locally as illustrated in Figure 1 (NAMC, 2014), suggesting that more returns are generated from export compared to processing and local market consumption.

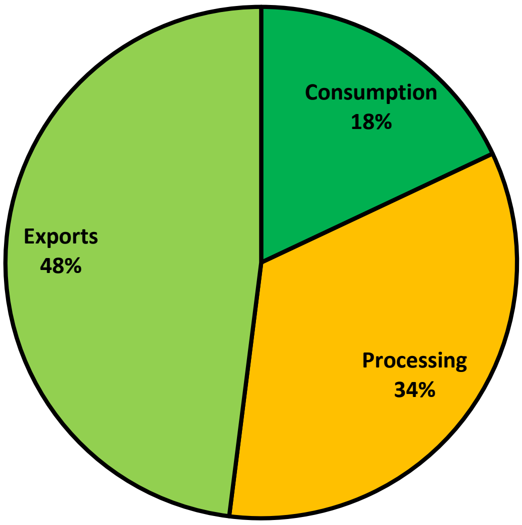


Figure 1. Distribution of South African pome fruit in the market: 2008-2012 (NAMC, 2014).

Pome fruit belong to the deciduous fruit group and comprise of apples and pears. South Africa accounted for 7.6% of world pome exports between 2008/2009 and 2012/2013. In 2013/2014, South Africa exported 203 660 tons and 339 321 tons of pears and apples, respectively (HORTGRO, 2014). The annual export volume of fresh pears is estimated at 203 660 tons (48% of the total production) with the value of R2 491 452. Pears contributed approximately 16% (R2 billion) of the total gross value for deciduous fruits (R12.6 billion) in South Africa during the 2012/2013 season (DAFF, 2014b).

Export markets generate a greater unit price than the local market, which contributes in making South Africa a major global exporter by volume, although pear production area is relatively small. Packham's Triumph is the leading pear cultivar produced in South Africa (Figure 2) planted at approximately 3 980 ha (HORTGRO, 2014) and also the major export pear, estimated at about 5 574 599/12.5kg equivalent cartons per annum (DAFF, 2014b; PPECB, 2014). 'Packham's Triumph' pears represents 33% of the total pears produced in South Africa from estimated planted area 12 211 ha (HORTGRO, 2014). The bulk of South African pears are exported to the EU and Russia (55%) followed by Far East and Asia (15%) and the Middle East (14%) (Figure 3). The main pear varieties exported are green pears which account for 80% of all pears exported.

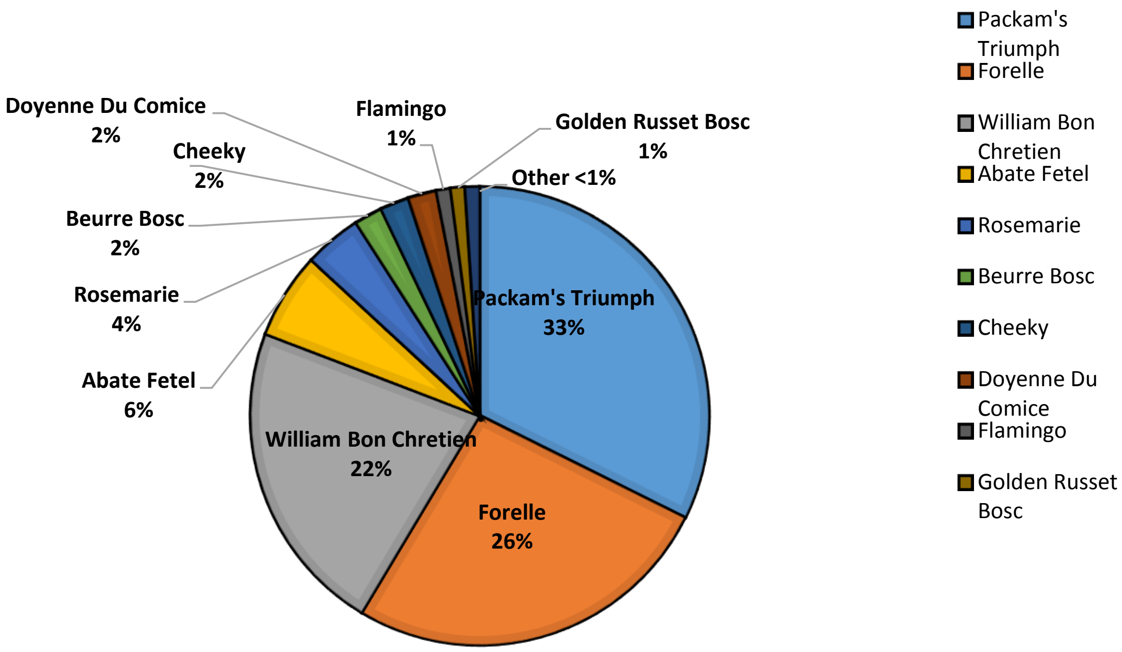


Figure 2. Leading pear cultivars planted (12 211ha) in South Africa during the 2014 season (HORTGRO, 2014).

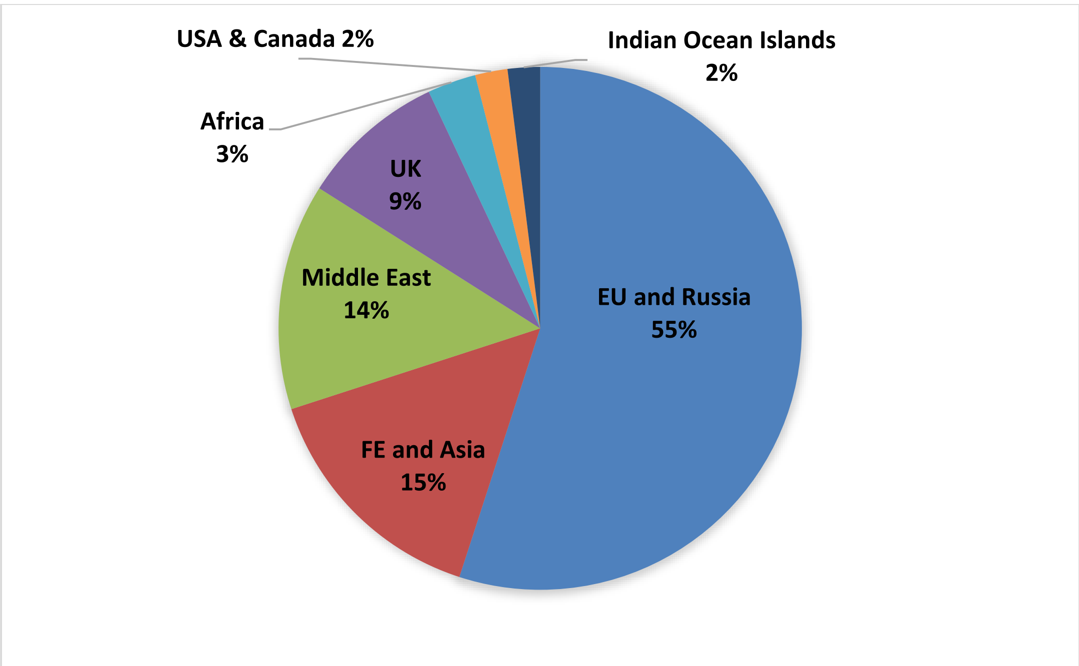


Figure 3. South African pear export volume per market in the 2014 season (DAFF and PPECB, 2014).

The export volume of fresh apples is estimated at 339 321 tons (42.7% of the total production) with the value of R4 768 856 (DAFF, 2014a; PPECB, 2014). 'Granny Smith' apples is the third major export apple at an estimated volume of 5 029 834/12.5kg equivalent cartons (PPECB, 2014). 'Granny Smith' apples is, however, the second most planted apples in South Africa at approximately 4218 ha amounting to 18% of the total area planted for apples (Figure 4) (HORTGRO, 2014). Apples account for an average export share of 66%. Africa commands a leading share of export destinations (32%), followed by markets in Asia (26%) and United Kingdom (21%) (Figure 5). For all the listed regions, 'Granny Smith' apples is the most demanded apple cultivar, with Australia importing only fruit from this cultivar from South Africa.

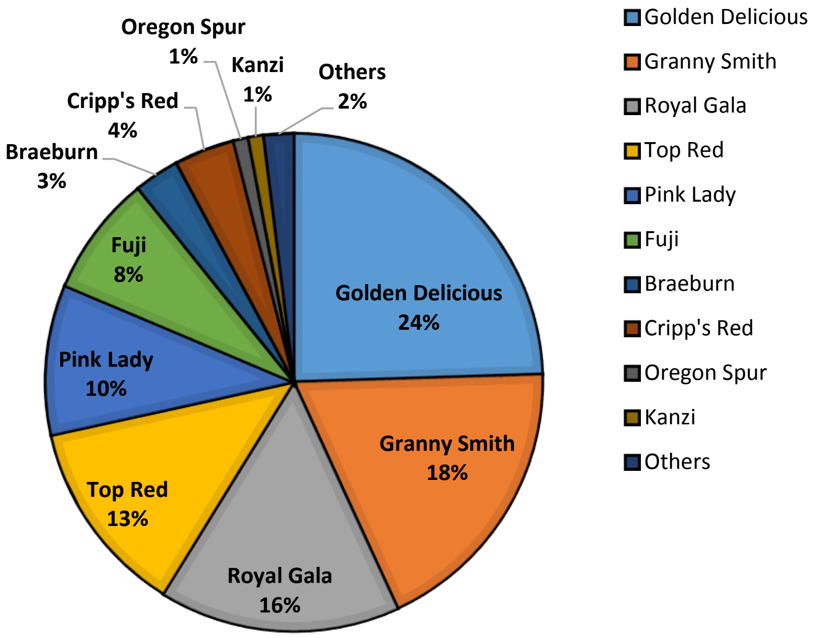


Figure 4. Leading apple cultivars planted (22 925 ha) in South Africa during the 2014 season (HORTGRO, 2014).

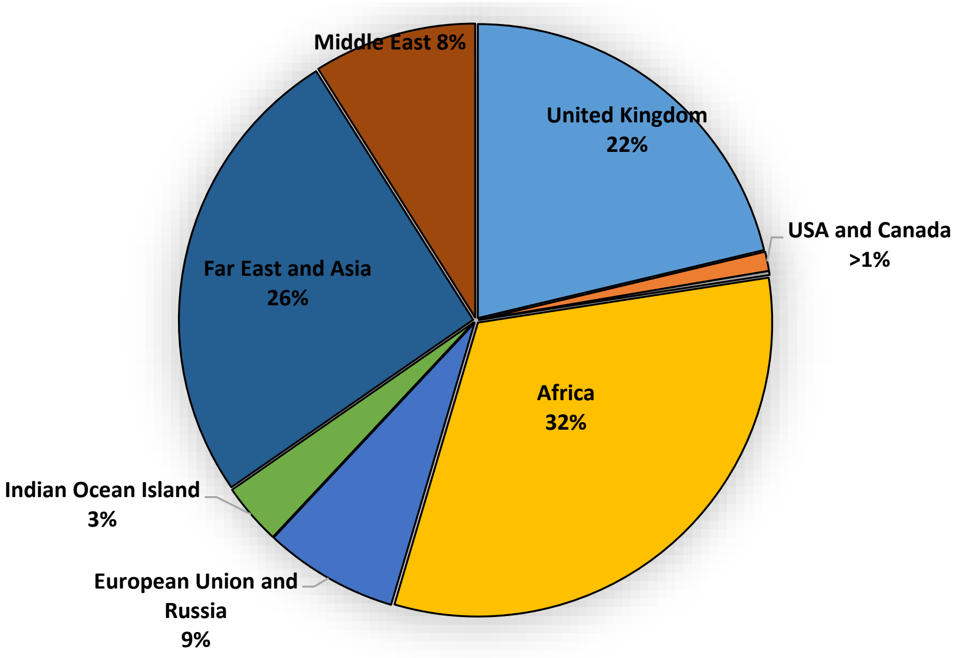


Figure 5. South African apple export volume per market for the 2014 season (DAFF and PPECB, 2014).

Securing and supporting the pome fruit industry sector for the country is important to ensure economic growth and promote the well-being of its people. Major fruit quality challenges exist currently within the pome fruit industry. The main causes are physiological (i.e. superficial scald), pathological (decay) and physical (mechanical injury) as many occur during transport and handling. These challenges require that the industry develops and adopts new technologies to ensure that it retains its market share and access to new markets for sustainability and future growth (Opara, 2009).

### Impact of superficial scald on export of pome fruit

Superficial scald, also referred to as storage scald, is a physiological disorder affecting several cultivars of apples and pears. It causes browning of the skin during or after long-term low temperature storage, thereby downgrading the appearance quality and fresh market value of the fruit (Ghahramani and Scott, 2000; Wang and Dilley, 1999 and 2000). The affected cells of the hypodermis die and dehydrate. Therefore, it is imperative that aspects of scald etiology (physical, physiology and biochemistry) and pre- and postharvest factors affecting its incidence and severity be understood. Better understanding of the mode of action of cold storage technologies in preventing development of the disorder will assist towards the development of cost-effective control strategies (Lurie and Watkins, 2012).

South Africa has been exporting fruit stored in CA since 1984 (Shepherd, 2005), and still remains an important role player in the Southern hemisphere, competing favourably with countries like Chile and Australia, especially in the early southern hemisphere apple season with the ability of extending the sale of attractive fruit beyond the end of July, with no competition from the European market (Shepherd, 2005; DAFF, 2014c). The success of South Africa in export of apples and pears to the EU has recently come under threat due to restrictions placed by the EU on the use of DPA, a synthetic antioxidant, in conjunction with CA to extend storage life of fruit and control undesirable physiological disorders.

Given the importance of the pome fruit sector in the economy, it is imperative that South Africa remains up to date in terms of compliance to EU legislation with respect to maximum residue levels (MRL) to ensure its competitiveness in the global market. For South Africa to access and retain existing export markets, fruit need to be kept in cold storage for extended periods and this lead to decay and ultimately development of physiological disorders. One of the major physiological disorders of pome fruit is superficial scald, which occur in storage after extended periods of exposure to lower temperatures. Superficial scald is one of the disorders not permissible in consignments destined for export markets.

'Granny Smith' apples and 'Packham's Triumph' pears are highly susceptible to superficial scald after extended period in storage. Superficial scald affects the peel, manifesting itself as brown to black patches on the epidermis of the fruit thereby reducing their market value (Lotze et al., 2003; Lurie and Watkins, 2012). Fruit affected by superficial scald can still be processed for juice or sliced bakery products as they still have acceptable sensory quality attributes, including taste and texture. Superficial scald on apples and pears appears after 2-3 months in cold storage (Anet and Coggiola, 1974; Emongor et al., 1994).

For decades postharvest drenching of pome fruit with DPA at a concentration of 1000-2000 mg $kg^{-1}$ in water has been used globally to inhibit superficial scald and other senescence related disorders (Wilkinson and Fidler, 1973; Zanella and Strüz, 2013). Recent restrictions by the EU Commission Regulation 772/2013 on the use of DPA has spurred a search into non-chemical alternatives for the control of superficial scald on pome fruit necessary to safeguard the South African pome fruit industry.

The MRL for DPA has been reduced from 5 ppm on apples and pears to negligible levels of 0.1 ppm effective from April 2014 (Robatscher et al., 2012; Commission Regulation

(EU) No 772/2013). It is therefore crucial for the South African apple and pear producers and exporters to have alternatives for scald control to safeguard the pome fruit industry by ensuring a continuous supply of quality fruit to its export markets and to provide consumers access to fresh fruit throughout the year.

## 2. Symptoms and physiology of superficial scald

Scald is a term loosely applied to a group of peel (cuticle, epidermis and hypodermis) disorders of apples and pears. It involves brown discoloration of irregularly shaped areas on the surface of the fruit during or following storage (Whitaker et al., 2009). Warm temperatures do not cause scald, but allow symptoms to develop from previous injury which occurred during cold storage. Symptoms may be visible in cold storage when injury is severe and in this case, the symptoms intensify upon warming the fruit (Ingle, 2001). Scald is usually not evident until after 3 months in storage (Figure 6). Scald can be more severe on the greener side of the fruit (Ingle and D'Souza, 1989; Ingle, 2001).

On apples, Wilkinson and Fidler (1973) described the following forms of scald:

- **Rugose scald**: Can affect almost all apple cultivars, especially those for which are liable to injury. Peel initially develops a faint bronze colour, but later these areas turn light brown to very dark brown. The surface layers of cells are dead and so they dry out and collapse, leaving a brown, sunken appearance. Usually many lenticels remain green, but stand out prominently from the sunken area.
- **Browning scald**: The lenticels do not remain green, the injury progressively invades deeper into the flesh, and areas often slough off because they remain moist.
- **Lenticel spot scald**: The injury is predominately around the lenticels, so that it appears as a spotting rather than a blotchy disorder (Brooks, 1968).
- **Stem-end browning**: The injury is primarily on the shoulder, radiating from the stem-end cavity, which remains relatively free of the disorder.

It appears that all these forms are expressions of the same problem, but specific cultivars are more prone to one form or another. On pears superficial scald appears as a sign of over-storage, however few important cultivars are especially susceptible to true scald which includes the 'Packham's Triumph' pear and 'd'Anjou' (Wilkinson and Fidler, 1973).

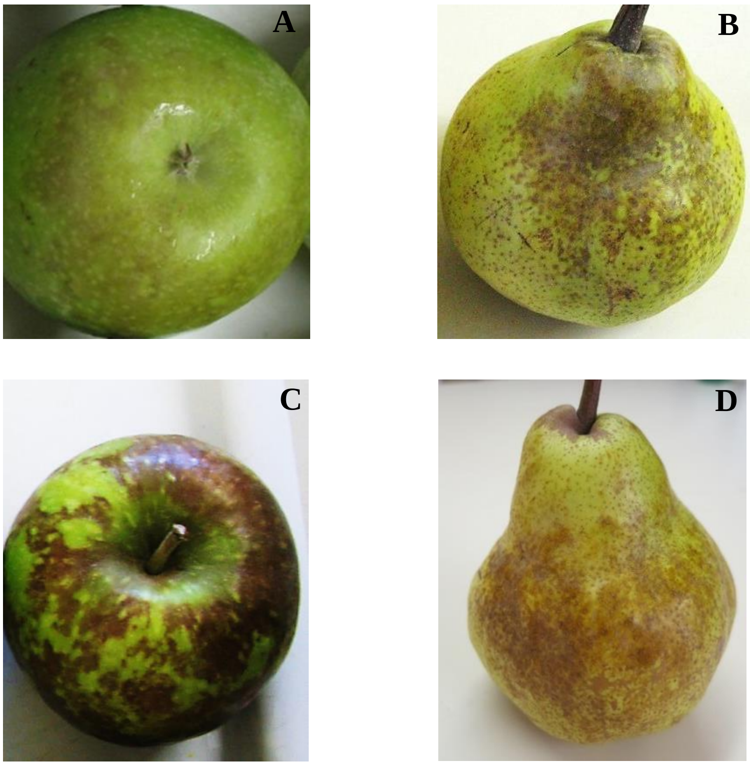


Figure 6. Symptoms of superficial scald physiological disorder on ‘Granny Smith’ apple (left) and ‘Packham’s Triumph’ pear (right). A = mild symptoms of superficial scald on ‘Granny Smith’ apple, B = mild symptoms of superficial scald on ‘Packham’s Triumph’ pear, C = severe symptoms of superficial scald on ‘Granny Smith’ apple, D = severe symptoms of superficial scald on ‘Packham’s Triumph’ pear.

It has been hypothesized that α-farnesene, a naturally occurring volatile terpene in apple fruit, is oxidized to a variety of products (conjugated trienes and 6-methyl-5-hepten-2-one) (MHO) (Meigh, 1969; Anet, 1972a; Mir et al., 1999). These oxidation products result in the injury to the cell membranes which eventually lead to cell death in the outermost cell layers of the conjugated trienes. Ethylene promotes the formation of α-farnesene and oxygen is required to oxidize α-farnesene to conjugated trienes (Ingle and D’Souza, 1989).

Some salient points that should be considered in understanding scald etiology and control are as follows: cultivars vary greatly in susceptibility to scald; growing region and seasons influence susceptibility; scald is usually more prevalent in earlier than later harvested fruit; low night temperatures in the period before harvest decrease the incidence of scald, and an inverse relationship between the number of days below the threshold temperature of 10°C and the incidence of scald has been noted (Lurie and Watkins, 2012).

## 3. Effects of pre-harvest factors on superficial scald of pome fruit

Pre-harvest factors during ontogeny greatly impact on the fruit quality at harvest; modify fruit responses to various treatments, affect the development of physiological disorders and the retention of fruit quality at the end of storage period. The development and severity of superficial scald in apple fruit is proportional to the amount of antioxidants in the peel and the extent of α-farnesene oxidation. Understanding the influence of pre-harvest factors on α-farnesene metabolism will assist in the development of reliable predictive models for scald susceptibility at harvest, prior to long-term storage (Emongor et al., 1994).

### 3.1.Climatic factors

Weather factors such as temperature, sunlight and rainfall are uncontrollable postharvest, although their variability throughout the season affects fruit quality and storage performance of apples. Shade-netting and kaolin particle film are effective in reducing sunburn on apples by reducing both irradiance and fruit temperature (Gindaba and Wand, 2005). Abnormal weather conditions during the growing season such as very high or very low temperatures and extended periods of cloudy, wet or dry conditions usually have undesirable effects on storage behaviour of fruits (Emongor et al., 1994).

Fruit grown in dry and warmer climatic conditions are generally more susceptible to superficial scald than fruit grown in cooler climates (Sharples, 1984; Wang and Dilley, 1999). Susceptibility of fruit to superficial scald may differ from region to region. Susceptibility of some cultivars such as ‘Bartlett’ pear has been reported to differ according to growing region. For instance, ‘Bartlett’ pears grown in central Washington (USA) were found to be less susceptible to superficial scald compared to those grown in northern California (Whitaker et al., 2009).

Pre-harvest temperatures below 10 °C showed a high correlation with reduced scald incidence of apples cvs. ‘Cortland’, ‘Delicious’, ‘Granny Smith’ and ‘Starkimson Delicious’)after storage (Bramlage and Meir, 1989; Blanpied et al, 1991; Thomai et al., 1998; Barden and Bramlage, 1994b, Diamantidis et al., 2002). This effect was positively correlated to increased content of unsaturated fatty acids oleic (C18:1) and linoleic (C18:2) acids. Exposure to sunlight affects the susceptibility of fruit to superficial scald. Brooks et al. (1923b) reported that fruit with more exposure to sunlight produces better colour and offer better resistance to scald development, however cultural practices such as shade netting are employed by most growers to control overexposure to sunlight as this may cause sunburn. Better colour is compromised for avoiding sunburn in fruit.

### 3.2. Cultivar effects

Cultivars have varying degrees of susceptibility to superficial scald physiological disorder. Some fruit cultivars, e.g. ‘Granny Smith’, ‘Delicious’, ‘McIntosh’, ‘Cortland’ and ‘Packham`s Triumph’, are particularly susceptible to superficial scald, while others, e.g. ‘Empire’,‘Golden Delicious’ and ‘Forelle’ show some natural resistance. This suggest that superficial scald has a genetic basis, as it affects most but not all cultivars of apples and pears (Wang and Dilley, 1999). Resistant cultivars like ‘Golden Delicious’ and ‘Forelle’ showed higher concentrations of α-farnesene in contrast to susceptible cultivars such as ‘Granny Smith’ and ‘Packham Triumph’ (Rupasinghe et al., 2000; Lotze et al., 2003).

### 3.3. Maturity

South African exporters are required to harvest fruit at less than ideal maturity stage due to necessity to ship fruit to long distance markets. Due to the demand for finest quality fruit by consumers, and the need to store fruit for extended periods of time, optimal picking dates are essential (Olivier, 1996). Pre-optimal harvested fruit are prone to postharvest physiological disorders including shrivelling (Kader, 1996 and 1999; Burger, 2005), scuffing disorder (Chen and Varga, 1996), inferior flavour quality (Kader, 1996) and later storage disorders such as superficial scald (van der Merwe et al., 2003). Scuffing disorder (fruit discolouration) in pears affects mostly small size pears and this disorder is one of the costly problems which remain unsolved in the pear industry (Chen and Varga, 1996). Over mature fruit are susceptible to softening, mealinesss, decay and some disorders such as senescence, internal breakdown as well as watery breakdown in pears (Kader, 1999; Mitcham and Mitchell, 2002; Lotze et al., 2003; Franck et al., 2007).

Fruit harvested at pre-optimal maturity (cvs. ‘Granny Smith’, ‘Stark Crimson’ apples) have been shown to be more susceptible to superficial scald than optimally harvested fruit (Anet, 1972a; Lotze, 1996; Lau, 1997; van der Merwe et al., 2003) due to lack of a sufficient, efficient antioxidant system needed to control the autoxidation of α-farnesene (Anet, 1972b). Pre-optimal harvested apples (cv. ‘Granny Smith’) were found to have more α-farnesene than optimally harvested fruit (Huelin and Coggiola, 1968). Fruit harvested at a later date showed less incidence and severity of superficial scald in 'Cortland' apples (Barden and Bramlage, 1994a).

Pear cultivars vary in scald susceptibility in relation to maturity at harvest. Optimally harvested 'Anjou' and 'Packham's Triumph' pear cultivars showed better resistance to superficial scald, however pre-optimal 'Bartlett' pears were more resistant to the disorder than optimally harvested fruit (Zoffoli et al., 1998).

### 3.4. Fruit colour

Scald is more likely to occur on the green area than on the well-coloured area of red coloured fruit, but occurs randomly on green fruit (Kupferman, 2001). This relationship is probably indirect as good exposure to sun is probably what reduces scald susceptibility, rather than red pigments alone (Kupferman, 2001). Thus, the production of red strains of susceptible cultivars largely obscures the fact that shaded areas on fruit and shaded fruit are more susceptible than exposed tree areas and exposed fruit. Excessive tree vigour and inadequate pruning increases scald susceptibility, while summer pruning reduces it (Barden and Bramlage, 1994b).

### 3.5. Fruit size

Smaller size apples are less susceptible to scald than larger ones (Brooks et al., 1923b); however, in a year when scald was severe even most small apples may scald. Similarly, large fruit from light-cropping trees frequently have lower calcium content and are more susceptible to storage disorders than smaller fruit from heavy-cropping trees (Emongor et al., 1994).

### 3.6. Nutrition

Plant nutrition is an important environmental factor which affects plant growth and development. Nutrient partitioning within apple trees at different stages of fruit growth and development may be of importance to scald development during storage. The concentrations of minerals such as calcium, magnesium and potassium in apple fruit at harvest can influence fruit condition during storage. Although orchard factors which control mineral flux into fruit during the growing season are poorly understood (Ferguson and Watkins, 1989), fruit size is an important determinant in mineral content (Emongor et al., 1994).

Apples with low calcium levels often develop more scald than those with high levels. Similarly lower calcium levels in fruit result in higher susceptibility to bitter pit in apples (Sharples, 1984; Lotze et al., 2003; Lotze and Theron, 2007), cork spot in pears (Wills et al., 2007), *Cloesporium* storage rot (Sharples, 1984) and senescent breakdown (Bramlage, 1993). Development of bitter bit in apples is also associated with high levels of potassium (K) in apples (Wills et al., 2007). Low calcium may indirectly enhance the occurrence of chilling injury by fostering rapid loss of membrane integrity under chilling stress (Bramlage, 1993). Raese and Drake (2000) reported that increased calcium content resulted in lesser fruit disorders and enhanced external fruit appearance and whiter fruit flesh colour in ‘Anjou’ pears.

Phosphorus content in apples may also influence scald susceptibility. Phosphorus fertilization tends to decrease α-farnesene content in the essential oil of some plant tissue such as chamomile (Emongor, 1988). Bramlage et al. (1982) reported a positive correlation of potassium with scald development of ‘McIntosh’ apples after storage. High levels of potassium have been associated with increased incidence of scald after storage of ‘Delicious’ apples (Weeks et al., 1965). Copper, iron and cobalt deficiencies induced symptoms similar to low temperature breakdown and superficial scald in apple, by acting as catalysts for enzyme systems which lead to enzymic browning of cut or damaged tissues that are exposed to air (Wills et al., 1989). Little is known on the effect of plant nutrition (macro- and micronutrients) on scald-promoting chemical substances in the apple skin (Emongor et al., 1994). The deficiency of boron leads to appearance of apple and pear drought spot (Kays, 1999).

### 3.7. Rootstocks/tree age

Rootstocks may influence the precocity, growth habit, size, and development of apple trees. Rootstocks can influence fruit quality before and during storage, and scald susceptibility. Apples from trees on M.26 rootstock developed less scald than fruits from seedling or trees on MM.111 rootstock (Drake et al., 1991), possibly due to differences in fruit maturity caused by the rootstocks. Fruit from trees on M.26 rootstock were more mature than apples on seedling and MM.111 rootstock (Emongor et al., 1994). Younger trees tend to produce fruit that are susceptible to a variety of postharvest disorders (Bramlage, 1993). Due to their higher vigour younger trees, regardless of rootstock, produce fruit with lower keeping quality and higher susceptibility to physiological disorders, because of their higher N content and lower Ca concentration (Watkins, 2009).

## 4. Postharvest factors affecting superficial scald incidence

Pre- and postharvest factors are interlinked in the development and control/ prevention of storage disorders. Superficial scald, like most storage disorders, may be reduced to a minimum level by managing pre-harvest factors including mineral nutrition and temperature; however, it can be effectively controlled by postharvest practices as shown in Figure 7 (Ferguson et al., 1999). Optimal storage technology should extend storage and shelf-life of pome fruit through a perfect balance of temperature, duration, relative humidity, and the concentration of gases ($O_2$, $CO_2$ and $C_2H_4$) (Saltveit, 2003) to delay ripening and senescence (Veltman et al., 2003).

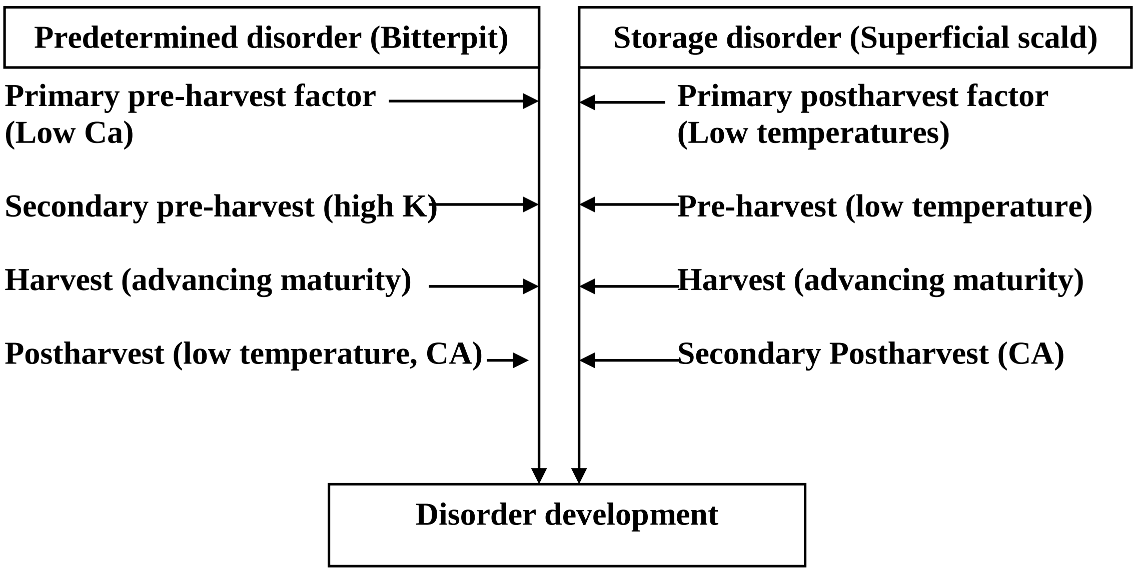


Figure 7. Model depicting the development of postharvest disorders in relation to pre-harvest factors. Superficial scald is induced by low temperature during storage but its severity is influenced by pre-harvest factors, including low temperature during fruit growth and development (Ferguson et al., 1999).

### 4.1. Storage temperature

Storage temperature is the main postharvest factor linked to scald development and severity. Superficial scald is a low temperature storage disorder (Lurie and Watkins, 2012), and hence scald occurrence and severity increases at low storage temperature conditions. In apples, scald can occur earlier at higher storage temperatures. The symptoms become more severe at lower storage temperatures. Like chilling injury, scald symptoms generally aggravate during shelf -life. Storage temperatures below 15 °C have been reported to be more conducive for scald development (Ingle, 2001).

Temperature control plays a crucial role in storage of fruits and vegetables in CA. Lower temperatures reduce postharvest respiration thereby extending storage life of stored produce (Taiz and Zeiger, 2010) and also slow down fungal growth (de Kock and Combrink, 1996). High temperatures advance the ripening of fruit and ultimately senescence. Pome fruits are the best horticultural fresh produce for long term CA storage, because of their excellent response to lower temperature storage and rapid cooling without danger of freezing or chilling injury (Wills et al, 1989; Mitcham and Mitchel, 2002). Apples are the most stored of pome fruit followed by pears. Most storage facilities are kept at about ±1 ºC of the desired temperature for the commodity being stored (Thompson, 2002).

Freezing and chilling injury may result as a consequence of temperatures held below optimal, whereas higher than optimal temperatures may shorten the storage life of the commodity (Thompson, 2002). The closer the temperature is to the freezing point, the slower the respiration rate and the longer the storage life (Findlay and Combrink, 1996). For most apple and pear cultivars as well as for nectarines, apricots and peaches optimal storage temperature is at -0.5 °C, with the exception of 'Granny Smith' which are stored at 0.0 to 0.5 °C to avoid the development of core flush which occurs at lower temperatures (van der Merwe, 1996).

### 4.2. Relative humidity (RH)

Relative humidity is necessary in storage rooms to minimise moisture loss, decay development, and loss of turgidity in tissues, thereby reducing incidence of shrivelling (Kader, 2002). Optimal relative humidity (amount of water vapour in the atmosphere as a percentage of saturation) is recommended between 90 and 95% for apple and pear storage (Hurndall and van der Merwe, 2005). At air relative humidity less than 95%, fruit stored in higher temperatures loose moisture faster (Mitcham and Mitchell, 2002). Shrivelling symptoms become visible after fruit has lost 3-5% of the original weight loss due to moisture loss. Retention of moisture is a key quality factor as shrivelled fruit has little or no market value (Wills et al., 1989), and this would also contribute to loss of mass, which amount to significant financial loss given that most fresh horticultural produce including apples and pears are sold on weight basis and appearance is a critical quality cue (Opara, 2009).

4.3. Gases ($O_2$, $CO_2$ and $C_2H_4$)

High $CO_2$ level can effectively decrease ethylene production (a ripening hormone) (Kader, 1989; Taiz and Zeiger, 2010; Blackenship and Dole, 2003), suppress respiration (Kader, 2002) and reduce loss of chlorophyll. However, it can also promote the development of anaerobic respiration, thereby increasing levels of alcohols and acetaldehydes in apples and pears which may result in off-flavours and $CO_2$ injury in some cultivars (Kader, 2002; Saltveit, 2003). Fruit susceptibility to elevated $CO_2$ levels is closely associated with temperatures and duration of exposure (Kader, 2002), the lower the storage temperature and the shorter the time of exposure the more tolerant the fruit will be to high $CO_2$ levels.

Lower $O_2$ levels may also stimulate anaerobic fermentation though they result in reduced respiration and ethylene action and synthesis (Saltveit, 2003). Lower $O_2$ levels (<0.5 $O_2$) favour the accumulation of acetaldehydes, ethanol, ethyl acetate and/or lactate which are responsible for formation of off-flavours in fruit, indicating a shift to anaerobic respiration (Kader, 1989 and 1995). Hypoxic conditions results in reduced $O_2$ available for respiration and this results in cells being unable to generate energy required for metabolic activities and this predispose membrane to damage by oxygen radicals (superoxide radical ($O_2^{\bullet-}$) hydrogen peroxide ($H_2O_2$) and reactive hydroxyl anion ($OH^{\bullet}$) (Veltman at al., 2003; Franck et al., 2007), and the membrane then loses ability to regenerate antioxidants to counteract these free radicals (Taiz and Zeiger, 2010). Loss of membrane integrity leads to development of some postharvest disorders including internal browning and superficial scald in susceptible cultivars (Taiz and Zeiger, 2010; Veltman at al., 2003; Franck et al., 2007).

The rate of fruit deterioration in storage is directly proportional to rate of respiration (Prange et al., 2005), and is hastened by the production of ethylene. Genetic and environmental factors including $CO_2$, $O_2$ and temperature influence ethylene synthesis (Kader, 2002; Taiz and Zeiger, 2010). Lower $O_2$ levels suppress the action of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase enzyme in the last step of ethylene synthesis pathway, the conversion of ACC which is a precursor of ethylene to ethylene (Taiz and Zeiger, 2010).

Lower oxygen pre-treatments at the storage temperature of 0 °C reduce the ethylene production (Kader, 1989) in the same way as 1-MCP treatment which is an ethylene inhibitor (Moggia et al., 2010), however, as soon as fruit is transferred to 20 °C (simulated ripening) the lower oxygen treatments gave a burst of ethylene but not the 1-MCP treated fruit (Pesis et al., 2010). Lower oxygen treatments effectively reduces bitter pit and superficial scald disorders in susceptible cultivars, and this is attributed to accumulation of volatile alcohols (Pesis et al., 2010).

4.4. Biological control agents

Fruit resistance to scald development is attributed to higher endogenous antioxidant activity (Anet and Coggiola, 1974; Barden and Bramlage, 1994a). Vitamin C (ascorbic acid) and vitamin E (α-tocopherol) have shown some potential to inhibit the incidence of superficial scald in ‘Granny Smith’ and ‘Red Delicious’ apples (Anet, 1974; Manseka and Vasilakakis, 1993).

## 5. Biochemistry of superficial scald

Superficial scald is the product of an oxidative process caused by the oxidation of α-farnesene into its oxidation products conjugated triene hydro peroxides, conjugated trienols, reactive oxygen species (ROS), and 6-methyl-5-hepten-2-one (Huelin and Coggiola, 1970a; Anet and Coggiola, 1974; Whitaker et al., 2000). The role of the volatile sesquiterpene α-farnesene and its products of oxidation the ketone 6-methyl-5-heptene-2-one (MHO), conjugated triene hydro peroxide, free radicals and conjugated trienols on superficial scald incidence have been confirmed by several researchers (Meigh, 1969; Meigh and Filmer, 1967 and 1971; Huelin and Coggiola, 1970a; Anet, 1969, 1972a and 1972b; Anet and Coggiola, 1974; Wang and Dilley, 1999 and 2000).

Researchers have shown that there is correlation between yield of conjugated trienes in ‘Granny Smith’ apples and ‘d’Anjou’ pears and the rate of scalding, which supported the theory that superficial scald is caused by autoxidation of α-farnesene to conjugated trienes (Huelin and Coggiola, 1970b; Anet, 1972b; Anet and Coggiola, 1974; Chen et al., 1990). It was further observed that at least 3 nmoles/cm$^2$ of conjugated trienes should be in the peel in order to induce superficial scald (Chen at al., 1990). Conjugated trienes concentrations of 2.5 nmoles/cm$^2$ may be used as one of the principal factors in predicting scald incidence of ‘d’Anjou’ pears during the marketing period (Chen et al., 1990). The ketone MHO which is the main product of α-farnesene autoxidation is toxic to fruit peel and has been shown to be positively correlated with higher scald incidence (Mir et al., 1999).

The naturally occurring unsaturated sesquiterpene hydrocarbon 3-7-11-trimethyldodecatetraene (α-farnesene) and its oxidation products 6-methyl-5-hepten-2-one (MHO), conjugated triene hydroperoxide free radicals, and conjugated trienols are found in the epidermis of the apple fruit (Huelin and Coggiola, 1968; Anet, 1969; von Mollendorf, 1996; Wang and Dilley, 1999; Rupasinghe et al., 2000). The terpene α-farnesene is a precursor for superficial scald (Filmer and Meigh, 1971), hence, its accumulation in the fruit does not cause scald development (Huelin and Coggiola, 1968).

MHO is the volatile end product of α-farnesene autoxidation associated with scald symptoms by causing discoloration and death of hypodermal cells (Meigh and Filmer, 1967; Anet, 1972a; Whitaker et al., 1999). During shelf-life conditions (22 °C) cumulative release of MHO from fruit peel for 2 to 72 hours followed the same pattern as the development of superficial scald in this fruit (Mir et al., 1999).

## 6. Current technologies used to control superficial scald and their mode of action

Available commercial treatments for the control of superficial scald on apples and pears include chemical treatments (namely the synthetic antioxidant DPA which is restricted to 0.1 ppm), ethoxyquin, 1-methylcyclopropene (1-MCP, an ethylene action inhibitor), and non-chemical treatments (such as various low oxygen controlled atmosphere protocols (CA, DCA and ILOS) (Huelin and Coggiola, 1970b; Calvo, 2003; Pesis et al., 2007; Watkins, 2008; Pesis et al., 2010). The use of controlled atmosphere technologies, including controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP), may offer alternatives to the use of postharvest chemicals on apples and pears for controlling some physiological disorders (Kader, 1995).

### 6.1. Diphenylamine (DPA) treatment

DPA drench has been used globally for decades to control superficial scald and prolong market life of apples and pears. DPA solutions have specific protocol for disposal as they are regarded as special waste (Lau, 1997) and hence the restriction on its use to 0.1 ppm as effective from April 2014 (EU comm.). DPA treated fruit show reduced α-farnesene synthesis, lower respiration rates and reduced ethylene production (Whitaker, 2000). The onset of accumulation of conjugated trienols was delayed by 5 weeks and accumulation was reduced by more than 2.5 folds in DPA treated fruit applied as a drench dissolved in water solution (Whitaker, 2000).

DPA inhibits superficial scald by preventing oxidation of α-farnesene to its toxic conjugated trienes, hence the incidence of superficial scald was found to be correlated with this oxidation process (Huelin and Coggiola, 1970b). Apart from inhibiting superficial scald DPA reduces other disorders such as bitter pit in susceptible cultivars (Ferguson, et al., 1999), however, its use is highly restricted in most export markets.

6.2. 1-Methylcyclopropene (1-MCP) treatment

1-MCP, an ethylene inhibitor, commercialised as SmartFresh$^{TM}$ is registered for use on both vegetables and fruit and it maintains postharvest quality (Watkins, 2008). It is one of the technologies effective in inhibiting superficial scald disorder in apples, however it is cultivar specific (Pesis et al., 2007; Watkins, 2008; Lee et al., 2012). It controls superficial scald by inhibiting ethylene action by binding irreversibly to the ethylene receptor (Taiz and Zeiger, 2010). 1-MCP is a non-toxic, easy to use compound effective at the gaseous phase therefore not requiring drenching like DPA (Zanella, 2003), commonly applied at 20-25 ºC (Blackenship and Dole, 2003).

In order to maximise benefits of the use of 1-MCP optimally harvested fruit need to be treated within 3 days of harvest (Jung and Watkins, 2008; Watkins, 2008), as delays of treatment will result in reduced effectiveness. Total soluble solids of 'Granny Smith' apples treated with 1-MCP were not influenced though the fruit found resulted in greater firmness and acidity after 14 days at shelf- life conditions of 20 °C (Zanella, 2003). 1-MCP treatment has an added advantage of reducing core flush (the major internal disorder of apples) incidence in 'Granny Smith' apples (Zanella, 2003), though it increased risk of carbon dioxide injury and flesh browning in some cultivars (Watkins, 2008).

1-MCP used at concentration as low as 0.1 ppm in 'Rocha' pears was effective in reducing superficial scald (Isidoro and Almeida, 2006), although other authors have reported serious concerns with regard to external $CO_2$ damage on 'Empire' apples (DeEll et al., 2005; Watkins and Nock, 2012). However, $CO_2$ damage can be reduced by eliminating or reducing $CO_2$ levels in rooms containing 1-MCP treated fruit (DeEll et al., 2005). The 1-MCP mode of action in the control of superficial scald in 'Rocha' pears appears to be by suppressing levels of conjugated trienols accumulation, and the α-farnesene levels were generally lower than control fruit (Isidoro and Almeida, 2006).

1-MCP treatment reduced the onset of senescence in 'McIntosh' apples (Watkins and Nock, 2012); however, it enhanced flesh browning at 3.3 °C than at 0.5 °C (Lee at al., 2012). Fruit treated with 1-MCP showed higher incidence of bitter pit (Pesis et al., 2010). Zanella et al., (2005) observed phytotoxic effects of 1-MCP related to the unusual hot and dry climate during the vegetative period and harvest. A spotwise browning developed on the surface of the affected fruit and subsequently changed into circular-shaped necrotic dark spots (2-4 mm diameter) (Zanella et al., 2005). Labelling of fruit treated with 1-MCP is not required in Europe, however, although it works well as a scald inhibitor on apples there are problems associated with ripening on pears treated with 1-MCP (Calvo, 2010).

6.3. Controlled atmosphere storage technologies and initial low oxygen stress

CA, DCA and ILOS technologies differ in their concentration of $O_2$ in the storage rooms (Figure 8). They are non-chemical technologies used to extend the storage life of the fruit and ensures the extension of the marketing window. Other benefits of CA technologies include flexibility and provision of ways to assist producers and exporters in planning pack house operations to avoid peaks in both packaging and delivery of fruit (Hurndall and van der Merwe, 2005).

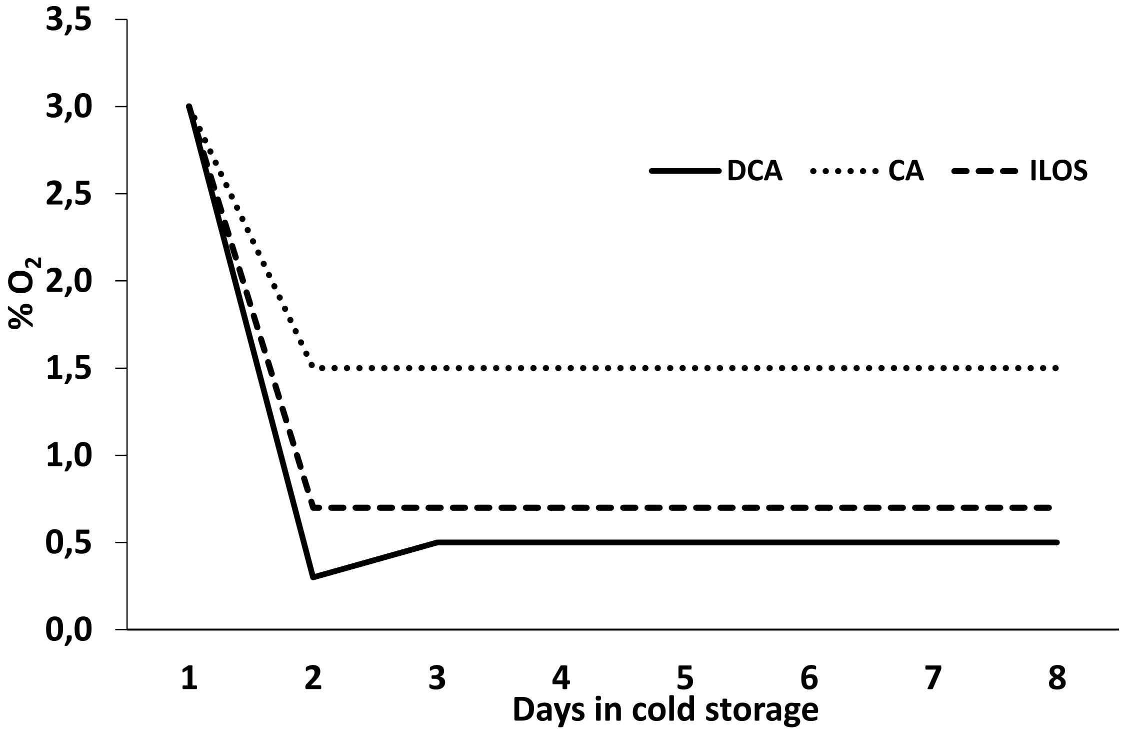


Figure 8. Examples of different levels of $O_2$ control for DCA, CA and ILOS storage technologies.

*6.3.1. Conventional controlled atmosphere (CA) (Low $O_2$ and high $CO_2$)*

Lower $O_2$ levels coupled with higher $CO_2$ inhibit the activity of certain enzymes in the glycolytic and Krebs cycle thereby reducing the respiration rate of the treated fruit (Kader, 1989). Accumulation of ethanol and acetaldehydes occur in lower oxygen treated fruit an indication of a shift to anaerobic fermentation state known as anaerobic compensation point (ACP) (Kader, 1989). The ripening process of ‘Bartlett’ pears and CA-stored fruit was delayed after transfer to shelf-life conditions compared to air-stored fruit (Kader, 1989). Storage in 0.7% $O_2$ + 1.0% $CO_2$ at 0 °C inhibited superficial scald and reduced water core induced breakdown in ‘Harold Red’, ‘Starking’ and ‘Starkrimson’ in research conducted in British Columbia (Lau, 1997).

Controlled atmosphere storage at low oxygen levels inhibits superficial scald by inhibiting synthesis α-farnesene and its subsequent conversion to its oxidation toxic product MHO (Wang and Dilley, 1999 and 2000; Pesis et al., 2010). Storage conditions may vary from country to country and even in every district within that country (Findlay and

Combrinck, 1996). Apples and pears account for approximately 97% of all fruit stored under CA conditions (van der Merwe, 1996). As cultivars respond differently to specific protocols, (Table 1) indicates recommended guidelines for ‘Packham’s Triumph’ pears and ‘Granny Smith’ apples in South Africa; however the fruit were treated with DPA prior to storage.

Table 1. Recommended CA gas regimes for storage of some apple and pear cultivars grown in South Africa with DPA treatment (van der Merwe, 2005).

| Cultivars | Tolerance level | $O_2$ | $CO_2$ | Storage period in months (with DPA) |
|---|---|---|---|---|
| **<u>Pears</u>** | | | | |
| ‘Packham’s Triumph’ | Optimum | 1.5 | 2.5 | 9 |
| | Minimum | 2.0 | 3.0 | |
| | Maximum | 1.0 | 1.0 | |
| ‘Forelle’ | Optimum | 1.5 | 0.0-1.5 | 7 |
| | Minimum | 2.0 | 1.5 | |
| | Maximum | 1.0 | 0.0 | |
| ‘Bon Chretien’ | Optimum | 1.0 | 0.0 | 4 |
| | Minimum | 1.5 | 0.0 | |
| | Maximum | 1.0 | 0.0 | |
| **<u>Apples</u>** | | | | |
| ‘Golden Delicious’ | Optimum | 1.5 | 1.5 | 9 |
| | Minimum | 2.0 | 3.0 | |
| | Maximum | 1.0 | 1.0 | |
| ‘Granny Smith’ | Optimum | 1.5 | 0.0-1.0 | 11 |
| | Minimum | 2.0 | 1.5 | |
| | Maximum | 1.0 | 0.0 | |
| ‘Red Delicious’ | Optimum | 1.5 | 1.5 | 9 |
| | Minimum | 2.0 | 3.0 | |
| | Maximum | 1.0 | 1.0 | |

*6.3.2. Initial low oxygen stress (ILOS)*

Initial low oxygen stress followed by CA has shown positive results in controlling superficial scald of optimally mature ‘Granny Smith’ apples for up to 6 months (Lotze, 1996). ILOS followed by CA has potential to inhibit scald in pre-optimal harvested 'Top Red' and 'Granny Smith' apples for 6 weeks, and for optimal harvested fruit 14 weeks and 16 weeks respectively (van der Merwe et al., 2003). Initial low oxygen stress has been shown to control superficial scald in 'Beurre'd Anjou' pears grown in Argentina and 'Top Red' apples grown in South Africa (Calvo et al., 2002; van der Merwe et al., 2003).

*6.3.3. Dynamic controlled atmosphere (DCA)*

Dynamic controlled atmosphere (DCA) (HarvestWatch ™ SAtlantic Inc. Halifax, N.S Canada) have shown great potential with inhibiting superficial scald (DeLong et al, 2004; Zanella et al., 2008; Prange et al., 2011). Commercial inception of DCA was implemented from 2004 (Prange et al., 2011) and it is used in many countries including Italy, South Africa, North America and Europe (Prange et al., 2011). DCA allows adaptation of atmospheric composition in the CA room in response to the actual physiological state of the fruit contrary to static CA storage conditions. DCA storage is already adapted in some countries including Italy's South Tyrol with 371 rooms with capacity of 129 807 tons of apples (Zanella and Strüz, 2013) and South Africa with 10 commercial rooms and 7 research rooms at Agricultural Research Council (van der Merwe, personal communication, March 2014). Different cultivars show specific response to the applied storage conditions (Zanella et al., 2008).

DCA is a non-destructive technology that allows the storage operator/technician to monitor the physiological response of the fruit using chlorophyll fluorescence in real time without disturbing the atmosphere in the storage room (DeLong et al., 2004; DeLong et al., 2007; Zanella et al., 2008). DCA offers competitive advantages as it is monitored electronically and can warn the operator of any equipment malfunction including CA equipment failure or refrigeration malfunction. It uses existing CA technology to offer extended storage life of fresh fruit (Prange et al., 2011). It has been reported that DCA maintains lower levels of methanol and acetyldehydes inside the fruit compared to conventional CA (1.5% $O_2$) (DeLong et al., 2004; Mattheis and Rudell, 2011), thereby suppressing physiological disorders, but still not shifting to anaerobic fermentation. DeLong et al. (2007) found that DCA treated fruit had higher levels of low-$O_2$ injury (e.g. epidermal purpling) compared to fruit treated with standard controlled atmosphere on organic 'Delicious' apples grown in the Annapolis Valley of Nova Scotia. Cellular $O_2$ deprivation may have occurred in low $O_2$ DCA treated fruit and this perhaps resulted in the purple discolouration of the fruit (DeLong et al., 2007).

Selection of fruit for the fluorescence interactive response monitor (FIRM) sensor (Figure 9) used in DCA technology is critical as this technology relies on a small representative sample of 6 fruit (Watkins, 2008). Mattheis and Rudell (2011) reported positive results on superficial scald control of ‘Beurre‘d Anjou’ pears exposed to DCA up to 8 months in storage. Lack of sufficient softening after 2 months on DCA treated fruit reported by Mattheis and Rudell (2011) on ‘Beurre‘d Anjou’ suggest that DCA treatment is well suited for long term, but not short term storage.

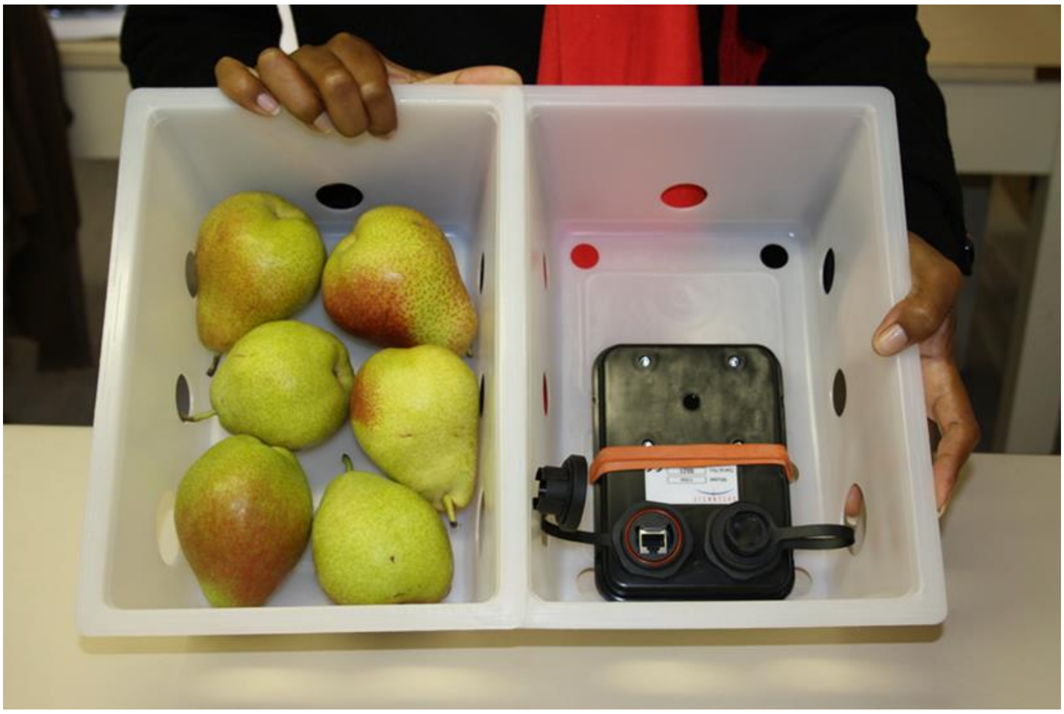

Figure 9. Fluorescence interactive response monitor (FIRM) used in DCA technology (taken at ARC Infruitec-Nietvoorbij, Stellenbosch).

6.4. Wrapping fruit with oils and dipping fruit in oils

Wrapping fruit using oil wraps is one of the oldest and considerably safe non-chemical techniques used in the control of superficial scald of pome fruit. This method dates as far back as 1925 (Brooks et al., 1923a), before the discovery of synthetic antioxidants such as diphenylamine. Brooks et al. (1923a) found that oil wrappers containing more than 15% of mineral oil (about 28g to each 100 wrappers) were more efficient in scald control than wrappers with less than 15% mineral oil. About seven (Wrapper numbers 1-7) different commercially available mineral oils were tested in oiled wrappers and all were efficient in controlling superficial scald.

The oiled wrappers Nos. 1, 1a and 1b were of paraffin origin and were treated with an oil having a yellow colour and a slight odour of kerosene. Wrapper no. 2 was from an oil from the same source as oiled wrappers 1, 1a and 1b, but with higher viscosity. The oil of wrapper 3 was from the same origin as 1 and 2, but with a higher specific gravity and a deeper colour. The oil for wrappers 4, 4a, 4b and 4c was odourless and colourless from a paraffin base. Wrapper no 5 was oiled with a little heavier oil which was odourless, tasteless and colourless. The oil for wrapper no 6 was also from a paraffin base, but a little lighter, but like wrapper no 5 the oil was odourless, tasteless and colourless. The oil from wrapper 7 was from an asphalt base, but with odourless, tasteless and colourless oil.

Apples treated with mineral oil wrappers resulted in normal in taste and appearance, but were generally firmer and crisper than unoiled wrapped oils (Brooks et al., 1923a). Care should be taken when using wrappers with free oil on their surface as this may cause slight injury to the skin of the apple, and they tend to stick together making it impractical for commercial use. Wrapping fruit with oils controlled superficial scald by transferring α-farnesene from the fruit to the wrap (Meigh and Filmer, 1969). Huelin and Coggiola (1968) found that oil wrapped fruit contained less α-farnesene than that of untreated fruit; however, the total content of α-farnesene in wraps and fruit was increased by wrapping due to partitioning of α-farnesene into the wraps (Wang and Dilley, 2000).

The type of oil used on dipped fruit prior to storage affect the taste and colour of fruit, and oil concentration may affect fruit texture (Nikkah and Moghadam, 2005). Nikkah and Moghadam (2005) tested the effects of canola, corn and sunflower oils on reducing decay and extending storage life on ‘Golden Delicious’ and ‘Red Delicious’ apples in France. Corn oil (2%) resulted in better sensory attributes on both ‘Golden Delicious’ and ‘Red Delicious’ apples after 6 months in storage (Nikkah and Moghadam, 2005).

## 7. Conclusions

Storage technologies involving low oxygen (DCA, ILOS and CA) and ethylene inhibitors have shown positive results in extending the storage life of horticultural commodities including apples and pears, and in controlling the superficial scald storage disorder. 1-MCP controls superficial scald and reduces senescence breakdown; however, it does not control other physiological disorders such as bitter pit, but may increase external $CO_2$ damage to the fruit peel. Another disadvantage of 1-MCP is that it is a chemical and it might experience some resistance from some consumers, especially with regard to organic markets. 1-MCP has challenges in ripening of pears as consumers prefer juicy and less firm pears, but achieved excellent results on apples as they remain green and firm, in accordance to consumer preferences.

DPA controls superficial scald and it reduces Bitter pit symptoms in susceptible cultivars; however, the use is limited due to its restriction in export markets. Lower oxygen treatments control superficial scald and reduce bitter pit symptoms in susceptible cultivars, however when fruit are transferred to ripening temperatures the increase in the burst of ethylene is evident which results in quick ripening, thereby reducing the shelf-life of the fruit. Lower oxygen treatments are, however, non-chemical and will be suitable for organic markets.

For the South African pome fruit industry to maintain its competitiveness as a major exporter, it is imperative that these alternative technologies be tested on South African fruit, to extend storage life and inhibit external physiological disorders such as superficial scald. Volatiles α-farnesene and MHO have been implicated in the incidence and severity of superficial scald and can be used to determine how treatments inhibit superficial scald in apples and pears. The accumulation of α-farnesene in fruit does not necessarily result in scald incidence as long as it is not oxidised to toxic autoxidation products such as conjugated trienes, reactive oxygen species and MHO during cold storage.

## 8. References

Anet, E.F.J.L. (1969). Autoxidation of α-Farnesene. *Australian Journal of Chemistry*, 22, 2403-2410.

Anet, E.F.J.L. (1972a). Superficial scald a functional disorder of stored apples. VIII. Volatile products from the autoxidation of α-farnesene. *Journal of the Science of Food and Agriculture*, 23, 605-608.

Anet, E.F.J.L. (1972b). Superficial scald a functional disorder of apples. IX. Effect of maturity and ventilation. *Journal of the Science of Food and Agriculture*, 23, 763-769.

Barden, C.L. & Bramlage, W.J. (1994a). Relationships of antioxidants in apple peel to changes in α-farnesene and conjugate trienes during storage, and to superficial scald development after storage. *Postharvest Biology and Technology*, 4, 23-33.

Barden, C.L. & Bramlage, W.J. (1994b). Separating the effects of low temperature, ripening, and light on loss of scald susceptibility in apples before harvest. *Journal of American Society of Horticultural Science*, 119, 54-58.

Blackenship, S.M. & Dole, J.M. (2003). 1-Methylcyclopropene: A review. *Postharvest Biology and Technology*, 28, 1-25.

Blanpied, G.D., Bramlage, W.J., Chu, C.L., Ingle, M., Kushad, M.M., Lau, O.L. & Lidster, P.D. (1991). A survey of the relationships among accumulated orchard hours below 10°C, fruit maturity, and the incidences of storage scald on 'Starkimson Delicious' apples. *Canadian Journal of Plant Science*, 71, 605-608.

Bramlage, W.J., Weis, A.S. & Drake, M. (1982). Contributions of macronutrient mineral concentration in fruit to losses of Massachusetts apples after storage. *Controlled atmosphere for storage and transport of perishable agricultural commodities*, 1, 193-200.

Bramlage, W.J. & Meir, S. (1989). A potential method for predicting susceptibility of apples to superficial scald. *Acta Horticulturae*, 258, 397-404.

Bramlage, W.J. (1993). Interactions of orchard factors and mineral nutrition on quality of pome fruit. *Acta Horticulturae*. 326, 15-28.

Brooks, C., Cooley, J. & Fisher, D. (1923a). Oiled wrappers, oil and waxes in the control of apple scald. *Journal of Agricultural Research*, XXVII, 513-536.

Brooks, C., Cooley, J. & Fisher, D. (1923b). Apple scald and its control. United States Department of Agriculture, *Farmers Bulletin*, 1380, 1-27.

Brooks, P.J. (1968). Pre-harvest lenticel spot of apples. *New Zealand Journal of Agricultural Research*, 11 (2), 237-244.

Burger, G.E. (2005). Factors affecting shrivelling and friction discolouration of pears (*Pyrus communis L*) MSc. Thesis, Stellenbosch University, Stellenbosch. http://scholar.sun.ac.za.

Calvo, G., Salvador, M.E. & Sanchez, E. (2002). Control of superficial scald in 'Beurre'd Anjou' pears with low oxygen levels. *Acta Horticulturae*, 596, 879-882.

Calvo, G. (2003). Effect of 1-Methylcyclopropene (1-MCP) on pear maturity and quality. *Acta Horticulturae*, 628, 203-209.

Calvo, G. (2010). Antioxidant use in apple and pear storage Part 3: Storage scald and 1-Methylcyclopropene (1-MCP), 1-5. http://postharvest.tfrec.wsu.edu/REP2010E.pdf.

Chen, P.M., Varga, D.M., Mielke, E.A., Facteau, T.J. & Drake, S.R. (1990). Control of superficial scald on d'Anjou' pears by ethoxyquin: Oxidation of α-farnesene and its inhibition. *Journal of Food Science*, 55, 272-275.

Chen, P.M. & Varga, D.M. (1996). Storage challenges with winter pears. In 1996 proceedings Storage challenges with winter pears, 1-5. http://postharvest.tfrec.wsu.edu/.

Commission Regulation (EU) N0 772/2013, (2013). *Official Journal of European Union*. Brussels.

DAFF, (2014a). A profile of the South African apple market value chain (2014). Department of Agriculture, Fisheries and Forestry 2011/12 retrieved on the 22nd of October 2015. www.daff.gov.za.

DAFF, (2014b). A profile of the South African pear market value chain (2014). Department of Agriculture, Fisheries and Forestry 2011/12 retrieved on the 22nd of October 2015. www.daff.gov.za.

DAFF, (2014c). Economic Review of the South African Agriculture (2014). Department of Agriculture, Forestry and Fisheries, Republic of South Africa retrieved 22nd of October 2015. www.daff.gov.za.

DAFF, (2014d). Food Trade SA. The South African food trade and supply chain directory, 13th ed., (2014). Department of Agriculture, Forestry and Fisheries, Republic of South Africa retrieved on the 22nd of October 2015. www.daff.gov.za.

DeEll, J.R., Murr, D.P., Mueller, L.W., Willey, L. & Porteous, M.D. (2005). Influence of 1-methylcyclopropene (1-MCP), diphenylamine (DPA), and $CO_2$ concentration during storage on 'Empire' apple quality. *Postharvest Biology and Technology*, 38, 1-8.

DeKock, S.L. & Combrink, J.C. (1996). Nature and origin of postharvest decay. In Combrink J.C. (Ed.), *Integrated management of postharvest quality* (pp. 50-56). ARC INFRUITEC: Paarl Printing, Paarl.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2004). A new technology that determines low-oxygen thresholds in controlled atmosphere stored apples. *HortTechnology*, 14, 262-266.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2007). Chlorophyll fluorescence based low-$O_2$ CA storage of organic 'Cortland' and 'Delicious' apples. *Acta Horticulturae*, 737, 31-37.

Diamantidis, Gr., Thomai, T., Genitsariotis, M., Nanos, G., Bolla, N. & Sfakiotakis, E. (2002). Scald susceptibility and biochemical/physiological changes in respect to low pre-harvest temperature in 'Starking Delicious' apple fruit. *Scientia Horticulturae*, 361-366.

Drake, S. R., Larsen, F. E. & Higgins, S.S. (1991). Quality and storage of 'Granny Smith' apples on seedling, M.26, and MM.111 rootstocks. *Journal of American Society of Horticultural Science,* 116, 261-264.

Emongor, V.E. (1988). The effect of nitrogen and phosphorous on growth, yield of flowers, and essential oil of chamomile (*Matricaria chamomilla*) growth under Kenyan conditions. Master of Science Thesis, Faculty of Agriculture, University of Nairobi.

Emongor, V.E., Murr, D.P. & Lougheed, E.C. (1994). Pre-harvest factors that predispose apples to superficial scald. *Postharvest Biology and Technology,* 4, 289-300.

Ferguson, I.B. & Watkins, C.B. (1989). Bitter pit in apple fruit. *Horticultural Reviews*, 11, 289-355.

Ferguson, I., Volz, R. & Woolf, A. (1999). Preharvest factors affecting physiological disorders of fruit. *Postharvest Biology and Technology*, 15, 255-262.

Filmer, A.A.E. & Meigh, D.F. (1971). Natural skin coating of the apple and its influence on scald in storage. IV. Oxidation products of α-farnesene. *Journal of the Science of Food and Agriculture,* 22, 188-190.

Findlay, J.S. & Combrink, J.C. (1996). Cold-chain management. In Combrink C. (Ed.) *Integrated management of post-harvest quality* (pp. 79-90). ARC INFRUITEC: Paarl Printing, Paarl.

Franck, C., Lammertyn, J., Ho, Q.T., Verboven, P., Verlinden B. & Nicolai, B.M. (2007). Browning disorder in pear fruit. Review. *Postharvest Biology and Technology*, 43, 1-13.

Ghahramani, F. & Scott, K.J. (2000). Effects of alcohol vapours and oxygen stress on superficial scald and red colour of stored 'Delicious' Apples. *HortScience*, 35, 1292-1293.

Gindaba, J. & Wand, S.J.E. (2005). Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience*, 40, 592-596.

HORTGRO, (2014). HORTGRO TREE CENSUS 2014 retrieved on the 22nd of October 2015. www.hortgro.co.za.

Huelin, F.E. & Coggiola, I.M. (1968). Superficial scald, a functional disorder of stored apples. IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of a-farnesene in the fruit. *Journal of the Science of Food and Agriculture*, 19, 298-301.

Huelin, F.E. & Coggiola, I.M. (1970a). Superficial scald, a functional disorder of stored apples. V. Oxidation of α-farnesene and its inhibition by diphenylamine. *Journal of the Science of Food and Agriculture,* 21, 44-48.

Huelin, F.E. & Coggiola, I.M. (1970b). Superficial scald, a functional disorder of stored apples. VII. –Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration of α-farnesene in the fruit. *Journal of the Science of Food and Agriculture*, 21,583-589.

Hurndall, R. & van der Merwe, J.A. (Eds.) (2005). *Operators manual for controlled atmosphere (CA) storage,* Deciduous Fruit Producers' Trust.

Ingle, M. & D' Souza, M. (1989). Physiology and control of superficial scald of apples: A review. *Journal of Food Science,* 24, 28-31.

Ingle, M. (2001). Physiology and biochemistry of superficial scald of apples and pears. *Horticultural Reviews*, 27, 228-261.

Isidoro, N. & Almeida, P.F. (2006). α-farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. *Postharvest Biology and Technology*, 42, 49-56.

Jung, S.K. & Watkins, C.B. (2008). Superficial scald control after delayed treatment of apple fruit with diphenylamine (DPA) and 1-methylcyclopropene (1-MCP). *Postharvest Biology and Technology*, 50, 45-52.

Kader, A. (1989). Mode of action of oxygen and carbon dioxide on postharvest physiology of 'Bartlett' pears. *Acta Horticulturae,* 88, 161-168.

Kader, A. (1995). Regulation of fruit physiology by controlled/modified atmospheres. *Acta Horticulturae*, 398, 59-69.

Kader, A. (1996). Maturity, ripening, and quality relationships of fruit-vegetables. *Acta Horticulturae*, 434, 249-255.

Kader, A. (1999). Fruit maturity, ripening and quality relationships. *Acta Hortiulturae*, 485, 203-208.

Kader, A. (2002). Postharvest biology and technology, an overview. In Kader, A. (Ed.), *Postharvest technology of horticultural crops*, (3rd ed., pp. 39-47). University of California Publication 3311.

Kays, J.S. (1999). Pre-harvest factors affecting appearance. *Postharvest Biology and Technology*, 15, 233-247.

Kupferman, E. (2001). Storage scald of apples. Postharvest information network, 1-7. http://postharvest.tfrec.wsu.edu/EMK2000C .

Lau, O.L. (1997). The effectiveness of 0.7% oxygen to attenuate scald symptoms in 'Delicious' apples is influenced by harvest maturity and cultivar strain. *Journal of American Society of Horticulture*, 122 (5), 691-697.

Lee, J., Cheng L., Rudell, D.R. & Watkins, C.B. (2012). Antioxidant metabolism of 1-methylcyclopropene (1-MCP) treated 'Empire' apples during controlled atmosphere storage. *Postharvest Biology and Technology*, 65, 79-81.

Lotze, E. (1996). Control of physiological postharvest disorders. In Combrink, C. (Ed.), *Integrated management of postharvest quality* (pp. 43-48). ARC INFRUITEC: Paarl Printing, Paarl.

Lotze, E., van der Merwe, K. & Rogalska, S. (2003). *Manual for the identification of post-harvest disorders of pome and stone fruit* (3rd rev ed., pp. 34-36). ARC: Infruitec-Nietvoorbij.

Lotze, E.M. & Theron, K.I. (2007). Evaluating the effectiveness of pre-harvest calcium applications for bitter pit control in 'Golden Delicious' apples under South African conditions. *South Africa Journal of Plant Nutrition*, 30, 471–485.

Lurie, S. & Watkins, C.B. (2012). Superficial scald, its etiology and control. A review. *Postharvest Biology and Technology*, 65, 46-60.

Manseka, V.S. & Vasilakakis, M. (1993). Effect of maturity, postharvest treatments and storage conditions on superficial scald and quality of apples. *Acta Horticulturae*, 326, 213-224.

Mattheis, J.P. & Rudell, D. (2011). Responses of 'd'Anjou' pear (*Pyrus communis* L.) fruit to storage at low oxygen set points determined by monitoring fruit chlorophyll fluorescence. *Postharvest Biology and Technology*, 60, 125-129.

Meigh, F.D. & Filmer, A.E. (1967). Natural skin coating of the apple and its influence on superficial scald. II. *Journal of the Science of Food and Agriculture*, 20, 140-143.

Meigh, D.F. (1969). Production of farnesene and incidence of scald in stored apples. *Qualitas Plantarum et Materiae Vegetabiles*, XIX, 1-3, 243-254.

Meigh, F.D. & Filmer, A.E. (1971). Natural skin coating of the apple and its influence on scald in storage. IV. Oxidation products of α-farnesene. *Journal of the Science of Food and Agriculture*, 22, 188-190.

Mir, N., Perez, R. & Beaudry, R.M. (1999). A post storage burst of 6-methyl-5-hepten-2-one (MHO) may be related to superficial scald development in 'Cortland' apples. *Journal of American Society of Horticultural Science*, 124, 173-176.

Mitcham, E.J. & Mitchell, F.G. (2002). Postharvest handling systems: Pome fruits. In Kader A.A. (Ed.), *Postharvest technology of horticultural crops* (3rd ed., pp. 333-344). University of California Publication 3311.

Moggia, C., Moya–León, M.A., Pereira, M., Yuri, J.A. & Lobos, G.A. (2010). Effect of DPA and 1-MCP on chemical compounds related to superficial scald on 'Granny Smith' apples. *Spanish Journal of Agricultural Research*, 8, 178-187.

NAMC, (2014). South African trade flow, 13, 2014. National agricultural and marketing council retrieved on the 18th of November 2014. www.namc.co.za.

Nikkah, S.H. & Moghadam, E.G. (2005). Effect of plant oils on reducing decay and extending storage life in 'Golden Delicious' and 'Red Delicious' apples. *Information and Technology for sustainable fruit and vegetable production*, 5, 61-68.

Olivier, O.J. (1996). Production factors affecting quality. In Combrink J.C., (Ed.) *Integrated management of post-harvest quality,* (pp. 8-13). ARC INFRUITEC: Paarl Printing, Paarl.

Opara, U.L. (2009). Quality management: An industrial approach to produce handling. In Florskowski, W.J., Shewfelt, R.L., Brueckner, B. & Prussia, S.E. (Eds.), *Postharvest handling: A systems Approach* ($2^{nd}$ ed., pp. 153-204). San Diego Academic Press.

Pesis, E., Ben-Arie, R., Feygernberg, O., Lichter, A., Gadiyeva, O., Antilofayev, I. & Uryupina, T. (2007). A simple pre-treatment with low oxygen alleviate superficial scald in 'Granny Smith' apples. *Journal of the Science of Food and Agriculture*, 87, 1836-1844.

Pesis, E., Ebeler, S.E., de Freitas, S.T., Padda, M. & Mitcham, E.J. (2010). Short anaerobiosis period prior to cold storage alleviates bitter pit and superficial scald in 'Granny Smith' apples. *Journal of the Science of Food and Agriculture*, 90, 2114-2123.

PPECB, (2014). Annual Report, 2013/2014. The Perishable Products Export Control Board retrieved on the $22^{nd}$ of October 2015. www.ppecb.com.

Prange, R.K., DeLong, J.M. & Harrison, P.A. (2005). Quality management through respiration control: Is there a relationship between lowest acceptable respiration, chlorophyll fluorescence and cytoplasmic acidosis? *Acta Horticulturae*, 682, 823-830.

Prange, R.K., DeLong, J.M. & Wright, A.H. (2011). Storage of pears using dynamic controlled atmosphere (DCA), a non-chemical method. *Acta Horticulturae*, 909, 707-717.

Raese, J.T. & Drake, S.R. (2000). Effect of calcium sprays, time of harvest, cold storage and ripeness on fruit quality of Ánjou' pears. *Journal of Plant Nutrition*, 23, 843-853.

Robatscher, P., Eisenstecken, D., Sacco, F., Pohl, H., Berger, J., Zanella, A. & Oberhuber, M. (2012). Diphenylamine residues in apples caused by contamination in fruit storage facilities. *Journal of Agricultural and Food Chemistry*, 60, 2205-2211.

Rupasinghe, H.P.V., Paliyath, G. & Murr, D.P. (2000). Sesquiterpene α-farnesene synthase: Partial purification, characterization and activity in relation to superficial scald development in apples. *Journal of American Society of Horticultural Science*, 125, 111-119.

Saltveit, M.E. (2003). Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology*, 27, 3-13.

Sharples, P.O. (1984). The influence of pre-harvest conditions on the quality of stored fruit. *Acta Horticulturae*, 157, 93-102.

Shepherd, P.A. (2005). The role of CA storage in the export of deciduous fruit. In Hurndall, R., & van der Merwe, J.A. (Eds.), *Operator's manual for controlled atmosphere for Controlled atmosphere (CA) storage* (p. 24). Deciduous Fruit Producers Trust, Paarl, South Africa.

Taiz, L., & Zeiger, E. (2010). *Plant Physiology* (5th ed., p. 330). Sunderland Massachusets U.S.A: Sinauer Associates Incorporated.

Thomai, T., Sfakiotakis, E., Diamantidis, Gr. & Vasilakakis, M. (1998). Effects of low pre harvest temperature on scald susceptibility and biochemical changes in 'Granny Smith' apple peel. *Scientia Horticulturae*, 76, 1-15.

Thompson, J.F. (2002). Storage systems. In Kader, A. (Ed.), *Postharvest Technology of Horticultural crops* (3rd ed., pp. 113-127). University of California Publication 3311.

Van der Merwe, J.A. (1996). Controlled atmosphere storage. In Combrink J.C. (Ed.) *Integrated management of post-harvest quality* (pp. 25-32). ARC INFRUITEC: Paarl Printing, Paarl.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on

South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae*, 600, 261-265.

Van der Merwe, J.A. (2005). Recommended temperature and gas regimes for cold storage of South African commercial apple and pear cultivar. In Hurndall, R. & van der Merwe, J.A. (Eds.), *Operator's manual for controlled atmosphere (CA) storage* (pp. 45-46). Deciduous fruit producers' Trust.

Veltman, R.H., Lenthe´ric, I., Van der Plas, L.H.W. & Peppelenbos, H.W. (2002). Internal browning in pear fruit (*Pyrus communis* L. cv. 'Conference') may be a result of a limited available energy and antioxidants. *Postharvest Biology and Technology*, 28, 295-302.

Veltman, R.H., Verschoor, J.A. & Ruijisch van Dugteren, J.H. (2003). Dynamic controlled system (DCS) for apples (*Malus domestica Borkh*. cv. Elstar): optimal quality through storage based on product response. *Postharvest Biology and Technology*, 27, 79-86.

Von Mollendorff, L.J. (1996). Development of physiological postharvest disorders. In Combrink, J.C. (Ed.), *Integrated management of post-harvest quality* (pp. 39-42). ARC INFRUITEC: Paarl printing, Paarl.

Wang, Z. & Dilley, D.R. (1999). Control of superficial scald of apples by low oxygen atmospheres. *HortScience.* 34, 1145-1151.

Wang, Z. & Dilley, D.R. (2000). Hypobaric storage removes scald-related volatiles during low temperature induction of superficial scald of apples. *Postharvest Biology and Technology*, 18, 191-199.

Watkins, C.B., Bramlage, W.J. & Cregoe, B.A. (1995). Superficial scald of 'Granny Smith' is a chilling injury. *Journal of American Society Horticultural Science,* 120, 88-94.

Watkins, C.B. (2008). Postharvest ripening regulation and innovation in storage technology. *Acta Horticulturae*, 796, 51-58.

Watkins, C.B. (2009). Postharvest physiological disorders and mineral nutrients. *New York Fruit Quarterly*, 17, 17-20.

Watkins, C.B. & Nock, J.F. (2012). Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. *Postharvest Biology and Technology*, 69, 24-31.

Weeks, W. D., Southwick, F. W., Drake, M. & Olanyk, G. W. (1965). Relation of differential N and K fertilization to tree performance, fruit quality and storage disorders of 'Delicious' apples. *Massachusetts Agricultural Experiment Station Bulletin*, 552, 1-20.

Whitaker, B.D. (2000). DPA treatment alters α-farnesene metabolism in peel of 'Empire' apples stored in air or 1.5% $O_2$ atmosphere. *Postharvest Biology and Technology*, 18, 91-97.

Whitaker, B.D., Nock, F.J. & Watkins, B.C. (2000). Peel tissue and α-farnesene and conjugated trienol concentrations during storage of 'White Angel' X 'Rome Beauty' hybrid apple selections susceptible and resistant to superficial scald. *Postharvest Biology and Technology*, 20, 231-241.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, E.J. & Mattheis, J.P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology*, 58, 43-50.

Wilkinson, B.G. & Fidler J.C. (1973). Physiological disorders in: *The biology of apple and pear storage*. Common Wealth Bureaux Research Review 3 (pp. 65-80). England.

Wills, R., McGlasson, B., Graham, D. & Joyce, D. (1989). *Postharvest an introduction to the physiology and handling of fruit, vegetables and ornamentals* (3rd edn. pp. 73-87). BSP professional books: A division of Blackwell Scientific publications Ltd.

Wills, R.B.H., McGlasson, W.B., Graham, D. & Joyce, D. (2007). Postharvest an introduction to the physiology and handling of fruit vegetables and ornamentals (5th edn. pp. 167-186). University of South Wales Press Ltd.

Zanella, A. (2003). Control of apple superficial scald and ripening- a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra-low oxygen storage. *Postharvest Biology and Technology*, 27, 69-78.

Zanella, A., Cazzanelli, P., Panarese, A., Coser, M., Cecchinel, M. & Rossi, O. (2005). Fruit fluorescence response to low oxygen stress: Modern storage technologies compared to 1-MCP treatment of apple. *Acta Horticulturae*, 682, 1535-1542.

Zanella, A., Cazzanelli, P. & Rossi, O. (2008). Dynamic controlled atmosphere (DCA) storage by means of chlorophyll fluorescence response for firmness retention in apple. *Acta Horticulturae*, 796, 77-82.

Zanella, A. & Stürz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10$^{th}$ of EU's apples producing area. *Acta Horticulturae,* 1012, 419-426.

Zoffoli, J.P., Richardson, D., Chen, P. & Sugar, D. (1998). Spectrophotometric characterization of superficial and senescent scald in pear fruits relative to different stages of maturity. *Acta Horticulturae,* 475, 543-558.

# PAPER 1: EFFECTS OF DYNAMIC CONTROLLED ATMOSPHERE AND INITIAL LOW OXYGEN STRESS ON PHYSIOLOGICAL DISORDERS AND INTERNAL QUALITY OF 'PACKHAM'S TRIUMPH' (*PYRUS COMMUNIS L.*) PEARS AFTER LONG TERM COLD STORAGE.

**Abstract**

'Packham's Triumph' pears are prone to development of superficial scald and shrivelling after extended periods in cold storage. The aim of this study was to evaluate the efficacy of controlled atmosphere technologies to inhibit the incidence of disorders (superficial scald, neck shrivelling and decay) and maintain internal quality of 'Packham's Triumph' pears. Assessment of external physiological disorders and measurement of internal quality parameters were carried out at the end of 2, 3, 5 and 7 months cold storage at -0.5 °C, and subsequently after 0, 3 and 7 days shelf life at 20 °C. The long term storage treatments incorporated periods of shipping simulation in regular atmosphere (RA) since these pears are shipped to the export markets. The trial was carried out over two harvest seasons: 2012 and 2013. It was found that dynamic controlled atmosphere (DCA) treatment inhibited superficial scald for up to 7 months, while controlled atmosphere (CA) storage preceded by initial low oxygen stress (ILOS) inhibited superficial scald for up to 5 months on 'Packham's Triumph' pears. DCA storage technology reduced decay by 19% compared to CA preceded by ILOS after 7 months in storage, followed by 7 days shelf life. Fruit had symptoms of neck shrivelling after 7 months in cold storage irrespective of treatment applied, however the percentages were below acceptable limit at the point of sale. There were no signs of internal breakdown on the 'Packham's Triumph' pears for both seasons (2012 and 2013). There was no significant difference on sugar: acid ratio after 7 months of storage, followed by 7 days shelf life, irrespective of treatment applied during storage. DCA, CA preceded by ILOS and CA storage treatments maintained total soluble sugars and titratable acid better than the control fruit after 7 months cold storage followed by 7 days shelf life.

## 1. Introduction

The major market for South African grown pears is the European Union (EU), requiring long term storage at lower temperatures and transportation to these markets without compromising quality of fruit. South Africa exports approximately 203 660 tons of pears per annum (PPECB, 2014), with 'Packham's Triumph' being the most exported at 33%.

Although cold storage extends the storage life of fruit it may result in physiological disorders. Physiological disorders are deviations from normal ripening and senescence processes characteristic with abnormal appearance (external and internal), or even abnormal flavours (Wilkinson and Fidler, 1973).

'Packham's Triumph' pears has longer storage potential, but this advantage is limited as the fruit is highly susceptible to physiological storage disorders including superficial scald (Wilkinson and Fidler, 1973; Calvo, 2003) and shrivelling (Burger, 2005). Superficial scald is an important physiological disorder as it reduces the market value of the pears (Wilkinson and Fidler, 1973; Wang and Dilley, 1999) for fresh produce market however severely affected fruit may be utilised for processing (Wilkinson and Fidler, 1973; Bauchot et al., 1999). Superficial scald affects the peel of the fruit without any effect on the underlying flesh (Wills et al., 1989).

Many consumers prefer fruits and vegetables with no chemical pesticides, and this has resulted with reviews of legislation on pesticide use, especially after harvest (Thompson, 2003). South Africa, like most developing countries, is under immense pressure to adhere to set maximum residue limit (MRL) to avoid loss of competitiveness in the global market. For decades the synthetic antioxidant diphenylamine (DPA) has been used as a drench to prolong the storage life of South African grown pears, however its use has been further restricted from 5 ppm to 0.1 ppm MRL, effective from April 2014 (Commission Regulation (EU) No. 772/2013 of 8 August 2013). It is therefore imperative that non-chemical technologies be investigated as alternatives to the use of the DPA to control superficial scald and prolong storage and shelf life of pears, without compromising eating quality.

Dynamic controlled atmosphere (DCA) technology is already implemented in most apple and pear growing countries including United States and South Tyrol in Italy (Zanella and Strüz, 2013). South African pome fruit industry faces a unique challenge as there is a need to incorporate handling and transportation time when developing and testing storage technologies as the industry is export orientated. Technologies that abide with legislation on MRL's are required to extend the storage life of pome fruit. DCA technology has shown potential on scald control of organic 'Uta' pears grown in St. Pölten, Lower Austria (Lafer, 2011), 'Granny Smith' apples grown in the orchards of the Adige valley, South-Tyrol (Italy) (Zanella et al., 2005), 'Granny Smith' and 'Red Delicious' apple cultivars grown in South Tyrol, Italy (Zanella and Strüz, 2013), organic 'Cortland' and 'Delicious' apples grown in

Nova Scotia, USA (DeLong et al., 2007), and on 'Cortland', 'Golden Delicious', 'Honey Crisp', 'Jonagold', 'McIntosh' and 'Delicious' apples (DeLong et al., 2004). DCA technology has not been tested on 'Packham's Triumph' pears and it is well documented in literature that each commodity's ideal storage conditions need to be determined for each cultivar. Various cultivars respond differently to gas combinations (Wills et al., 1989).

CA preceded by ILOS has shown positive results on superficial scald control on 'Beurre d' Anjou' pear, 'Granny Smith', 'Law Rome, 'Idared' and 'Red Delicious' apples (Key et al., 1990; Wang and Dilley, 2000; Little and Holmes, 2000; Calvo et al., 2002; van der Merwe et al., 2003). The main advantage of ILOS is the inhibition of superficial scald which is achieved without the use of synthetic antioxidants (Little and Holmes, 2000) and no added costs for installation of software as is the case with the use of DCA. Australian researchers have found that 'Packham's Triumph' pears responded well to ILOS (Little and Holmes, 2000). The pull down to desired low oxygen level should be achieved in the first five to ten days otherwise delays may render the treatment ineffective (Little and Holmes, 2000).

The aim of the study was to investigate controlled atmosphere technologies such as DCA, CA preceded by ILOS, CA, RA preceded by ILOS to determine their efficacy to control superficial scald and maintain quality of 'Packham's Triumph' pears at long term storage to offer an alternative to the use of DPA for the South African growers and exporters. DPA was however not utilised in this trial to avoid cross contamination in the storage rooms (Robatscher et al., 2012).

## 2. Materials and methods

### 2.1. Fruit supply

'Packham's Triumph' pears were harvested at optimal maturity on 13-15 February (Table 1) during the 2012 and 2013 seasons from Grabouw production area (34.1500°S, 19.0167°E), Western Cape Province, South Africa. Fruit were transported in bulk bins to the Agricultural Research Council Infruitec-Nietvoorbij laboratory and sorted immediately on arrival to remove fruit with external defects such as sunburn, mechanical damage and decay. After sorting, fruit were put into crates, with each treatment in triplicate, and each replicate consisted of 90 – 120 fruit.

## 2.2. Storage treatments and periods

Fruit were stored for up to 7 months at -0.5 °C in 5 storage treatments at various $O_2$ and $CO_2$ combinations (Table 2).

### *2.2.1. Regular atmosphere preceded by initial low oxygen stress (RA preceded by ILOS)*

In RA preceded by ILOS fruit were stressed with low oxygen at 0.7% for 10 days as a preconditioning treatment (Table 3), before being transferred into the RA room. For the 2 months storage period fruit were kept in ILOS for 10 days only, and were followed for 8 weeks in RA to simulate shipment and handling. 'Granny Smith' apples and 'Packham's Triumph' pears destined for the export market, are first stored under CA conditions between 8 and 12 weeks before being packed and exported under regular atmosphere as a general handling and shipment protocol in South Africa (Crouch, 2003). For 3 months storage period fruit were kept in ILOS for 10 days only and thereafter kept in RA for 12 weeks. For 5 months storage period fruit were kept in ILOS for 10 days and followed by 22 weeks in RA. For 7 months storage period fruit were kept in ILOS for 10 days only and thereafter followed by 30 weeks of RA to extend the marketing window.

### *2.2.2. Controlled atmosphere preceded by initial low oxygen stress (CA preceded by ILOS)*

Fruit treated with CA preceded by ILOS were kept for 10 days in initial low oxygen stress before transfer into CA. For the two months storage period fruit were stored in ILOS for 10 days and transferred into CA for 8 weeks (Table 3). For three months storage period fruit were stored in ILOS for 10 days followed by 6 weeks in CA and thereafter transferred to RA for 6 weeks to simulate shipment and handling. After the 5 months storage period the fruit were stored in ILOS for 10 days and thereafter transferred to CA for 16 weeks followed by 6 weeks in RA to simulate shipment and handling. For 7 months storage period fruit were preconditioned with ILOS for 10 days and transferred into CA for 24 weeks followed by RA for 6 weeks to simulate shipment and handling (Table 3).

### *2.2.3. Dynamic controlled atmosphere (DCA)*

DCA treated fruit were kept for 10 days in DCA followed by 8 weeks in RA to simulate shipment and handling for the 2 months storage period (Table 3). For the 3, 5 and 7 months storage periods the DCA treated fruit were kept in the DCA room for 6, 16 and 24 weeks respectively, and then transferred into RA for 6 weeks to simulate shipment and handling. The DCA treatment facility was fitted with HarvestWatch™ (SAtlantic Inc. Halifax, N.S., Canada) fluorescence interactive response monitor sensor (FIRM) to monitor fruit response to low $O_2$ levels hourly. The selection of representative sample of 6 fruit for each FIRM sensor is crucial (Zanella et al., 2005), because the selected fruit represent the whole batch in the treatment room. The fruit were loaded into cold rooms and the desired fruit core temperature of -0.5 °C was achieved within 48 to 96 hours after harvest. The spiking for lower oxygen limit was set at 0.3% $O_2$ for both harvest seasons (2012 and 2013), and it was thereafter adjusted to 0.5% $O_2$ for the rest of the storage period as explained by DeLong et al. (2007). The DCA protocol and the pull down process should be completed within few days after receiving the fruit (Figure 1).

*2.2.4. Controlled atmosphere (CA)*

CA fruit were kept for 10 days in CA followed by 8 weeks of RA for the 2 months storage period (Table 3). For the 3, 5 and 7 months storage period, fruit were kept in CA for 6, 16 and 24 weeks respectively and thereafter transferred to RA for 6 weeks to simulate shipment and handling.

*2.2.5. Regular atmosphere (RA)*

RA was the control treatment for the trial. RA fruit were kept solely in RA for the duration of storage of 2, 3, 5 and 7 months (Table 3).

2.3. Physiological disorders.

*2.3.1 Superficial scald intensity (scald index)*

Fruit affected by superficial scald were counted at the end of each storage period, and after 7 days of shelf life for each replicate (90-120) and the number of fruit affected was recorded as percentage. Each fruit was inspected visually and the percentage area of the fruit surface affected (intensity) with the disorder was recorded. The intensity of the disorder was evaluated and graded according to an index used by Magwaza et al. (2012) on rind breakdown of 'Nules Clementine' mandarin, based on the percentage surface area of fruit affected (Figure 2), resulting in the following categories: Grade 0 = no scald (0%), Grade 1 = Slight scald (1-25%), Grade 2 = Moderate scald (26-50%), Grade 3 = Severe scald (51-75%), Grade 4 = Extreme scald (76-100%). The inspection of fruit was done after each storage interval at 0 day, and after 7 days shelf life at 20 °C in the 2012 season, however in 2013 the fruit were evaluated at 0 day, 3 and 7 days shelf life.

*2.3.2. Neck shrivelling*

Fruit were evaluated for shrivelling and the total of fruit affected was recorded for each replicate as a percentage. The incidence of neck shrivelling was evaluated at the end of storage life at 0 and 7 days shelf life at 20 °C.

*2.3.3. Decay incidence*

The incidence of decay (%) was evaluated during each evaluation after 2, 3, 5 and 7 months of cold storage followed by a shelf life period of 0 and 7 days, after each storage period.

*2.3.4. Internal breakdown*

A total of 10 fruit per replicate was cut into half and evaluated for internal breakdown. Number of fruit with the internal breakdown disorder was then recorded as a percentage.

2.4. Internal quality

A total of 10 fruit were from each replicate of 90-120 fruit was randomly sourced and evaluatated for total soluble solids (TSS) and titratable acidity (TTA). Each fruit weighed 50-60 g, however only 20 g of the fruit segments were cut and placed into a domestic juicer for extraction of juice for TSS and TTA measurements.

*2.4.1. Total soluble solids (TSS) (°Brix)*

Fruit segments (20 g) were cut from each of the 10 fruit from each replicate and blended, and the juicing was done using a domestic juice extractor (Mellerware Liquafresh juice extractor III), and filtered through a muslin cloth. A drop of the juice was placed onto the calibrated refractometer (Palette PR 32 α (˚Brix 32) ATAGO Co. LTD, Japan) and the reading recorded. The instrument was standardised before taking readings with distilled water which has a refractive index of 0. The refractometer was cleaned between readings.

*2.4.2. Titratable acidity (TTA) (% malic acid)*

Fruit segments of 20 g were cut from each of the 10 fruit were blended and 10 mL of the representative juice sample titrated with a 0.1N sodium hydroxide solution to a pH of 8.2 using titrator (Crison Titromatic, Crison Instruments E 08238, Allela, Barcelona Spain). The volume in millilitres of NaOH needed is used to calculate the TTA and the percentage TTA was expressed as percentage of malic acid and can be calculated as follows: % acid = (mL NaOH x 0.067)/ (mL juice titrated x 100) where 0.067 is the acid milliequivalent factor for malic acid, the predominant organic acid in pears.

*2.4.3. Sugar to acid ratio (TSS/TTA)*

The sugar to acid ratio was quantified using the following formula:

$$\text{TSS/TTA ratio} = \frac{\text{Total soluble solids}}{\text{Titratable acidity}} \qquad (1)$$

2.5. Trial design and statistical analysis

The trial design was a completely randomised three–way factorial with treatment (5), storage periods (4) and shelf life period (3) as sources of variation (5 storage treatments x 4 storage periods x 3 shelf life periods). Five treatments evaluated were: dynamic controlled atmosphere (DCA), controlled atmosphere (CA), regular atmosphere (RA) or control, controlled atmosphere preceded by initial low oxygen stress (ILOS+CA), and regular atmosphere preceded by initial low oxygen stress (ILOS+RA) (Table 2). Fruit were evaluated after each storage interval period: after 2, 3, 5 and 7 months in storage as shown in (Table 3). Shelf life periods for 2012 harvest season were 0 and 7 days; however, in 2013 harvest season fruit were evaluated at 0 day and after 3 and 7 days of shelf life at 20 °C.

Categorical data (superficial scald, neck shrivelling and decay incidence) were arcsin transformed prior to statistical analysis. Data was subjected to analysis of variance (ANOVA) using General linear model (GLM) (SAS Enterprise Guide 5.1) Separation of means (*p=0.05)* were performed using the Least significance difference (LSD) method. Pearson correlations were performed using SAS enterprise guide 5.1. Pearson correlations were used to quantify the relationships among assessed parameters.

## 3. Results and discussion

### 3.1. Fruit quality at harvest

Fruit quality is determined for a maturity stage at harvest. It is therefore important to harvest the fruit at the correct maturity stage (van der Merwe, 1996). Harvesting fruit at the correct maturity stage ensures the ability of the fruit to maintain an acceptable market quality for extended periods under controlled atmosphere storage protocols (van der Merwe, 1996). This aspect is particularly crucial if there is an extended period between harvest and consumption such as the case of the South African market where the bulk of the fruit is destined for export markets and spends long periods in transit. The fruit in 2012 were harvested at more advanced maturity than the fruit during the 2013 season (Table 1).

### 3.2. Superficial scald incidence (%) and intensity (scald index)

After 2 months in cold storage there was no superficial scald on 'Packham's Triumph' pears for both 2012 and 2013 (data not shown) seasons, irrespective of applied storage treatments (DCA, CA, RA, RA preceded by ILOS, CA preceded by ILOS) (Table 4). The onset of superficial scald on 'Packham's Triumph' pears for 2012 season was after 7 days of shelf life preceded by 3 months of cold storage on control (RA) fruit with only 3.8% of the fruit affected (Table 4). This results is in agreement with Zanella et al. (2005) that shelf life period of 7 days at 20 °C fostered the development of superficial scald on 'Granny Smith' apples grown in South Tyrol, Italy. Similar results of onset of superficial scald after 3 months cold storage were reported by Whitaker et al. (2009) on 'Bartlett' pears grown in California and by Boonkiyat et al. (1987) on 'd'Anjou' pears. Symptoms of superficial scald on 'Packham's Triumph' pears appeared after transfer of fruit to warmer temperatures of 20 °C for the retailer's shelf life simulation. Similar pattern of superficial scald development has been observed on ' Law Rome', 'Delicious', 'Gala' and 'Cortland' apples, the lesions became darker after transfer to warmer temperatures of 22 °C (Ingle, 2001).

There was no superficial scald on RA preceded by ILOS, CA preceded by ILOS, DCA and CA fruit after 3 months cold storage followed by 7 days shelf life during the 2012 season (Table 4). During 2013 season there was no superficial scald on applied treatments (RA preceded by ILOS, CA by preceded ILOS, DCA, CA and RA) after 3 months followed by 7 days shelf life (data not shown). After 5 months cold storage during the 2012 season there was superficial scald incidence on RA preceded by ILOS, RA, and CA treated 'Packham's Triumph' pears after 7 days shelf life, and the same trend was observed in the

2013 harvest season after 3 days shelf life. However there was no scald incidence on CA treated fruit after 7 days shelf life.

Ju and Curry (2002) observed that CA treated fruit which developed scald after 5 months in storage as observed in this study. However it should be noted that in this study the fruit were kept in CA for 4 months (16 weeks) and subsequently for 6 weeks in RA, to simulate the shipment and handling period required for export markets. In other countries CA is applied as a storage treatment throughout due to close proximity of their destined markets. It was also observed that 'Packham's Triumph' pears treated with RA preceded by ILOS had the higher superficial scald severity index of (3.62) than RA (2.43) treated fruit. This observation implies that ILOS followed by RA offers no suppression of superficial scald on 'Packham's Triumph' pears. Alternatively the ILOS treatment in this study was too short to render any effectiveness.

The onset and intensity of superficial scald in pome fruit may vary from season to season (von Mollendorff, 1996). During the 2012 season the onset scald started at 3 months cold storage followed by 7 days shelf life on RA (control) treated fruit, whereas in 2013 the superficial scald started after 5 months followed by 3 days shelf life on RA preceded by ILOS and RA (control) treated fruit. RA preceded by ILOS suppressed scald up to the end of storage (0 day) of 5 months cold storage in 2012, consistent with 2013 results. RA fruit developed superficial scald earlier in 2012 season during 3 months storage followed by 7 days shelf life.

During the 2013 season the disorder started after 5 months cold storage followed by 3 days shelf life on RA preceded by ILOS RA and RA treated fruit. During 2012 season the superficial scald onset was 5 months cold storage followed by 7 days shelf life on CA treated fruit, however during the 2013 harvest season CA treated fruit developed superficial scald at 5 months cold storage followed by 3 days shelf life.

The severity index was lower during 2013 than 2012 in all treatments (RA, RA preceded by ILOS and CA) showing symptoms of superficial scald (Table 4). During the 2012 harvest season RA preceded by ILOS treated 'Packham 's Triumph' pears had the highest scald index of 3.62 with about 44% of the 'Packham's Triumph' pears affected whereas in 2013 the scald index was only 0.90 and 40% of the fruit was affected. The similar trend was observed on RA fruit as it showed scald index of 2.43 with 51.39% of the fruit affected during the 2012 harvest season, whereas during the 2013 season the scald index was 0.33 and only 16% of the fruit was affected. CA treated fruit had superficial scald during the 2012 harvest season after 5 months followed by 7 days shelf life, however there was no superficial scald on the same period in during 2013 season, although some fruit showed signs of superficial scald after 3 days in shelf life.

After extended periods in cold storage the symptoms of superficial scald may appear whilst fruit are still in cold storage (Lotze et al., 2003, Lurie and Watkins, 2012). RA (control) and RA preceded by ILOS treated fruit had superficial scald after 7 months in cold storage during the 2012 harvest season. DCA treatment suppressed the incidence of superficial scald up to 7 months and 7 days shelf life in the 2012 season (Table 4). The positive results of DCA technology in suppressing superficial scald has been reported by Lafer (2011) on organic 'Uta' pears grown in Austria, Zanella and Strüz (2013) on 'Granny Smith' and 'Red Delicious' apples grown in South Tyrol, Italy, DeLong et al. (2004) on 'Cortland', 'Delicious', 'McIntosh', 'Golden delicious', 'Jonagold' and 'Honeycrisp' and by DeLong et al. (2007) on organic 'Cortland' and 'Delicious' apples grown in Nova Scotia, USA.

CA preceded by ILOS effectively suppressed superficial scald symptoms on 'Packham's Triumph' pears for up to 5 months in cold storage for both the 2012 and 2013 harvest season. The effectiveness of CA preceded by ILOS has been reported on 'Beurre d' Anjou' pears grown in Argentina by Calvo et al. (2002) and on 'Granny Smith' and 'Top Red' apples grown in South Africa (van der Merwe et al., 2003). The CA preceded by ILOS treated fruit had superficial scald after 7 months cold storage followed by 7 day shelf life during the 2012 season , thus it is can be effective up to 5 months (short term storage) cold storage, but not for long term storage (7 months). ILOS treatments can be considered as an alternative short term superficial scald inhibitors without the use of chemicals (Wang and Dilley, 2000; Zanella and Strüz, 2013).

The incidence and intensity of superficial scald increased with days of shelf life on 'Packham's Triumph' pears in this study. RA preceded by ILOS treated fruit had no superficial scald (scald index = 0) after 5 months at the end of storage life at 0 day, however at the end of shelf life after 7 days it was 3.62 during the 2012 season. The same trend of an increase in the severity index from 0 to 7 days was observed for the 2013 season (Table 4). During the 2012 season, RA fruit had scald index of zero at the end of storage life (0 day), but this increased to 2.43 after 7 days shelf life after 5 months cold storage. Similar trends of increase of scald intensity with increased period in shelf life were observed by Ju and Curry (2002) on ‘Delicious’ and ‘Granny Smith’ apples.

Development of superficial scald started from the stem end of the fruit and later spreads toward the blossom end with increase in severity and the same pattern was observed for shrivelling in ‘Packham’s Triumph’ pears (Figure 2 and Figure 3). Defillipi et al. (2006) reported stem to blossom end pattern of scald development on ‘Wonderful’ pomegranates.

### 3.3. Neck shrivelling

Statistical analysis showed significant interaction for the main effects (storage treatment, storage period and shelf life) $Pr>F$ = 0.0002 during the 2012 harvest season (Table 5). There was no neck shrivelling observed on treatments (RA preceded by ILOS, CA preceded ILOS, DCA, CA and RA) at all storage periods of 2, 3, 5 and 7 months at the end of storage life on 0 d, however, shrivelling (Figure 3) was observed at the end of shelf life at 7 days for 3, 5 and 7 months of storage (Table 5). The findings in this study confirm that higher temperatures during storage and ripening increase susceptibility of fruit to neck shrivelling (Burger, 2005; Buchner, 2006).

There was no neck shrivelling on the ‘Packham’s Triumph’ pears in all treatments both at 0 and 7 days at 20 °C after 2 months of cold storage. This might be attributable to perhaps the shorter time the fruit spent in cold storage. After the 3 months storage period the control fruit had symptoms of neck shrivelling. ‘Packham’s Triumph’ pears treated with CA and RA preceded by ILOS had significantly higher neck shrivelling after 5 months and shelf life on 7 days at 20 °C, with CA treated fruit showing the highest percentage of neck shrivelling at 2.2% (Table 5). After 7 months cold storage followed by 7 days shelf life all treatments had neck shrivelling, with treatments, RA preceded by ILOS and DCA showing highest incidence of neck shrivelling. ‘Packham’s Triumph’ pears have been reported to be prone to neck (stem-end) shrivelling (Burger, 2005). Fruit harvested earlier and smaller fruit are more susceptible to shrivelling (Burger, 2005), however late harvested fruit might also be susceptible to shrivelling (Buchner, 2006).

Burger (2005) reported that shrivelling may be attributed to as little as 2.5% loss in weight from ‘Packham‘s Triumph’ pears due to moisture loss. Some researchers reported 3-4% water loss in pears to cause shrivelling (Mitcham and Elkins, 2007). Although DCA treatment effectively suppresses the incidence of superficial scald on ‘Packham’s Triumph’ pears, fruit tend to be prone to shrivelling due to loss of moisture. There was incidence of neck shrivelling noted on the 2012 fruit but none on the 2013 fruit (data not shown) perhaps due to seasonal variation and maturity level. During the 2013 season fruit were firmer (71.74 N) than 2012 season (63.90 N) (Table 1). ‘Packham’s Triumph’ pears used during the 2012 season were not at optimal maturity as required by the South African standards of 72.68 N (von Mollendorff, 1996), and that might have attributed to neck shrivelling during the 2012 season.

Fruit should be harvested at the optimal maturity in order to ensure satisfactory quality and extended storage life. Neck shrivelling due to moisture loss may be reduced by use of internal packaging (Watkins, 2008; Berry, 2013), edible coatings such as kafirin (Buchner, 2006), sealing of the fruit stem (Burger, 2005), and/or humidifying the storage room (Mitcham and Elkins, 2007) to prevent loss of moisture from the fruit. Internal packaging might provide a physical barrier thereby reducing air movement around the fruit and reducing water loss (Wills et al., 1989), however this method might have limitations because in higher temperature such as during ripening, high humidity and high temperature might lead to decay of fruit.

## 3.4. Decay incidence

### 2012 harvest season

There was significant interaction ($Pr>F$ = <0.0001) among main effects (storage treatments, storage period and shelf life). Decay incidence was influenced by storage treatment, storage period and shelf life. At the beginning of shelf life, during the 2012 season, the decay incidence of DCA, CA, RA and CA preceded by ILOS treated fruit were not significantly different after 2 months in storage (Table 6). Although fruit stored in RA and fruit stored in RA preceded by ILOS treatment showed the highest decay incidence after 7 days shelf life, it was not significantly different from CA, DCA and CA preceded by ILOS treated fruit (Table 6). After 7 days shelf life of the 3 months storage period, RA (control) stored fruit had the significantly highest decay incidence (12.2%), compared to DCA (3.64%), although at 0 day the decay incidence was lower in RA than in DCA fruit.

DCA treatment suppressed decay incidence better compared to CA and CA preceded by ILOS treatments over extended shelf life periods. DCA technology was able to reduce fruit rot on organically grown 'Uta' pears by 41% compared to fruit stored only in CA (Lafer, 2011). At the end of the shelf life after 7 months, following 7 days in shelf life DCA treated fruit had a significantly lower decay incidence of 1.55% compared to CA and CA preceded by ILOS treated fruit which had 10.84% and 8.38% decay incidence respectively (Table 6). However the incidence of decay in DCA treated fruit was not significantly different from RA (2.69%) and RA preceded by ILOS (1.36%) fruit after 7 days shelf life of the 7 month storage period.

**2013 harvest season**

There was no significant interaction between treatment, storage period and shelf life during the 2013 harvest season $Pr>F$ = 0.6185. Storage period had the main effect on decay incidence during 0 day shelf life, with 5 month storage period showing significantly higher decay incidence than 2 and 3 months storage intervals (Table 7). Decay in stored fruit affects external appearance and eating quality of the fruit negatively. Decay can result in major losses in long term CA storage (Lennox et al., 2004). Susceptibility of fruit to decay incidence increases with the onset of ripening during shelf life conditions (Kader, 1989). DCA and CA had significantly higher postharvest decay incidence after 7 days of shelf life during the 2013 season (Table 7).

It was observed that after 5 months of cold storage fruit had the highest incidence of decay (Table 7). Generally no significant difference in decay incidence after 2 and 3 months in cold storage were noted. Storage treatment had significant effect on decay incidence with DCA fruit having the highest decay incidence at 7 days of shelf life although not significantly different from CA treated fruit. CA treatment was not significantly different from RA and CA preceded by ILOS. RA preceded by ILOS treated fruit had the lowest decay incidence after 7 days shelf life. Similar results of ineffectiveness of DCA treatment to suppress decay incidence on fruit has been reported by DeLong et al. (2007) on 'Cortland' and 'Delicious' apples. At 0 day and after 3 days shelf life during the 2013 harvest there was no significant difference in occurrence of decay in fruit (Table 7).

### 3.5. Internal breakdown

There was no incidence of internal breakdown during ripening on the 'Packham's Triumph' pears for both 2012 and 2013 season (data not shown). These findings suggest that 'Packham's Triumph' pears are not prone to an internal breakdown disorder. Lotze et al. (2003) reported that 'Packham's Triumph' pears are resistant to internal breakdown.

3.6. Effects of treatment and storage period on titratable acidity (TTA) of 'Packham's Triumph' pears

**2012 harvest season**

The organic acid content of fleshy fruits is estimated by the TTA (Pasquirello et al., 2013). In pears the composition of organic acids is more varied than that of TSS (Pasquirello et al., 2013). There was a significant interaction among main effects (storage treatment, storage period and shelf life – T X P X D *Pr>F* = <0.0001). However, statistical analysis showed (Table 8) that treatment and storage period (T X P) were highly significant at 0 day and after 7 days shelf life during the 2012 season (*Pr>F* = 0.0055) at 0 day and at day 7 (*Pr>F* = <0.0001) .

After 2 months in cold storage TTA levels decreased significantly from 0 day to 7 days shelf life in 'Packham's Triumph' pears, irrespective of treatment applied. There was no significant difference on TTA content on 0 day of the 2 months storage period irrespective of treatment applied (Table 8). However after 3 months of cold storage the TTA concentration of 'Packham's Triumph' pears treated with DCA, CA and control was significantly higher than that of RA preceded by ILOS and CA preceded by ILOS fruit on 0 day. After 7 days in shelf life of the 3 months cold storage there was no significant difference in TTA content, irrespective of treatment applied.

TTA values were higher during the 2 and 3 months cold storage periods and lower after 5 and 7 months of cold storage on the RA fruit. The decrease in TTA with storage period might be due to the breakdown of organic substrate for the respiratory metabolism in fruit during the postharvest period (Pasquirello et al., 2013). After 7 months in cold storage the TTA levels of control fruit were significantly lower than that of DCA, CA, CA preceded by ILOS and RA preceded by ILOS fruit.

**2013 harvest season**

There was no significant interaction between treatment and storage periods at 0 day on TTA level of 'Packham's Triumph' pears during the 2013 season *Pr>F* = 0.8235 (Table 9). TTA changes were as a result of the combined effects of storage period and treatment at 0 and 3 days at shelf life conditions, whereas significant interaction effect of the main factors influenced TTA after 7 days shelf life (Table 9 and Table 10). After 0 day the TTA was highest in CA preceded by ILOS, DCA and CA and the lowest in RA and RA preceded by ILOS; however TTA in CA preceded by ILOS, DCA and control were not significantly different.

The maintenance of TTA by DCA has been reported by Lafer (2011) on ‘Uta’ pears. However in this study there was no significant difference in TTA among CA preceded by ILOS, CA and DCA treated fruit after 3 and 5 months during the 2013 season indicating that both treatments maintained TTA of ‘Packham’s Triumph’ pears. The effectiveness of low oxygen levels in maintaining TTA values on ‘Beurre’d Anjou’ pears has been reported by Calvo et al. (2002).

During ripening and maturation fruit tend to loose acidity rapidly (Wills, et al., 1989), however, TTA or TSS alone is not the best indicators of maturity. TTA was lowest in RA preceded by ILOS, DCA, CA preceded by ILOS and highest in CA treated fruit. Palatability of the fruit is often related to total soluble solids/acid ratio (Wills et al., 1989). TTA decreased significantly with earlier storage period of 2 months having the highest level of TTA, and the level declined after 3 and 5 months. There was no significant difference between the TTA level after 3 and 5 months in storage treatments after 3 days in shelf life (Table 9).

There was no significant change of TTA on the fruit after 2, 3 and 5 months in storage followed by 7 days of shelf life on DCA and CA treated fruit during the 2013 harvest season (Table 10). These results indicate that there was TTA maintenance by DCA and CA treatments. TTA was higher after 2 months of cold storage but declined after 3 and 5 months on RA preceded by ILOS, RA, and CA preceded by ILOS during the 2013 season (Table 10). Thus although CA preceded by ILOS and CA treated fruit had the same level of TTA after 2 months of cold storage followed by 7 days, there was a decline of TTA level after 3 and 5 months on CA preceded by ILOS but remained the same on CA treated fruit.

### 3.7. Effects of treatment and storage period on total soluble solids (TSS) (°Brix) of 'Packham's Triumph' pears

**2012 harvest season**

There was significant interaction among main effects (treatment, storage period and shelf life) *Pr>F* = <0.0001. After storage, the effects on TSS content of 'Packham's Triumph' pears were as a result of significant interaction between storage period and treatment during the 2012 season (Table 11). 'Packham's Triumph' pears stored in CA preceeded by ILOS and RA preeded by ILOS had significantly higher TSS content than DCA, CA and RA fruit on 0 day of the 2 months storage period (Table 11). After 7 days in shelf life there was no significant difference in DCA and CA treated fruit's TSS content. Although the CA preceded by ILOS and RA preceded by ILOS fruit had significantly higher TSS content, it was not significantly different from TSS in CA stored fruit after 7 days of the 2 months storage period (Table 11).

After 5 months period (0 day) there was no significant difference in TSS of the 'Packham's Triumph' pears between DCA, CA preceded by ILOS, CA treated fruit (Table 11), however RA preceded by ILOS fruit had significantly higher TSS although not significantly different from RA and CA treated fruit. After 5 months storage period followed by 7 days shelf life CA preceded by ILOS and RA preceded by ILOS fruit had significantly higher TSS than DCA, CA and RA treated fruit (Table 11).

On 0 day of the 7 months storage period, there was no significant difference of TSS between DCA and RA preceded by ILOS fruit, although they were significanlt higher that that of fruit stored in CA preceded by ILOS, CA and RA. After 7 months in cold storage followed by 7 days shelf life DCA treated fruit had sigbificantly higher TSS than CA and RA fruit, although not significantly different from RA preceded by ILOS and CA precede by ILOS fruit (Table 11). These results confirm that DCA treated fruit maintained higher TSS than RA (DeLong et al., 2007). Similar trend was observed by Zanella et al., (2005) on 'Granny Smith' apples.

**2013 harvest season**

During the 2013 harvest season, there was no interaction among the main effects (treatment, storage period and shelf life) on TSS of 'Packham's Triumph' pears *Pr >F* =

0.4272. After the 2 month storage period at 0 day TSS content of CA preceded by ILOS treated ‘Packham’s Triumph’ pears was significantly higher than DCA, CA, RA preceded by ILOS and RA storeed fruit during the 2013 harvest season (Table 12). There was no significant difference in TSS change after 2 and 3 months on 0 day during the 2013 harvest season, although CA precede by ILOS fruit had the highest TSS content (Table 12). After 5 months in storage there was no significant difference in TSS of ‘Packham’s Triumph’ pears between DCA and CA preceded by ILOS, although DCA treated fruit had significantly higher TSS than CA treated fruit. These findings corroborate 2012 harvest season‘s findings. At 0 day of the 5 month storage period RA fruit had the significantly higher TSS than that of RA preceded by ILOS fruit. RA preceded by ILOS treatment had the lowest TSS content.

After 3 days in shelf life the CA preceded by ILOS treated fruit had the significantly higher TSS content than DCA, CA and RA treated fruit, however RA preceded by ILOS fruit had the lowest TSS content (Table 13). After 7 days shelf life there was no significant difference among fruit treated with CA preceded by ILOS, DCA, CA and RA fruit, but the RA preceded by ILOS fruit had the lowest TSS content (Table 13).

3.8. Effects of treatment and storage period on sugar to acid ratio of ‘Packham’s Triumph’ pears

**2012 harvest season**

The interaction among main effects (storage treatment, period and shelf life) was highly significant during the 2012 harvest season $Pr>F$ = 0.0005. The sugar to acid ratio in DCA treated fruit increased with shelf life period, with the lowest at 0 day and highest after 7 days shelf life for all storage periods tested (Table 14). DCA treated fruit had the significantly highest sugar to acid ratio of 93.39 after 7 days of the 2 months storage period during the 2012 harvest season than that of CA (57.23) and CA preceded by ILOS (70.68), although not significantly different from RA preceded by ILOS (74.34). RA preceded by ILOS treated fruit had significantly higher sugar to acid ratio than RA fruit, although there was no significant difference in sugar to acid ratio amongst CA preceded by ILOS and CA treated fruit. After the 3 months storage period, followed by 7 days shelf life the sugar to acid ratio of DCA fruit was not significantly different from all other treatments (RA preceded by ILOS, RA, CA preceded by ILOS and CA).

At 5 months storage period followed by 7 days shelf life DCA treated fruit had significantly highest sugar to acid ratio than CA preceded by ILOS treated fruit although not significantly different from CA treated fruit. However CA treated fruit showed significantly higher sugar to acid ratio than CA preceded by ILOS treated fruit. After 7 months storage period followed by 7 days shelf life there was no significant difference in sugar to acid ration content in all treatments.

**2013 harvest season**

There was no significant interaction among treatments applied, storage period and shelf life during the 2013 season $Pr>F$ = 0.1435. At the end of storage life (0 day) during the 2013 season the CA treated fruit had the lowest sugar: acid level, whereas RA preceded by ILOS had the highest sugar to acid ratio (Table 15). The sugar to acid ratio in DCA, CA preceded by ILOS and RA fruit. However the sugar to acid ratio increased steadily with storage period, lowest at the earlier period of 2 months and highest after 5 months in cold storage. At the end of storage period after 2 months in cold storage 'Packham's Triumph' pears had the lowest sugar to acid ratio indicating that the fruit were not yet ripe hence the low soluble sugars and the sugar acid ratio increased with storage time the highest after 5 months of cold storage.

RA preceded by ILOS treated 'Packham's Triumph' pears had higher sugar: acid ratio compared to all other treatments at 0 and 3 days. The sugar to acid ratio was not significantly different among treatments after 7 days (Table 16). At 0 d there was significant interaction between storage treatment and storage period. RA preceded by ILOS had the highest sugar: acid ratio at the end of storage period whereas the level was the same for CA preceded by ILOS, DCA and RA with CA showing the lowest ratio. At 3 and 7 days the change of sugar: acid ratio was influenced by both storage treatment and storage period hence the significant interaction between storage treatment and storage period. There was a strong significant correlation between TTA and sugar to acid ratio ($R^2$ = -0.92) ($p$<0.0001).

The sugar to acid ratio increased with storage period for 3 and 5 months storage periods whilst it remained the same for 2 months for both 3 and 7 days shelf life in CA treated fruit (Table 16). In control fruit sugar: acid ratio increased with period in shelf life higher at 7 days than at 3 days for 2 and 3 months storage period; however remained the same for 2 months storage period. Sugar to acid ratio is an important indication of maturity and a minimum level is often included in processing specification commonly in certain horticultural commodities such as citrus and pineapple.

**4. Conclusions**

The onset and severity of superficial scald in pome fruit may vary from season to season. During the 2012 harvest season the onset of superficial scald on 'Packham's Triumph' pears was after 3 months followed by 7 days shelf life, whereas in 2013 season superficial scald started after 5 months on RA stored fruit. During the 2012 harvest season fruit treated with ILOS for 10 days before long term RA storage had the highest severity index of 3.62 with 44% of the fruit affected, however, during the 2013 season fruit scald index was only 0.90% while 40% of the fruit was affected by the physiological disorder.

The superficial scald symptoms may appear after transfer to warmer temperatures. Fruit treated with ILOS for 10 days followed by long term storage in RA showed no symptoms of superficial scald at the end of 5 months storage period, but at the end of shelf life (7 days) the scald index was 3.62 and 100% of the fruit was affected during the 2012 season. When storage period with RA and ILOS preceded by ILOS was extended to 7 months symptoms of superficial scald appeared during storage on 'Packham's Triumph' pears. At the end of 7 months storage there were symptoms of superficial scald on stored fruit under RA and RA preceded by ILOS during the 2012 season, however during the 2013 season superficial scald appeared at the end of 5 months storage.

'Packham's Triumph' pears appear to be prone to shrivelling disorder. The shrivelling on 'Packham's Triumph' pears started from the neck (stem-end) for the fruit and later spread toward the blossom end with increase in severity and the same pattern was evident with the incidence of superficial scald on the fruit. Similar pattern of superficial scald development was observed on 'Wonderful' pomegranates by Defillipi et al., (2006). Fruit from all low oxygen treatments: DCA, CA, CA preceded by ILOS, RA preceded by ILOS and RA storage had neck shrivelling after 7 months in cold storage. DCA treatment suppressed decay of 'Packham's Triumph' pears better than treating fruit with ILOS for 10 days before long term CA. DCA, CA preceded by ILOS and CA treatments maintained TSS and TTA better than the control (regular atmosphere) stored 'Packham's Triumph' pears after 7 months cold storage followed by 7 days shelf life.

DCA treatment offers a promising alternative to the use of DPA as a long term storage scald inhibitor on 'Packham's Triumph' pears for South African exporters and growers. Storing fruit in DCA effectively suppressed superficial scald symptoms on 'Packham's Triumph' pears for up to 7 months in cold storage followed by 7 days in shelf life. Storing fruit under ILOS for 10 days, prior to long term CA storage effectively controlled superficial scald on 'Packham's Triumph' pears up to 5 months. CA treatment preceded by 10 days under ILOS offered an alternative as a short term treatment to inhibit superficial scald on 'Packham's Triumph' pears to the use of DPA. Without the use of ILOS treatment, CA effectively controlled superficial scald for 3 months only on 'Packham's Triumph' pears.

## 5. References

Bauchot, A.D., Reid, S.J., Ross, G.S. & Burmeister, D.M. (1999). Induction of apple scald by anaerobiosis has similar characteristics to naturally occurring superficial scald in ‘Granny Smith’ apple fruit. *Postharvest Biology and Technology*, 16, 9–14.

Berry, T.M. (2013). Resistance to airflow and the effects on cooling efficiency of multi scale ventilated pome fruit packaging. MSc. Stellenbosch University. http://scholar.sun.ac.za.

Boonkiyat, D., Chen, P.M., Spotts, R.A. & Richardson, D.G. (1987). Effect of harvest maturity on decay and post-harvest life of ‘d'Anjou’ pear. *Scientia Horticulturae*, 31, 131-139.

Buchner, S. (2006). Coating of pears (var. ‘Packham’s Triumph’) with kafirin protein and its effect on postharvest physiology and shelf life. MSc. Thesis University of Pretoria, Pretoria.

Burger, G.E. (2005). Factors affecting shrivelling and friction discolouration of pears (*Pyrus communis L*). MSc. Thesis, Stellenbosch University, Stellenbosch. http://scholar.sun.ac.za.

Calvo, G., Salvador, M.E. & Sanchez, E. (2002). Control of superficial scald in ‘Beurre d’ Anjou’ pears with low oxygen levels. *Acta Horticulturae*, 596, 879-882.

Calvo, G. (2003). Effect of 1-Methylcyclopropene (1-MCP) on pear maturity and quality. *Acta Horticulturae*, 62, 203-211.

Commission Regulation (EU) No. 772/2013 of 8 August 2013. *Official Journal of European Union.*

Crouch, I. (2003). 1-Methylcyclopropene (SmartFresh™) as an alternative to modified atmosphere and controlled atmosphere storage of apples and pears. *Acta Horticulturae*, 600, 433-436.

Defillipi, B.G., Whitaker, B.D., Hess-Pierce, B.M. & Kader, A.A. (2006). Development and control of superficial scald on ‘Wonderful’ pomegranates during long-term storage. *Postharvest Biology and Technology*, 41, 234-243.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2004). A new technology that determines low-oxygen thresholds in controlled atmosphere stored apples. *HortTechnology,* 14, 262-266.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2007). Chlorophyll fluorescence based low-$O_2$ CA storage of organic 'Cortland' and 'Delicious' apples. *Acta Horticulturae*, 737, 31-37.

Ingle, M. (2001). Physiology and biochemistry of superficial scald of apples and pears. *Horticultural Reviews*, 27, 228-261.

Ju, Z. & Curry, E.A. (2002). Effects of 6-methyl-5-hepten-2-one vapour on peel browning of 'Delicious' and 'Granny Smith' apples: open vs. closed system. *Postharvest Biology and Technology*, 25, 265-272.

Kader, A.A. (1989). Mode of action of oxygen and carbon dioxide on postharvest physiology of 'Bartlett' pears. *Acta Horticulturae*, 88, 161-167.

Ke, D., van Gorsel, H. & Kader, A.A. (1990). Physiological and quality responses of 'Bartlett' pears to reduced $O_2$ and enhanced $CO_2$ levels and storage temperature. *Journal of American Society of Horticultural Science*, 115, 453-439.

Lafer, G. (2011). Effect of different CA storage conditions on storability of 'Uta' pear. *Acta Horticulturae,* 909, 757-760.

Lennox, C.L., Spotts, R.A. & Booyse, M.A. (2004). Incidence of decay of 'D Anjou' pear and control with a thiabendazole drench. *Plant disease,* 88, 474-478.

Little, C.R. & Holmes, R.J. (2000). Controlled atmosphere storage. In *Storage technology for apples and pears: A guide to production, postharvest treatment and storage of pome fruit in Australia* (pp. 420-515). Knoxfield: Highway Press Pty Ltd.

Lotze, E., van der Merwe, K. & Rogalska, S. (2003). *Manual identification of post-harvest disorders of pome and stone fruit* (3rd rev ed., pp. 34-36). ARC Infruitec-Nietvoorbij.

Lurie, S. & Watkins, B. (2012). Superficial scald etiology and control. *Postharvest Biology and Technology,* 65, 44-60.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J., Nieuwoudt, H., Mouazen, AM., Saeys, W. & Nicolai, B.M. (2012). Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. *Postharvest Biology and Technology*, 74, 1-10.

Mitcham, E.J. & Elkins, R.B. (2007). *Pear production and handling manual* (p. 159). University of California Agriculture and Natural Resources Publication 3483.

Mitcham, E.J. & Mitchell, F.G. (2002). Postharvest handling systems: Pome fruits. In Kader A.A. (Ed.), *Postharvest technology of horticultural crops* (3rd ed., pp. 333-344). University of California Publication 3311.

Pasquariello, M.S., Rega, P., Migliozzi, T., Capuano, L.R., Scortichini, M. & Petriccione, M. (2013). Effect of cold storage and shelf life on physiological and quality traits of ripening pear cultivars. *Scientia Horticulturae,* 162, 341-350.

PPECB, (2014). Annual Report 2013/2014. The Perishable Products Export Control Board, retrieved on the 22nd of October 2015. www.ppecb.com.

Robatscher, P., Eisenstecken, D., Sacco, F., Pohl, H., Berger, J., Zanella, A. & Oberhuber, M. (2012). Diphenylamine residues in apples caused by contamination in fruit storage facilities. *Journal of Agricultural and Food Chemistry,* 60 (9), 2205-2211.

Thompson, A.K. (2003). *Disease control in fruit and vegetables harvesting, handling and storage*. (2nd ed., pp. 71-85). Kent: Backwell Publishing.

Van der Merwe, J.A. (1996). Maturity standards for deciduous fruit. In Combrink, J.C (Eds.) *Integrated management of postharvest quality* (pp. 25-32). ARC Infruitec-Nietvoorbij: Paarl Printing.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae,* 600, 261-265.

Von Mollendorf, L.J., 1996. Development of physiological post-harvest disorders. In Combrink, J.C. (Ed.), *Integrated management of post-harvest quality* (pp. 39-42). ARC Infruitec-Nietvoorbij: Paarl Printing.

Wang, Z. & Dilley, D.R. (1999). Control of superficial scald of apples by low oxygen atmospheres. *HortScience*, 34 (7), 1145-1151.

Wang, Z. & Dilley, D.R. (2000). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology*, 18, 201-213.

Watkins, C.B. (2008). Postharvest ripening regulation and innovation in storage technology. *Acta Horticulturae*, 796, 51-57.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, E.J. & Mattheis, J.P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology*, 58, 43-50.

Wilkinson, B.G. & Fidler J.C. (1973). Physiological disorders. In *The biology of apple and pear storage* (pp. 65-80). Common Wealth Bureaux Research review 3, England.

Wills, R.B.H., McGlasson, W.B., Graham, D., Lee, T.H. & Hall, E.G. (1989). *Postharvest: An introduction to the physiology and handling of fruit and vegetables*, (3$^{rd}$ edn. p. 57). BSP Professional books: A division of Blackwell Scientific Publications Ltd.

Zanella, A., Cazzanelli, P., Panarese, A., Coser, M., Cecchinel, M. & Rossi, O. (2005). Fruit fluorescence response to low oxygen stress: Modern storage technologies compared to 1-MCP treatment of apple. *Acta Horticulturae*, 682, 1535-1542.

Zanella, A. & Strüz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10$^{th}$ of EU's apples producing area. *Acta Horticulturae*, 1012, 419-426.

Table 1. Maturity indices for 'Packham's Triumph' pears harvested for the growing seasons 2012/2013 according to the standard maturity indices used in South Africa (van der Merwe, 1996).

| Maturity indices | Harvest season | | Standard |
|---|---|---|---|
| | 2012 | 2013 | |
| Firmness (N) | 63.90 | 71.74 | 72.61 |
| Skin colour | 2.99 | 2.56 | 2.10 |
| TSS (°Brix) | 12.63 | 12.06 | 12.50 |
| TTA (g malic acid/100g juice) | 0.37 | 0.22 | 0.25 |
| TSS/TTA ratio | 34.14 | 54.82 | 50.00 |

Table 2. $O_2$ and $CO_2$ concentrations associated with storage treatments of 'Packham's Triumph' pears during or before storage at -0.5 °C

| Storage treatment | Gas composition (%) | |
|---|---|---|
| | % $O_2$ | % $CO_2$ |
| DCA | 0.5 | 1 |
| CA | 1.5 | 2.5 |
| RA (control) | 20.95 | 0-1 |
| ILOS+CA | 0.5 | 2.5 |
| ILOS+RA | 0.5 | 0-1 |

Note: DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, For ILOS treatments, the gas composition refers only to the initial low gas stress condition and not the subsequent CA or RA applied.

Table 3. Summary of low oxygen storage treatments and duration applied to simulate typical South African shipping and handling periods for 'Packham's Triumph' pears.

| | | Breakdown of storage period | | |
|---|---|---|---|---|
| Storage treatment | Storage period (months) | Preconditioning (days) | Treatment | Shipment & handling simulation (weeks) |
| DCA | 2 | None | 10d* | 8w** |
| | 3 | | 6w * | 6w** |
| | 5 | | 16w * | 6w** |
| | 7 | | 24w* | 6w** |
| CA | 2 | None | 10d* | 8w** |
| | 3 | | 6w* | 6w** |
| | 5 | | 16w* | 6w** |
| | 7 | | 24w* | 6w** |
| Control (RA) | 2 | None | | 8w** |
| | 3 | | | 12w** |
| | 5 | | | 22w** |
| | 7 | | | 30w** |
| ILOS+CA | 2 | 10d* | | 8w** |
| | 3 | 10d* | 6w* | 6w** |
| | 5 | 10d* | 16w* | 6w** |
| | 7 | 10d* | 24w* | 6w** |
| ILOS+RA | 2 | 10d* | | 8w** |
| | 3 | 10d* | | 12w** |
| | 5 | 10d* | | 22w** |
| | 7 | 10d* | | 30w** |

Note: ILOS is a preconditioning once off 10d treatment, * Treatment period, ** Shipment and handling period, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Treatment periods were followed by 0, 3 and 7 days of shelf life at 20 °C.

Table 4. Effect of different storage treatments on superficial scald severity index (SI) and superficial scald incidence (%) of 'Packham's Triumph' pears after designated post storage intervals and after retailer's conditions during the 2012 and 2013 harvest seasons.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| | | SI | % | SI | % | SI | % | SI | % | SI | % |
| | | | | | | *2012 season* | | | | | |
| 3 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.03±0.08 | 3.82±5.15 |
| 5 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 7 | 3.62±0.26 | 44.04±2.82 | 0.00 | 0.00 | 0.00 | 0.00 | 0.53±0.46 | 0.33±0.57 | 2.43±0.12 | 51.39±10.63 |
| 7 | 0 | 3.60±0.36 | 4.60±3.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.77±0.10 | 2.42±1.42 |
| | 7 | 3.87±0.11 | 99.39±2.08 | 0.83±0.21 | 8.27±1.99 | 0.00 | 0.00 | 1.13±0.34 | 17.36±3.65 | 3.34±0.16 | 97.32±3.93 |
| | | | | | | *2013 season* | | | | | |
| 5 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 3 | 0.09±0.05 | 5.57±2.36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01±0.01 | 0.62±1.08 | 0.20±0.07 | 10.13±3.27 |
| | 7 | 0.90±0.10 | 40.07±1.74 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.33±0.12 | 16.26±6.01 |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere.

Table 5. Effect of different storage treatments on neck shrivelling (%) of ‘Packham’s Triumph’ pear after designated post storage intervals and after retailer’s shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| | | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0 | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| | 7 | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| 3 | 0 | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| | 7 | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.30$^{cd}$ |
| 5 | 0 | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| | 7 | 1.43$^{b}$ | 0.61$^{bc}$ | 0.00$^{d}$ | 2.20$^{a}$ | 0.00$^{d}$ |
| 7 | 0 | 0.00$^{d}$ | 0.29$^{cd}$ | 0.29$^{cd}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| | 7 | 1.43$^{b}$ | 0.29$^{cd}$ | 1.14$^{b}$ | 0.59$^{cd}$ | 0.00$^{d}$ |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | *0.0665* | | | | | |
| *Period* | *0.0001* | | | | | |
| *Days* | *<0.0001* | | | | | |
| *TXP* | *0.0028* | | | | | |
| *TXD* | *0.0288* | | | | | |
| *PXD* | *0.0002* | | | | | |
| *T X P X D* | *0.0014* | | | | | |

Note: ILOS is a preconditioning once-off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = Storage treatment x storage period x shelf life period. Means with the same letter are not significantly different at $p<0.05$.

Table 6. Effect of different storage treatments on decay (%) of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| | | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0 | 0.00 $^{i}$ | 0.00 $^{i}$ | 0.84 $^{e-i}$ | 0.30 $^{ji}$ | 0.00 $^{i}$ |
| | 7 | 3.84 $^{c-h}$ | 1.41 $^{d-i}$ | 2.62 $^{d-i}$ | 2.38 $^{d-1}$ | 4.32 $^{c-g}$ |
| 3 | 0 | 0.00 $^{i}$ | 0.58 $^{g-i}$ | 2.09 $^{d-i}$ | 2.48 $^{d-i}$ | 0.51 $^{g}$ |
| | 7 | 0.81 $^{f-i}$ | 4.06 $^{c-h}$ | 3.64 $^{c-i}$ | 0.59 $^{g-i}$ | 12.20 $^{a}$ |
| 5 | 0 | 1.76 $^{d-i}$ | 2.83 $^{d-i}$ | 1.74 $^{d-i}$ | 0.30 $^{h-i}$ | 2.76 $^{d-i}$ |
| | 7 | 4.44 $^{c-f}$ | 4.83 $^{b-d}$ | 1.82 $^{d-i}$ | 1.98 $^{d-i}$ | 4.67 $^{b-e}$ |
| 7 | 0 | 1.91 $^{d-i}$ | 1.66 $^{c-i}$ | 3.84 $^{c-i}$ | 2.53 $^{d-i}$ | 2.67 $^{d-i}$ |
| | 7 | 1.36 $^{d-i}$ | 8.38 $^{ab}$ | 1.55 $^{d-i}$ | 10.84 $^{a}$ | 2.69 $^{d-i}$ |

| *Significance level Pr>F* | |
|---|---|
| *Treatment* | *0.0678* |
| *Period* | *0.0027* |
| *Shelf life (days)* | *<0.0001* |
| *P X D* | *<0.0001* |
| *T X P* | *0.0002* |
| *T X P X D* | *<0.0001* |
| *LSD $_{(5\%)}$* | *3.8372* |

Note: ILOS is a pre-conditioning once-off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = Storage treatment x storage period x shelf life period. Means with the same letter are not significantly different at $p<0.05$.

Table 7. Effect of different storage treatments on decay incidence (%) of 'Packham's Triumph' pears after designated post storage intervals at the end of storage life (0 day), and at the end of shelf life (3 and 7 days) during the 2013 harvest season.

| | Shelf life (days) | | |
|---|---|---|---|
| Storage treatment | 0 | 3 | 7 |
| ILOS+RA | 3.6[ns] | 1.3[ns] | 2.2[c] |
| ILOS+CA | 6.0 | 2.8 | 4.9[bc] |
| DCA | 5.7 | 4.1 | 9.1[a] |
| CA | 2.4 | 2.5 | 6.7[ab] |
| RA | 3.7 | 3.0 | 5.0[bc] |
| *LSD* $_{(5\%)}$ | *ns* | *ns* | *3.7* |
| Storage period (months) | | | |
| 2 | 3.3[b] | 2.7[ns] | 4.3[ns] |
| 3 | 4.3[b] | 3.8 | 7.6 |
| 5 | 9.6[a] | 1.7 | 4.9 |
| *LSD* $_{(5\%)}$ | 3.45 | *ns* | *ns* |
| *Significance level Pr>F* | | | |
| *Treatment* | *0.2902* | *0.2911* | *0.0107* |
| *Period* | *<0.0001* | *0.1149* | *0.0575* |
| *Days* | *0.0010* | | |
| *T X D* | *0.3099* | | |
| *P X D* | *0.0006* | | |
| *T X P* | *0.8091* | *0.6212* | *0.2834* |
| *T X P X D* | *0.6185* | | |

Note: ILOS is a preconditioning once-off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means with the same letter within columns are not significantly different at $p<0.05$. ns = non-significant.

Table 8. Effect of storage treatments on titratable acidity (TTA) content of 'Packham's Triumph' pears juice after designated storage intervals and after retailer's shelf conditions during the 2012 harvest season.

| | | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| Storage period (months) | Shelf life (days) | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0 | 0.38 [bc] | 0.37 [bc] | 0.36 [bc] | 0.36 [bc] | 0.39 [b] |
| | 7 | 0.21 [h] | 0.23 [e-h] | 0.16 [h] | 0.26 [d-g] | 0.28 [d-g] |
| 3 | 0 | 0.39 [b] | 0.34 [bc] | 0.46 [a] | 0.45 [a] | 0.47 [a] |
| | 7 | 0.34 [b-d] | 0.26 [b-g] | 0.33 [b-d] | 0.31 [b-d] | 0.30 [b-e] |
| 5 | 0 | 0.29 [e-g] | 0.37 [bc] | 0.37 [bc] | 0.30 [d-g] | 0.26 [gh] |
| | 7 | 0.30 [b-e] | 0.33 [bc] | 0.21 [gh] | 0.22 [f-h] | 0.24 [d-g] |
| 7 | 0 | 0.33 [c-e] | 0.31 [de] | 0.30 [b-e] | 0.28 [fg] | 0.21 [h] |
| | 7 | 0.24 [d-g] | 0.25 [d-g] | 0.31 [b-e] | 0.24 [d-g] | 0.21 [h] |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | | *0.2490* | | | | |
| *Period* | | *<0.0001* | | | | |
| *Shelf life (days)* | | *<0.0001* | | | | |
| *T X D* | | *0.3223* | | | | |
| *P X D* | | *<0.0001* | | | | |
| *T X P* | | *<0.0001* | | | | |
| *T X P X D* | | *0.0086* | | | | |
| *LSD $_{(5\%)}$* | | *0.0786* | | | | |

Note: ILOS treatment is a once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D= storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 9. Effect of different storage treatments on titratable acidity (% malic acid) of 'Packham's Triumph' pears juice after designated post storage intervals and after retailer's shelf conditions during the 2013 harvest season.

| | TTA | |
|---|---|---|
| | Shelf life (days) | |
| Storage treatment | 0 | 3 |
| ILOS+RA | 0.28 [c] | 0.26 [c] |
| ILOS+CA | 0.37 [ab] | 0.41 [bc] |
| DCA | 0.37 [ab] | 0.41 [bc] |
| CA | 0.41 [a] | 0.50 [a] |
| RA | 0.34 [b] | 0.37 [c] |
| *$LSD_{(5\%)}$* | *0.05* | *0.08* |
| Storage period (months) | | |
| 2 | 0.45 [a] | 0.44 [ns] |
| 3 | 0.39 [b] | 0.41 |
| 5 | 0.34 [b] | 0.36 |
| *$LSD_{(5\%)}$* | *0.05* | *0.06* |
| *Significance level Pr>F* | | |
| *Treatment* | *0.2578* | |
| *Period* | *0.3598* | |
| *Days* | *0.4145* | |
| *T X D* | *0.0484* | |
| *P X D* | *0.3481* | |
| *T X P* | *0.4407* | |
| *T X P X D* | *0.8235* | |

Note. ILOS treatment is once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period. Means within column followed by different letters are significantly different at $p<0.05$.

Table 10. Effect of different storage treatments on titratable acidity (% malic acid) of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf conditions (7 days) during the 2013 harvest season.

| | Storage treatment | | | | |
|---|---|---|---|---|---|
| | TTA | | | | |
| Storage period (months) | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0.34 $^{a\text{-}c}$ | 0.44 $^{a}$ | 0.27 $^{b\text{-}d}$ | 0.33 $^{a\text{-}c}$ | 0.46 $^{a}$ |
| 3 | 0.18 $^{d}$ | 0.29 $^{b\text{-}d}$ | 0.30 $^{b\text{-}d}$ | 0.37 $^{ab}$ | 0.28 $^{b\text{-}d}$ |
| 5 | 0.23 $^{cd}$ | 0.28 $^{b\text{-}d}$ | 0.36 $^{a\text{-}c}$ | 0.33 $^{a\text{-}c}$ | 0.23 $^{b\text{-}d}$ |
| *Significance level Pr>F* | | | | | |
| | *TTA* | | | | |
| *Treatment* | *0.2072* | | | | |
| *Period* | *0.0297* | | | | |
| *Days* | *0.4145* | | | | |
| *T X P* | *0.1431* | | | | |
| *LSD $_{(5\%)}$* | *0.1365* | | | | |

Note. ILOS treatment is a once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 11. Effect of different storage treatments on total soluble solids (°Brix) content of 'Packham's Triumph' pears juice after designated post storage intervals and after retailer's shelf conditions during the 2012 harvest season.

| | | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| Storage period (months) | Shelf life (days) | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0 | 15.27 $^{a}$ | 15.43 $^{a}$ | 13.73 $^{f\text{-}h}$ | 13.93 $^{c\text{-}f}$ | 13.90 $^{c\text{-}g}$ |
| | 7 | 15.43 $^{a}$ | 15.00 $^{a}$ | 14.00 $^{ef}$ | 14.77 $^{a\text{-}d}$ | 13.70 $^{e\text{-}h}$ |
| 3 | 0 | 14.40 $^{b\text{-}e}$ | 13.50 $^{f\text{-}i}$ | 13.63 $^{f\text{-}h}$ | 13.77 $^{e\text{-}h}$ | 13.90 $^{c\text{-}g}$ |
| | 7 | 14.93 $^{ab}$ | 14.03 $^{ef}$ | 13.97 $^{e\text{-}g}$ | 13.77 $^{e\text{-}h}$ | 13.80 $^{e\text{-}h}$ |
| 5 | 0 | 13.90 $^{c\text{-}g}$ | 13.57 $^{f\text{-}i}$ | 13.87 $^{d\text{-}g}$ | 13.80 $^{e\text{-}h}$ | 13.20 $^{h\text{-}k}$ |
| | 7 | 15.10 $^{a}$ | 15.20 $^{a}$ | 13.83 $^{e\text{-}h}$ | 13.80 $^{e\text{-}h}$ | 13.17 $^{f}$ |
| 7 | 0 | 14.70 $^{ab}$ | 13.77 $^{e\text{-}h}$ | 14.50 $^{b\text{-}d}$ | 13.17 $^{h\text{-}k}$ | 12.97 $^{i\text{-}k}$ |
| | 7 | 14.20 $^{d\text{-}f}$ | 13.53 $^{f\text{-}h}$ | 14.23 $^{c\text{-}e}$ | 13.30 $^{gh}$ | 12.40 $^{i}$ |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | | *<0.0001* | | | | |
| *Period* | | *<0.0001* | | | | |
| *Days* | | *<0.0001* | | | | |
| *T X D* | | *0.0103* | | | | |
| *P X D* | | *0.0006* | | | | |
| *TXP* | | *<0.0001* | | | | |
| *T X P X D* | | *<0.0001* | | | | |
| *LSD $_{(5\%)}$* | | *0.6483* | | | | |

Note: ILOS treatment is a once off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 12. Effect of different storage treatments on total soluble solids (°Brix) of 'Packham's Triumph' pears after designated post storage intervals and after the retailer's shelf conditions (0 day) during the 2013 harvest season.

| | Storage treatment | | | | |
|---|---|---|---|---|---|
| | TSS (˚Brix) | | | | |
| Storage period (months) | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 12.93 $^{e\text{-}g}$ | 15.03 $^{a}$ | 14.00 $^{cd}$ | 13.17 $^{d\text{-}f}$ | 13.50 $^{c\text{-}e}$ |
| 3 | 13.27 $^{de}$ | 15.23 $^{a}$ | 14.20 $^{bc}$ | 13.90 $^{cd}$ | 13.73 $^{c\text{-}e}$ |
| 5 | 12.37 $^{fg}$ | 14.23 $^{bc}$ | 14.93 $^{b}$ | 13.40 $^{c\text{-}e}$ | 13.37 $^{c\text{-}e}$ |
| *Significance level Pr>F* | | | | | |
| *Treatment* | *<0.0001* | | | | |
| *Period* | *<0.0001* | | | | |
| *T X P* | *0.4428* | | | | |
| *T X P X D* | *0.4272* | | | | |
| *LSD $_{(5\%)}$* | *0.8894* | | | | |

Note: ILOS treatment is a once-off preconditioning 10d treatment, ILOS+RA = Regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 13. Effect of different storage treatments on total soluble solids (°Brix) of 'Packham's Triumph' pears juice after designated post storage intervals and after retailer's shelf conditions during the 2013 harvest season.

| | TSS (˚Brix) | |
|---|---|---|
| | Shelf life (days) | |
| Storage treatment | 3 | 7 |
| ILOS+RA | 13.10 [c] | 13.23 [b] |
| ILOS+CA | 14.39 [a] | 14.81 [a] |
| DCA | 13.72 [b] | 14.19 [a] |
| CA | 13.68 [b] | 14.11 [a] |
| RA | 13.76 [b] | 14.39 [a] |
| *LSD (5%)* | *0.39* | *0.74* |
| Storage period (months) | | |
| 2 | 13.68 [ns] | 13.80 [b] |
| 3 | 13.70 | 14.09 [ab] |
| 5 | 13.80 | 14.55 [a] |
| *LSD (5%)* | *0.30* | *0.57* |
| *Significance level Pr>F* | | |
| *Treatment* | *<0.0001* | *0.0027* |
| *Period* | *0.6828* | *0.0357* |
| *Shelf life (days)* | *<0.0001* | |
| *T X P* | *0.0919* | *0.5980* |
| *T X D* | *0.4461* | |
| *P X D* | *0.3734* | |
| *T X P X D* | *0.4272* | |

Note: ILOS treatment is once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means within column followed by different letters are significantly different at $p<0.05$.

Table 14. Effect of different storage treatments on sugar to acid ratio of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment: ILOS+RA | ILOS+CA | DCA | CA | RA |
|---|---|---|---|---|---|---|
| 2 | 0 | 41.10 [e-h] | 36.94 [e-h] | 38.22 [g-i] | 38.97 [f-h] | 36.07 [h-j] |
| | 7 | 74.34 [ab] | 70.68 [bc] | 93.39 [a] | 57.23 [b-f] | 49.70 [c-f] |
| 3 | 0 | 36.94 [g-j] | 39.11 [f-h] | 29.91 [k] | 31.04 [jk] | 29.58 [k] |
| | 7 | 44.10 [ef] | 54.04 [b-f] | 42.01 [f] | 44.28 [ef] | 47.08 [d-f] |
| 5 | 0 | 47.68 [b-d] | 37.34 [g-i] | 37.58 [g-i] | 45.51 [b-e] | 51.57 [b] |
| | 7 | 52.78 [c-f] | 46.67 [ef] | 75.88 [ab] | 68.70 [b-d] | 56.25 [b-f] |
| 7 | 0 | 44.79 [c-f] | 44.75 [c-f] | 48.12 [bc] | 47.72 [b-d] | 63.18 [a] |
| | 7 | 58.62 [b-f] | 55.04 [b-f] | 46.56 [ef] | 56.53 [b-f] | 60.09 [b-f] |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | | *0.5282* | | | | |
| *Period* | | *<0.0001* | | | | |
| *Shelf life (days)* | | *<0.0001* | | | | |
| *T X D* | | *0.0678* | | | | |
| *P X D* | | *<0.0001* | | | | |
| *T X P* | | *<0.0001* | | | | |
| *T X P X D* | | *0.0005* | | | | |
| *LSD $_{(5\%)}$* | | *6.0867* | | | | |

Note. ILOS treatment is a once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 15. Effect of different storage treatments on sugar to acid ratio of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf conditions (0 day) during the 2013 harvest season.

| | TSS/TTA ratio |
|---|---|
| Storage treatment | |
| ILOS + RA | 47.22 [a] |
| ILOS + CA | 41.22 [b] |
| DCA | 40.04 [b] |
| CA | 35.87 [c] |
| RA | 41.54 [b] |
| *LSD $_{(5\%)}$* | *3.60* |
| Storage period (months) | |
| 2 | 32.00 [c] |
| 3 | 37.01 [b] |
| 5 | 41.46 [a] |
| *LSD $_{(5\%)}$* | *3.49* |
| *Significance level Pr>F* | |
| *Treatment* | *<0.0001* |
| *Period* | *<0.0001* |
| *Shelf life (days)* | *0.0666* |
| *T X D* | *0.6702* |
| *P X D* | *0.1288* |
| *T X P* | *0.1198* |
| *T X P X D* | *0.1435* |

Note: ILOS is a once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D= storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

Table 16. Effect of different storage treatments on sugar to acid ratio of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf conditions (3 and 7 days) during the 2013 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| | | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 3 | 43.11 [b-d] | 29.07 [de] | 28.62 [de] | 34.51 [de] | 30.23 [e] |
| | 7 | 38.89 [cd] | 33.35 [d] | 53.43 [b-d] | 54.90 [b-d] | 32.64 [d] |
| 3 | 3 | 52.94 [ab] | 37.14 [cd] | 33.08 [de] | 25.75 [e] | 34.00 [de] |
| | 7 | 79.35 [a] | 54.43 [b-d] | 46.93 [b-d] | 37.25 [cd] | 56.56 [a-c] |
| 5 | 3 | 59.21 [a] | 50.37 [a-c] | 26.37 [de] | 27.00 [e] | 57.14 [ab] |
| | 7 | 58.26 [a-c] | 54.32 [b-d] | 41.35 [b-d] | 46.48 [b-d] | 63.99 [ab] |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | 3 | *<0.0001* | | | | |
| | 7 | *0.3064* | | | | |
| *Period* | 3 | *0.0047* | | | | |
| | 7 | *0.0472* | | | | |
| *T X D* | | *0.6702* | | | | |
| *P X D* | | *0.1288* | | | | |
| *T X P* | 3 | *0.0358* | | | | |
| | 7 | *0.0185* | | | | |
| *T X P X D* | | *0.1435* | | | | |
| *LSD (5%)* | 3 | *14.83* | | | | |
| | 7 | *23.10* | | | | |

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere, T X P X D = Storage treatment x storage period x shelf life. Means with different letters are significantly different at $p<0.05$.

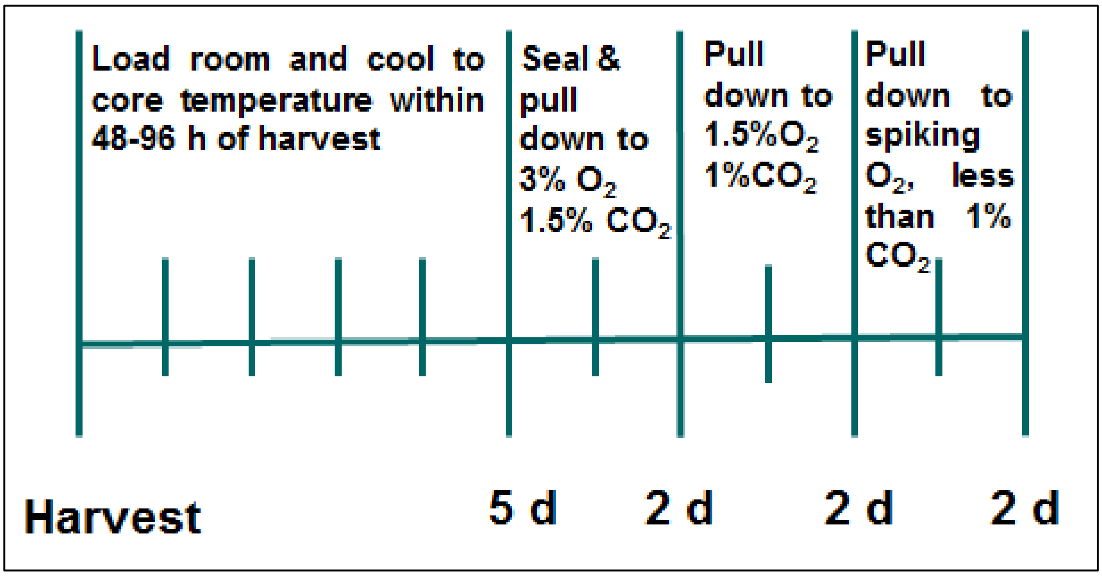


Figure 1. Dynamic controlled atmosphere (DCA) protocol for ‘Packham's Triumph’ pear (van der Merwe, 2013).

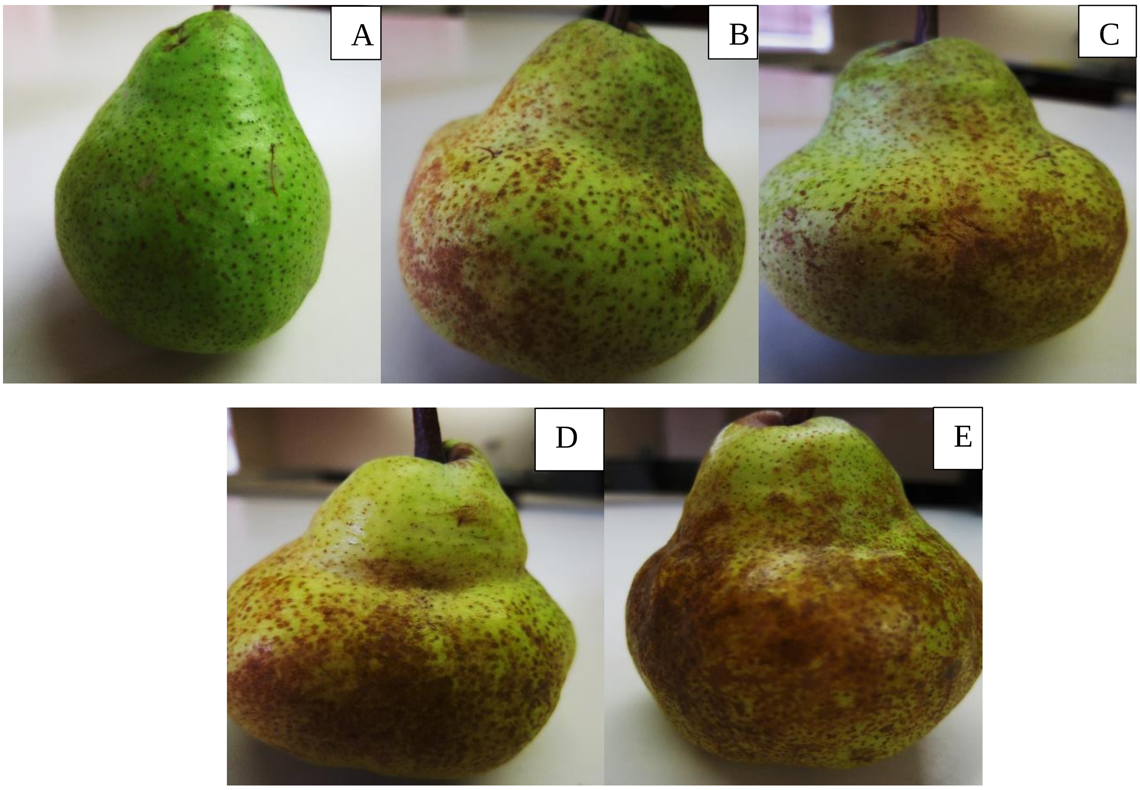


Figure 2. Grading of superficial scald severity on ‘Packham’s Triumph’ pears A = Grade 0 (no scald), B = Grade 1 (light scald 1-25%), C = Grade 2 (moderate scald 26-50%), D = Grade 3 (severe scald 50-75%) and E = Grade 4 (extreme scald 76-100%).

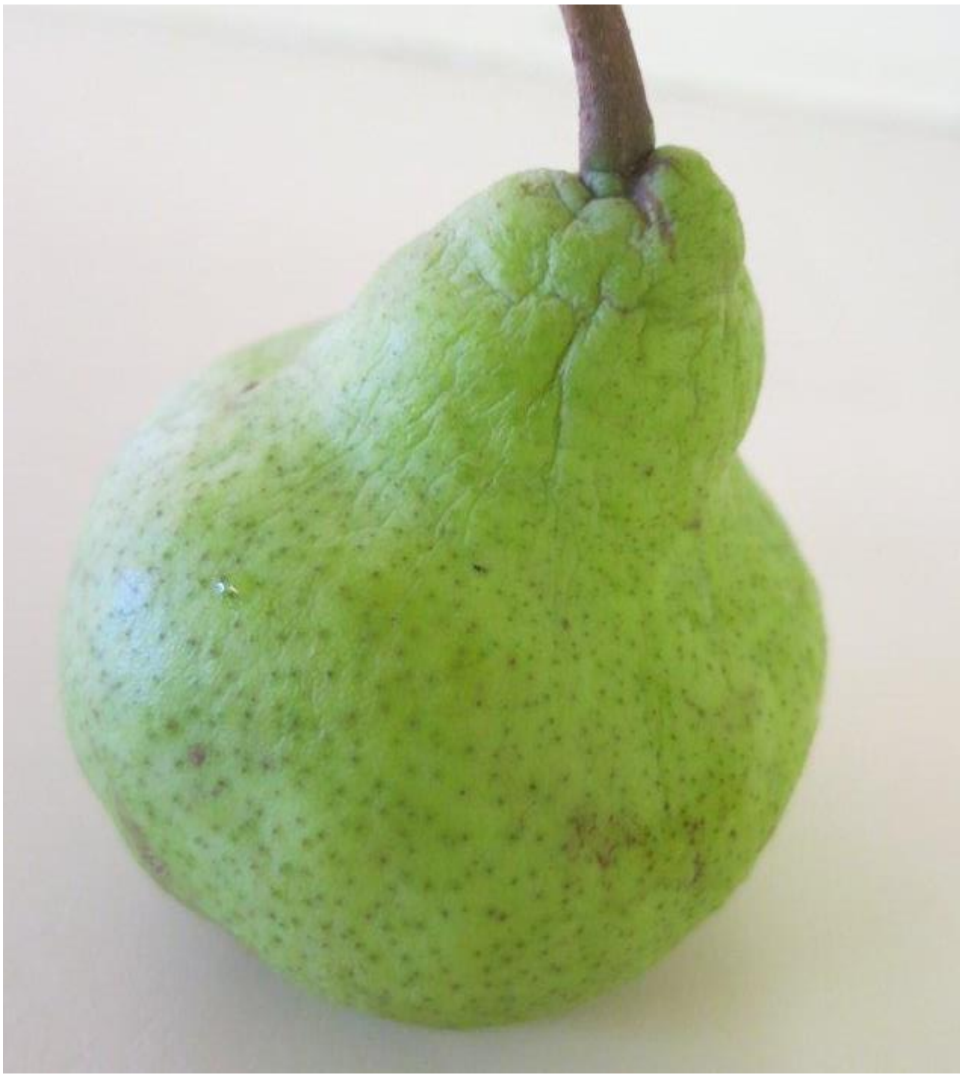

Figure 3. Neck shrivelling on 'Packham's Triumph' pear at the end of storage life at -0.5 °C after 3 months of storage on DCA treated fruit.

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere.

# PAPER 2: EFFECTS OF DYNAMIC CONTROLLED ATMOSPHERE AND INITIAL LOW OXYGEN STRESS TECHNOLOGIES ON PHYSICO-CHEMICAL PROPERTIES AND SENSORY PARAMETERS OF 'PACKHAM'S TRIUMPH' (*PYRUS COMMUNIS L.)* PEARS

## Abstract

'Packham's Triumph' pears are kept in storage for extended periods to maximise the window period for export market and to ensure continuous availability of fruit for the local market. Like most climacteric fruit pears loose firmness and change colour from green to yellow during ripening after cold storage. Firmness and skin colour are commonly used parameters in quality control programmes of pack houses which handle large quantities of apples and pears. 'Packham's Triumph' pears were stored in various low oxygen regimes: DCA, CA preceded by ILOS, RA preceded by ILOS, CA and RA as a control were applied and for each storage treatment fruit were stored for 2, 3, 5 and 7 months at -0.5 °C followed by 0, 3 and 7 days shelf life. DCA treated fruit showed better postharvest quality retention than CA preceded by ILOS, CA, RA preceded by ILOS and RA (control), thus better skin colour retention. DCA technology offers an alternative to the use of chemicals for long term storage (7 months) and maintain better appearance and taste on 'Packham's Triumph' pears.

## 1. Introduction

Controlled atmosphere technologies store fruit at low oxygen levels and this might lead to accumulation of alcohols and acetaldehydes, ethanol, ethyl acetate and/or lactate which might cause off flavours, indicating a shift to anaerobic respiration (Kader, 1989). It is therefore necessary to investigate whether the effects of these treatments on taste and texture of the fruit after storage. Although the effects of ILOS on superficial scald has been well documented (Wang and Dilley, 2000; Calvo et al., 2002; van der Merwe et al., 2003) reports on effects of this technology on the quality of the fruit are limited. It is important to assess quality of fruit after storage to ensure that consumers are sold satisfactory products. Firmness and skin colour are commonly used parameters in quality control programmes of pack houses which handle large quantities of apples and pears. The primary textural attribute measure in fruits and vegetables is firmness (Shewfelt, 2009). 'Uta' pears treated with DCA were reported to be firmer than pears treated with CA (Lafer, 2011). Skin colour is one of the most important quality parameters in fresh produce industry as it determines the overall appearance of the fruit and determines acceptability of products by consumers.

DCA and CA preceded by ILOS has shown potential in controlling superficial scald on 'Packham's Triumph' pears (paper 1), however their effectiveness on retaining firmness, skin colour and sensory quality parameters on 'Packham's Triumph' pears are not documented in literature. The aim of this paper was to investigate the effects of low oxygen storage (DCA and CA) preceded by initial low oxygen stress (ILOS) on maintaining physico-chemical properties and sensory parameters of 'Packham's Triumph' pears.

The objectives of this study were to:

(1) Investigate the effects of ILOS, CA and DCA treatments on skin colour and firmness retention of 'Packham's Triumph' pears after long term storage, and

(2) Study the effects of initial low oxygen stress (ILOS), controlled atmosphere (CA) and dynamic controlled atmosphere (DCA) on sensory quality parameters (taste, texture and appearance) of 'Packham's Triumph' pears after long term storage.

## 2. Materials and methods

### 2.1. Fruit supply

Fruit were supplied as described in Chapter 1, Section 2.1.

### 2.2. Storage treatments and periods

Fruit were stored for up to 7 months at -0.5 °C in 5 storage treatments at various $O_2$ and $CO_2$ combinations (Table 1).

#### *2.2.1. Regular atmosphere preceded by initial low oxygen stress (RA preceded by ILOS)*

In RA preceded by ILOS fruit were stressed with low oxygen at 0.7% for 10 days as a preconditioning treatment (Table 2), before being transferred into the RA room. For the 2 months storage period fruit were kept in ILOS for 10 days only, and were followed for 8 weeks in RA to simulate shipment and handling. 'Granny Smith' apples and 'Packham's Triumph' pears destined for the export market, are first stored under CA conditions between 8 and 12 weeks before being packed and exported under regular atmosphere as a general handling and shipment protocol in South Africa (Crouch, 2003). For 3 months storage period fruit were kept in ILOS for 10 days only and thereafter kept in RA for 12 weeks. For 5 months storage period fruit were kept in ILOS for 10 days and followed by 22 weeks in RA.

For 7 months storage period fruit were kept in ILOS for 10 days only and thereafter followed by 30 weeks of RA to extend the marketing window.

### *2.2.2. Controlled atmosphere preceded by initial low oxygen stress (CA preceded by ILOS)*

Fruit treated with CA preceded by ILOS were kept for 10 days in initial low oxygen stress before transfer into CA. For the two months storage period fruit are stored in ILOS for 10 days and transferred into CA for 8 weeks (Table 2). For three months storage period fruit were stored in ILOS for 10 days followed by 6 weeks in CA and thereafter transferred to RA for 6 weeks to simulate shipment and handling. After the 5 months storage period the fruit were stored in ILOS for 10 days and thereafter transferred to CA for 16 weeks followed by 6 weeks in RA to simulate shipment and handling. For 7 months storage period fruit were preconditioned with ILOS for 10 days and transferred into CA for 24 weeks followed by RA for 6 weeks to simulate shipment and handling (Table 2).

### *2.2.3. Dynamic controlled atmosphere (DCA)*

DCA treated fruit were kept for 10 days in DCA followed by 8 weeks in RA to simulate shipment and handling for the 2 months storage period (Table 2). For the 3, 5 and 7 months storage periods the DCA treated fruit were kept in the DCA room for 6, 16 and 24 weeks respectively, and then transferred into RA for 6 weeks to simulate shipment and handling. The DCA treatment facility was fitted with HarvestWatch™ (SAtlantic Inc. Halifax, N.S., Canada) fluorescence interactive response monitor sensor (FIRM) to monitor fruit response to low $O_2$ levels hourly. The selection of representative sample of 6 fruit for each FIRM sensor is crucial (Zanella et al., 2005), because the selected fruit represent the whole batch in the treatment room. The fruit were loaded into cold rooms and the desired fruit core temperature of -0.5 °C was achieved within 48 to 96 hours after harvest. The spiking for lower oxygen limit was set at 0.3% $O_2$ for both harvest seasons (2012 and 2013), and it was thereafter adjusted to 0.5% $O_2$ for the rest of the storage period as explained by DeLong et al. (2007).

### *2.2.4. Controlled atmosphere (CA)*

CA fruit were kept for 10 days in CA followed by 8 weeks of RA for the 2 months storage period (Table 2). For the 3, 5 and 7 months storage period, fruit were kept in CA for 6, 16 and 24 weeks respectively and thereafter transferred to RA for 6 weeks to simulate shipment and handling.

### *2.2.5. Regular atmosphere (RA)*

RA was the control treatment for the trial. RA fruit were kept solely in RA for the duration of storage of 2, 3, 5 and 7 months (Table 2).

## 2.3. Physico-chemical properties

### *2.3.1. Firmness (N)*

A total of 10 fruit were used to evaluate firmness for each replicate (30 fruit for each treatment). Measurements for firmness were taken with a mounted penetrometer (GUSS fruit texture analyser, South Africa), using a plunger of 11.1 mm in diameter. Each fruit was peeled at opposite sides and the plunger pressed on to the peeled flesh and the firmness reading recorded.

### *2.3.2. Skin colour*

Skin ground colour using (Chroma Meter CR 400/410 Konica Minolta Sensing Inc. Japan) and each treatment was in triplicate. For each replicate 10 measurements were taken as each fruit represented a replicate, thus 10 replicates were used for skin colour measurements. The skin colour index was calculated (Equation 1) (van der Merwe, 2013).

$$\frac{L*.a*.b*}{3} - 2.8 \quad (1)$$

L* = CIE lightness coordinate, a* = CIE red (+)/green (-) colour attribute, b* = CIE yellow (+)/blue (-) colour attribute.

The hue angle (H*) and the yellowing index (YI) were calculated Equations (2) and (3) respectively (Pathare et al., 2013).

$$H^* = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

$$YI = \left[\frac{142.86b*}{L*}\right] \quad (3)$$

## 2.4. Sensory quality attributes (external appearance, taste and texture)

A trained panel of five highly experienced members' assessed the sensory attributes of 'Packham's Triumph' pears at the Agricultural Research Council (ARC) postharvest laboratory, ARC Infruitec-Nietvoorbij, Stellenbosch South Africa. The panel was comprised of three females and two males with more than five years of experience in conducting sensory evaluations on 'Packham's Triumph' pears grown in South Africa. Panel members are employed by the ARC postharvest laboratory and are regular pear consumers. One of the panel members had more than 20 years experience in pears postharvest assessment. Three of the panel members had five years pears postharvest assessment.

Thirty 'Packham's Triumph' pears per treatment (storage treatment × storage period × shelf life period) were used for sensory analysis. A total number of 10 randomly selected fruit from each replicate (thus 30 fruit from each treatment) were placed on white trays and evaluated, according to appearance score (1-10), texture score (1-10) and taste scores (1-10) and recorded. Appearance of the fruit was evaluated first, and thereafter fruit were cut and evaluated tor taste and texture. Codes were used for treatments applied to eliminate bias from the judges. Mineral water was used as palate cleanser between samples.

### *2.4.1. Appearance scores of 'Packham's Triumph' pears*

'Packham's Triumph' pears were evaluated and appearance score (1-10) recorded after each storage interval and after shelf life intervals of 3 and 7 days. External appearance of 'Packham's Triumph' pears was scored based on freedom from defects, decay, mechanical damage, scuff marks, uniformity of colour and physiological disorder incidence. The score of 1-3 represented poor appearance (unacceptable), a score of 4-6 represented limited marketability, whereas a score of 7-10 was considered desirable, with high marketability.

### *2.4.2. Texture scores of 'Packham's Triumph' pears*

After evaluating the fruit for external appearance the fruit were cut and evaluated for texture. Texture of the 'Packham's Triumph' pears was scored based on crunchiness and crispiness of the first bite. Texture scores were from 1-10. The texture scores from 1-3 represented poor texture (unacceptable), texture scores of 4-6 represented good texture, whereas the scales of 7-10 represented excellent texture.

*2.4.3. Taste scores of 'Packham's Triumph' pears*

'After evaluating the fruit for texture the fruit used were tasted by panel members and scored from 1-10. The taste score of 1-3 represented unacceptable taste, and scores of 4.1 - 6 represented acceptable taste, whereas scores of 7.1 - 10 represented excellent taste. Taste was scored based on pear flavour development and the presence of off-flavours. Unacceptable taste was mostly due to the presence of an alcoholic taste in the fruit leading to off flavours or the presence of bland flavour due to lack of pear flavour development.

3. **Results and Discussion**

3.1. Fruit firmness (N)

**2012 harvest season**.

The firmness of the fruit is one of the important physico-chemical properties used to monitor fruit quality during storage, distribution and ripening (Chen and Opara, 2013; Pasquirello et al., 2013). There was significant interaction among effects (storage treatment, storage period and shelf life) on the firmness retention of 'Packham's Triumph' pears grown during the 2012 harvest season $Pr>F$ = 0.0001 (Figure 1). There was no significant difference in firmness retention among RA preceded by ILOS, CA preceded by ILOS, DCA and CA treated fruit, however RA preceded by ILOS fruit were significantly firmer than RA fruit after 2 months of cold storage (Figure 1). The loss of firmness was rapid after the transfer of fruit from cold storage to 20 ºC for 7 days and this trend increased with storage duration (Figure 1 and Figure 2). Similar findings were reported on 'Estruca', 'Carmen', 'Precoce of Fiorana', 'Coscia', 'Turandot' and 'Tosca' pears by Pasquirello et al. 2013.

Fruit treated with RA preceded by ILOS and CA respectively had the highest percentage loss of at 22.22% and 20.68% respectively, higher than that of the control (RA) fruit at 20.27% during the 2012 season (Figure 1). DCA treated fruit had the lowest percentage loss of firmness from 0 day to 7 days at 16.53% compared to CA treated fruit which had 19.43% loss of firmness after 7 days shelf life. Similar trend was observed after 3, 5 and 7 months.

The firmness retention was above acceptable level of 50 N for end of storage life as stipulated by Little and Holmes (2000) for 'Packham's Triumph' pears for fresh produce markets of Australia, meaning that at the end of storage fruit were still of acceptable quality, except for CA preceded by ILOS at 7 months storage. At 7 days shelf life of the 2 months storage period there was no significant difference in firmness retention among the low oxygen storage treatments. The rapid loss of firmness after removal from cold storage into the ripening conditions in this trial confirm results reported on ‘Bartlett’ pears by Mitcham and Mitchell (2002) and loss of firmness on ‘Packham’s Triumph’ pears by Sugar and Basile (2014). Loss of firmness during ripening may be attributed to softening by pectinase enzymes (Little and Holmes, 2000) by dissolving the middle lamellae of cell walls (Wills et al., 1989). Softening of the fruit is directly correlated with the rate of degradation of pectic substances (Wills et al., 1989). Pectins prevent aggregation and collapse of the cellulose network as they form a hydrated gel phase in which the cellulose-hemi-cellulose network is embedded (Taiz and Zeiger, 2010).

DCA treated ‘Packham’s Triumph’ pears were firmer than RA and RA preceded by ILOS treated fruit, although not significantly different from CA and CA preceded by ILOS treated fruit after 3 months in cold storage (Figure 1). After 7 days for the 3 months storage period the firmness retention fell below 20 N for all treatments, the same trend that was observed during the 2 months storage period.

‘Packham’s Triumph’ pears treated with DCA were significantly firmer than CA preceded by ILOS, CA, RA preceded by ILOS and RA at 5 months storage period after removal from storage on 0 day (Figure 1). Lafer (2011) observed that ‘Uta’ pears treated with DCA were firmer than pears treated with CA. All other treatments were not significantly different. At 7 days after 5 months storage period there was no significant difference in firmness retention of ‘Packham’s Triumph’ pears, irrespective of treatment applied during storage. Same trend was observed during the 2 and 3 months storage period.

‘Packham’s Triumph’ pears stored in DCA and RA treatments were significantly firmer than fruit from all treatments after 7 months in storage (Figure 1). Zanella et al. (2008) showed that DCA retains firmness better than ultra-low oxygen treatment followed by CA. Firmness level of ‘Packham’s Triumph’ pears treated with CA preceded by ILOS was 45.10 N and CA fruit was 48.08 N and this was below minimum acceptable level after storage of 50 N as stipulated by Little and Holmes (2000). RA fruit were firmer than CA and RA preceded by ILOS fruit after 0 day after 7 months of cold storage, and CA preceded by ILOS fruit had the lowest firmness retention. At 7 days during the 7 months period firmness level for the fruit fell below minimum acceptable level of 20 N the same trend observed during 2, 3 and 5 months storage period irrespective of storage treatment.

**2013 harvest season**.

There was significant interaction among effects (storage treatment, storage period and shelf life) on the firmness retention of ‘Packham’s Triumph’ pears grown during the 2013 harvest season $Pr>F$ = 0.0001 (Figure 2). Similar trend was observed during the 2012 harvest season (Figure 1). Firmness decreased with prolonged shelf life period, irrespective of the treatment applied, following 2, 3 and 5 months cold storage during the 2013 season (Figure 2*)*. Firmness was below 20 N irrespective of storage treatment after 7 days shelf life for all storage periods (2, 3 and 5 months). There was no significant difference at the beginning of shelf life and after 3 days in shelf life after 5 months among DCA, CA and CA preceded by ILOS treated fruit.

3.*2. Skin colour*

**2012 harvest season**.

There was significant interaction between storage treatment, storage period and shelf life. During the 2012 harvest season after 2 months in cold storage on 0 day there was no significant difference in skin colour irrespective of treatment applied, however RA preceded by ILOS treated ‘Packham’s Triumph’ pears were of unacceptable score of skin colour of 4.04-full yellow (FY) (Table 3). There was significant increase in skin colour on DCA treated fruit from 0 day to 7 days after 2 months in cold storage, however the skin colour score was green yellow yellow (GYY) which is of acceptable colour to consumers. Irrespective of applied treatment skin colour scores increased significantly from 0 day to 7 days (Table 3).

After 3 months in cold storage ‘Packham’s Triumph’ pears DCA treated fruit had significantly better skin colour on 0 day better than CA, CA preceded by ILOS, RA and RA preceded by ILOS fruit. The score was 2.85 class green yellow (GY). After 7 days shelf life of the 3months storage period there was no significant difference in skin colour, irrespective of treatment applied, however the skin colour score was still of acceptable colouring. The same pattern of increase in skin colour after 3 months cold from 0 day to 7 days was observed similar to the 2 month storage period (Table 3).

DCA, CA preceded by ILOS and CA treated ‘Packham’s Triumph’ pears were significantly different in skin colour scores (equation 1) and yellowing index (equation 3) from RA preceded by ILOS and RA fruit and showed better skin colour retention than the control treatments after 5 months storage (Table 3). The same trend was observed after 3 and 7 days in shelf life for CA, CA preceded by ILOS and DCA treated fruit had better skin colour retention than RA and RA preceded by ILOS treated ‘Packham’s Triumph’ pears. In addition to suppressing superficial scald and decay DCA, CA preceded by ILOS and CA storage technologies maintain skin colour better than RA preceded by ILOS and RA on during long term storage of ‘Packham’s Triumph’ pears.

DCA and CA treated ‘Packham’s Triumph’ pears had better skin colour retention on 0day of the 5 month period (skin colour score of (2-2.99 = GY) than CA preceded by ILOS, RA preceded by ILOS and RA. There was no significant difference in skin colour retention after 7 days of the 5 months storage period, irrespective of treatment applied. The same pattern of increase in skin colour form 0 day to 7 days was observed as during the 2 and 3 months storage period.

Generally the yellowness of the fruit is associated with degradation of product by light, chemical exposure and processing (Pathare et al., 2013). DCA, CA and CA preceded by ILOS treated ‘Packham’s Triumph’ pears were much greener at 0 day than RA preceded by ILOS and control fruit (Table 3 and Table 4). RA preceded by ILOS treated fruit was more yellow than RA treated fruit. There was significant correlation ($p$=<$0.0001$) between firmness retention and skin colour retention ($R^2$ = -0.63). The yellow the fruit (higher the skin colour score) the lower the firmness. Skin colour was also negatively correlated with texture score $R^2$ = -0.64 ($p$=<$0.0001$). Changes in skin colour are as a result of both storage treatments and shelf life conditions. DCA, CA and CA preceded by ILOS treated fruit had better skin colour retention than RA and RA preceded by ILOS treated fruit.

DCA treated ‘Packham’s Triumph’ pears had better skin colour retention than CA preceded by ILOS, RA preceded by RA and RA, however it was not significantly different from CA treated fruit based on hue angle and skin colour scores after 7 months in storage during the 2012 season (Table 5). RA and RA preceded by ILOS had the highest skin colour score (more yellow) based on both the hue angle and the skin colour score. Skin colour index of ‘Packham’s Triumph’ pears increases thus changes from green to yellow as during ripening. By end of storage period after 7 months the skin colour index was more yellow in all treatments supporting findings by Whitaker et al. 2009 on Bartlett pears. Skin colour increases with period in shelf life conditions (20 °C) in all treatments, with treatment driving the change in skin colour and firmness tend to decrease as skin colour increases.

**2013 harvest season.**

The interaction among effects (storage treatment, storage period and shelf life) on the skin colour of ‘Packham’s Triumph’ pears grown during the 2013 harvest season was not significant *Pr>F* = 0.2346 (Table 4). The effect of days in shelf life was significant Pr>F = 0.0003, implying that the changes in skin colour were as a result of number of days in shelf life conditions at 20 °C. Neither treatment applied during storage nor storage period had significant effect on skin colour of the ‘Packham’s Triumps’ pears.

There were no significant changes in colour based on scores (Table 4) for all treatments (DCA, CA, CA preceded by ILOS, RA and RA preceded by ILOS) during the 2 and 3 months storage period. The ‘Packham’s Triumph’ pears stored in RA preceded by 10 days ILOS treatment were more yellower after 3 and 7 days shelf life during the 5 month storage period compared to fruit stored in other treatments (DCA, CA, CA preceded by ILOS and RA). ILOS followed by RA treatment seems to exacerbate the yellowing of ‘Packham’s Triumph’ pears that control (RA) treatment. Yellowness of the fruit is often associated with degradation of product by light, chemical exposure and processing (Pathare et al., 2013), indicating that ILOS treatment followed by long term RA storage did not delay the yellowing of ‘Packham’s Triumph’ pears.

After 7 months (0 day) in cold storage ‘Packham’s Triumph’ pears treated with DCA and CA had significantly higher Hue angle than CA preceded by ILOS, RA preceded by ILOS and control fruit (Table 5). The skin colour score was also significantly lower (greener) on DCA and CA treated fruit than on CA preceded by ILOS, RA preceded by ILOS and RA treated fruit.

*Pearson correlations between firmness and skin colour retention of ‘Packham’s Triumph’ pears (2012 season)*

Firmness retention was negatively correlated with skin colour retention ($R^2$ = -0.63) ($p$ = <*0.0001*), thus the more yellow the fruit (higher the skin colour score) the lower the firmness, thus the less firm the fruit the yellower the fruit. Skin colour was also negatively correlated with texture score $R^2$ = -0.64 (*p* = *<0.0001*) implying that as the fruit texture score decreased the fruit skin colour score increased meaning the fruit became more yellow.

## 3.3. Sensory attributes

### *3.3.1. Appearance scores of 'Packham's Triumph' pears after storage*

**2012 harvest season**.

The appearance of fruit influences the market value and consumer acceptance, making it one of the important quality parameters. Consumers are likely to use appearance to inform their choices at the point of purchase (Jaeger et al., 2003). There was significant interaction among effects (storage treatment, storage period and shelf life) on the appearance scores of 'Packham's Triumph' pears grown during the 2012 harvest season *Pr>F* = 0.0001 (Figure 3). At 0 day of the 2 months storage period, RA fruit had significantly better appearance scores than other treatments (DCA, CA, CA preceded by ILOS and RA preceded by ILOS); however all the treatments had scores of excellent appearance (between 7 and 10) (Figure 3). After 7 days in shelf life of 2 months storage period DCA treated fruit had significantly better appearance scores than all the other treatments (CA preceded by ILOS, CA, RA preceded by ILOS and RA), and the similar trend was observed after the 3 months storage period at 0 day. There was no significant difference of the appearance scores of CA preceded by ILOS, CA and RA treated fruit at 0 day of the 3 months storage period

After the 5 months storage period at 0 day, DCA treated 'Packham's Triumph' pears had significantly higher appearance scores than other CA and CA preceded by ILOS fruit (Figure 3). However CA and CA preceded by ILOS treated fruit were not significantly different, although significantly higher than RA and RA preceded by ILOS treated fruit. Fruit from all treatments had acceptable appearance scores of between 4 and 6. After 7 days following the 5 months period, DCA, CA and CA preceded by ILOS had significantly higher appearance scores than RA and RA preceded by ILOS fruit. The appearance score for control (RA) fruit were significantly lower after 7 days following the 5 months storage period and were of poor appearance perhaps due to the visible symptoms of superficial scald. Superficial scald affects the external appearance of the fruit reducing its fresh market value. The presence of physiological disorders greatly reduces the appearance score of fruit (Ke and Kader, 1992).

At 0 day of the 7 months storage period DCA and CA had significantly higher appearance scores than CA preceded by ILOS, RA preceded by ILOS and RA (Figure 8). However RA and RA preceded by ILOS showed signs of superficial scald, the fruit were still of limited marketability with the scores of between 4 and 6. After 7 days shelf life during the 7 months period RA, RA preceded by ILOS and CA fruit has the significantly lowest appearance scores due to the presence of superficial scald on the fruit. The appearance score was of poor quality (between 0 and 3).

**2013 harvest season**.

There was significant interaction among effects (storage treatment, storage period and shelf life) on the appearance of ‘Packham’s Triumph’ pears grown during the 2013 harvest season $Pr>F$ = 0.0001 (Figure 4). At 0 day of the 2 months storage period, there were no significant differences irrespective of applied treatment (DCA, CA, CA preceded by ILOS, RA preceded by ILOS and RA) for the 2013 harvest season (Figure 4), however the appearance scores were of excellent quality (scores between 7 and 10). There were no significant differences in appearance scores of ‘Packham’s Triumph’ pears on DCA and CA fruit on 0, 3 and 7 days shelf life after the 2 months storage period. After 3 days of shelf life the appearance score for RA fruit was significantly lower than those of RA preceded by ILOS, CA preceded by ILOS, DCA and CA stored fruit. After 7 days of shelf life the DCA and CA fruit had significantly higher appearance scores than CA preceded by ILOS, RA preceded by ILOS and RA stored fruit.

After 3 months in storage at 0 day, the fruit stored in DCA, CA and CA preceded by ILOS had significantly higher appearance scores of excellent appearance (between 7 and 10), whereas RA preceded by ILOS and RA had the lower appearance score of acceptable appearance (Figure 4). The appearance scores of RA preceded by ILOS were higher than the scores of RA stored fruit. After 3 days in shelf life, the appearance scores of DCA and CA were significantly higher than scores for CA preceded by ILOS treated fruit. CA preceded by ILOS fruit had significantly higher appearance scores than RA preceded by ILOS and RA, although the scores were all of acceptable appearance (between 4 and 6).

After 5 months in cold storage the DCA and CA treated fruit had significantly higher appearance scores than CA preceded by ILOS fruit, and the scores were of excellent quality (Figure 4). The appearance scores for DCA and CA were not significantly different after 0, 3 and 7 days shelf life following the 5 months storage period. After 3 days shelf life the RA preceded by ILOS treated fruit appearance scores were significantly higher than those of RA stored fruit, however the scores were of limited marketability (between 4 and 6). RA and RA preceded by ILOS treated fruit had the significantly lowest scores due to the appearance of symptoms of superficial scald after 7 days of the 5 months storage period.

*3.3.2 Texture scores of 'Packham's Triumph' pears after storage*

**2012 harvest season**.

According to Chen and Opara (2013) food texture is used widely to measure quality attributes during postharvest handling, processing, and consumption. There was significant interaction among treatment, storage period and shelf life on texture scores of 'Packham's Triumph' pears during the 2012 harvest season $Pr>F$ = <0.0001. Texture score was higher at 0 day compared to 7 days for all storage periods (2, 3, 5 and 7 months), irrespective of the applied treatment during storage (DCA, CA, CA preceded by ILOS, RA preceded by ILOS and RA) during the 2012 season (Figure 5). At 0 day of the 2 months storage period, the texture scores were of excellent texture quality (scores between 7 and 10) irrespective of applied treatment (Figure 5).

RA preceded by ILOS and CA preceded by ILOS treated 'Packham's Triumph' pears had significantly higher texture scores than DCA, CA and RA treated fruit at 0 day of the 2 months storage period. After 7 days in shelf life there was no significant difference in texture scores on DCA, CA, CA preceded by ILOS and RA preceded by ILOS however the fruit had significantly higher texture scores than the control fruit.

After 3 months in storage on 0 day, there was no significant difference among treatments (RA preceded by ILOS, CA preceded by ILOS, DCA, CA and RA) on texture scores (Figure 5). However after 7 days in shelf life the DCA treated fruit had significantly higher texture scores than CA preceded by ILOS and CA fruit. The RA preceded by ILOS treated fruit had significantly higher texture score than RA fruit.

At 0 day of the 5 months storage period, DCA treated fruit had significantly higher texture scores than all other treatments (CA, CA preceded by ILOS, RA preceded by ILOS and RA) (Figure 5). The texture scores on CA and RA preceded by ILOS were not significantly different, however were significantly higher than scores for RA and CA preceded by ILOS fruit. After 7 days DCA treated fruit had significantly higher texture scores than all other treatments (CA, CA preceded by ILOS, RA preceded by ILOS and RA). There was no significant difference in texture scores among fruit treated with CA, CA preceded by ILOS, RA and RA preceded by ILOS after 7 days shelf life following the 5 month storage period (Figure 5).

The texture scores of DCA treated fruit were significantly higher at 0 day of the 7 months storage period compared to other treatments (CA preceded by ILOS, CA, RA preceded by ILOS and RA) (Figure 5), and the texture score was of excellent quality. The texture scores of CA fruit had the significantly lowest texture score, although the score was still of acceptable quality (between 4 and 6). After 7 days following the 7 months storage period, RA preceded by ILOS had significantly higher texture scores compared to DCA and CA preceded by ILOS fruit, and the texture score was of excellent quality (between 7 and 10). The texture scores for DCA and CA preceded by ILOS were not significantly different although they were higher than those of RA and CA.

**2013 harvest season**.

The interaction for texture scores was significant $Pr>F$ = <0.0001 among main effects (storage treatment, storage period and shelf life) during the 2013 harvest season (Figure 6). There was no significant difference in texture scores of 'Packham's Triumph' pears after 2 months storage period and 0 day, irrespective of applied storage treatment. The texture scores fell from 0 day, 3 to 7 days shelf life on all treatments except for CA where the texture scores for 0 day and 3 days were not significantly different. After 7 days in shelf life DCA, CA and RA fruit had significantly higher texture scores than RA preceded by ILOS and CA preceded by ILOS treated fruit, however the scores were of acceptable quality.

After 3 months in storage the texture scores fell significantly form 0 day, 3 and 7 days on RA preceded by ILOS, CA and RA fruit, however for DCA and CA preceded by ILOS the scores fell only from 0 day to  days and thereafter the scores remained significantly the same (Figure 6). After the 7 day shelf life period DCA and CA fruit had significantly higher texture scores than CA preceded by ILOS, RA preceded by ILOS and RA fruit, however the scores were of acceptable quality of texture. RA fruit had significantly higher texture scores than RA preceded by ILOS fruit.

At 0 day of the 5 months storage period, there were no significant differences in the texture scores of all treatments (RA preceded by ILOS, CA preceded by ILOS, DCA, CA and RA) (Figure 6). After 3 days shelf life following the 5 months storage period, CA treated 'Packham's Triumph' pears had significantly higher texture scores than DCA treated fruit and both treatments had significantly higher scores than CA preceded by ILOS, CA and RA stored fruit. The texture scores for CA and DCA were of excellent quality (between 7 and 10) (Figure 6). The appearance scores for CA preceded by ILOS, RA and RA preceded by ILOS were still of acceptable taste (between 4 and 6). After 7 days in shelf life during the 5 months storage period, DCA and CA treated fruit had significantly higher texture scores than CA preceded by ILOS fruit. 'Packham's Triumph' pears stored in CA preceded by ILOS were significantly higher than scores of fruit stored in RA preceded by ILOS, although not significantly different from the control.

There were no significant differences in texture at 0 day of 2, 3 and 5 months storage interval irrespective of treatment. Echeverria et al. (2004) found no significant difference on texture scores of 'Fuji' apples treated with low oxygen treatments and control after 1 day following cold storage.

*3.3.3. Taste scores of 'Packham's Triumph' pears after storage*

**2012 harvest season**.

There was significant interaction among main effects $Pr>F$ = <0.0001 (storage treatment, storage period and shelf life) on taste scores of 'Packham's Triumph' pears during the 2012 harvest season. The taste scores increased with shelf life period 0 day and 7 days after 2 months in storage for RA preceded by ILOS, CA preceded by ILOS, DCA and CA treated fruit except for control (Figure 7). The taste scores for control remained the same after 0 and 7 days shelf life of the 2 months cold storage period. The taste scores were acceptable (scores ranging from 4-6) after 2 months storage period.

Taste scores increased with shelf life period from 0 day to 7 days in CA preceded by ILOS, CA, DCA and RA except for RA preceded by ILOS treated fruit which had no significant difference in taste scores at day 0 and at 7 days after 3 months in cold storage; however the scores remained acceptable for human consumption.

The taste scores for CA preceded by ILOS, DCA and CA treated 'Packham's Triumph' pears increased at 7 days of 5 months in cold storage, and for RA preceded by ILOS treated fruit the taste score was not significantly different, however it decreased in control fruit. Although the taste scores of 'Packham's Triumph' pears were significantly lower after 7 days of the 3 months storage period, the scores were of acceptable taste quality.

After 7 months in cold storage, the taste scores decreased in CA preceded by ILOS, DCA, CA, RA and RA preceded by ILOS fruit the increased from 0 day to 7 days retailer's shelf conditions. DCA treated fruit had a better taste score than RA and RA preceded by ILOS treated fruit, perhaps due to preservation of aroma compounds during long term storage by DCA treatment (Raffo et al., 2009).

**2013 harvest season**.

There was significant interaction among main effects $Pr>F$ = <0.0001 (storage treatment, storage period and shelf life) on taste scores of 'Packham's Triumph' pears during the 2013 harvest season. At 0 day of the 2 months storage period there was no significant difference in taste scores among treatments (DCA, CA preceded by ILOS, RA preceded by ILOS, CA and RA) (Figure 8). After 3 and 7 days shelf life of the 2 months storage period RA preceded by ILOS treated 'Packham's Triumph' pear had significantly lower taste scores than RA stored fruit (Figure 8), however the scores were still of acceptable texture. After 7 days shelf life DCA, CA and RA fruit had significantly higher scores than initial low oxygen treatments. Initial low oxygen treatments (CA and RA) had the lowest taste scores after shelf life condition of 3 and 7 days after all storage periods (2,3 and 5 months) (Figure 8).

After 3 months in storage at 0 day, RA preceded by ILOS treated fruit had the lowest score of 3 which are regarded as of poor taste; however the RA treated fruit had a score of 5.5 which is of acceptable taste (Figure 8). The taste score for RA fruit were significantly higher than those of other treatments (DCA, CA, CA preceded by ILOS and RA preceded by ILOS) after 7 days in shelf life after the 3 months storage period. DCA, CA and CA preceded by ILOS maintained taste scores of acceptable human consumption after long term storage. Similar results have been reported on 'Fuji' apples by Echeverria et al. (2004).

After 5 months in storage the taste scores of DCA and CA treated fruit increased from 3 days to 7 days shelf life, although the scores were not significantly different after 0 and 3 days (Figure 8). The taste scores for CA preceded by ILOS treated fruit were not significantly different after 0, 3 and 7 days shelf life after 5 months in cold storage. On RA stored fruit the taste scores increased from 0 day to 3 days, but remained the same after 7 days shelf life during the 5 month storage period. The taste scores for all treatments (DCA, CA, CA preceded by ILOS, RA and RA preceded by ILOS) were of acceptable quality after 0, 3 and 7 days of the 5 month storage period (Figure 8).

There were no off flavors in DCA treated fruit resulting with higher taste scores than control fruit which had lower taste scores. Similar results of off flavours in fruit due to accumulation of acetaldehydes and ethanol as a result of anaerobic fermentation caused by hypoxic conditions in control fruit have been reported on peaches by Bonghi et al. (1999) and on ‘Bartlett’ pears by Ke et al. (1990).

*Pearson correlations between firmness, texture and appearance scores (2012 season)*

There was strong positive correlation between texture and firmness $R^2$ = 0.74 ($p$ = <*0.0001*), the firmer the fruit the higher the texture scores. Firmness was measured objectively using the penetrometer and texture was measured subjectively by the tasting panel however there was strong positive correlation. Although firmness was measured objectively and texture was measured subjectively by the tasting panel the correlation show a strong positive correlation confirming that both the subjective and objective measurements got the same results. Texture and appearance $R^2$ = 0.65 ($p$ = <0.0001). Texture was positively correlated with appearance score of the fruit, the higher the texture scores the better the appearance of the fruit. Texture was positively correlated with taste score of the fruit ($R^2$ = 0.58 ($p$ = <0.0001), the better the texture the better the taste of the fruit.

## 4. Conclusions

Loss of firmness is a limiting parameter for long term storage in low oxygen treated ‘Packham’s Triumph’ pears. DCA and RA treated ‘Packham’s Triumph’ pears had significantly better firmness retention than CA preceded by ILOS, RA preceded by ILOS and CA after 7 months as it was above the minimum acceptable limit of 50 N immediately after cold storage. The firmness level fell below acceptable human consumption (20 N) after 7 days of 2, 3 5 and 7 months’ storage periods, irrespective of treatment applied during storage. Apart from suppressing superficial scald on Packham’s Triumph’ pears DCA technology retains firmness better after extended storage of 7 months.

DCA and CA treated fruit had better retention of skin colour after 7 months in storage compared to CA preceded by ILOS treated fruit. CA and CA preceded by ILOS treated

‘Packham’s Triumph’ pears were not significantly different in skin colour retention after 7 months. CA preceded by ILOS treated fruit had the highest yellowing index (YI) but were not significantly different from RA preceded by ILOS and DCA fruit. DCA and RA preceded by ILOS treated ‘Packham’s Triumph’ pears had better taste scores than CA, CA preceded by ILOS and RA treated fruit after 7 months during the 2012 season. RA fruit had the lowest taste score below acceptable levels for human consumption due to the presence of off flavours in the fruit. DCA and CA treated ‘Packham’s Triumph’ pears had better appearance than fruit treated with CA preceded by ILOS, RA preceded by ILOS and control. Control fruit had the lowest appearance score at the of the 5 months storage period. Texture score decreases with increased shelf life. DCA, CA and CA preceded by ILOS generally maintained sensory quality attributes (appearance, texture and taste) of ‘Packham’s Triumph’ pears. Echeverria et al. (2004) reported similar findings by ultra-low oxygen on ‘Fuji’ apples.

## 5. References

Bonghi, C., Ramina, A., Ruperti, B., Vidrih, R. & Tonutti, P. (1999). Peach fruit ripening and quality in relation to picking time, and hypoxic and high $CO_2$ short-term postharvest treatments. *Postharvest Biology and Technology*, 16, 213-222.

Calvo, G., Salvador, M.E. & Sanchez, E. (2002). Control of superficial scald in 'Beurre d' Anjou' pears with low oxygen levels. *Acta Horticulturae*, 596, 879-882.

Chen, L. & Opara, U.L. (2013). Texture measurement approaches in fresh and processed foods-A review. *Food Research International*, 51, 823–835.

Crouch, I. (2003). 1-Methylcyclopropene (SmartFresh™) as an alternative to modified atmosphere and controlled atmosphere storage of apples and pears. *Acta Horticulturae*, 600, 433-436.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2007). Chlorophyll fluorescence based low-$O_2$ CA storage of organic 'Cortland' and 'Delicious' apples. *Acta Horticulturae*, 737, 31-37.

Echeverria, G., Lara, I., Fuentes, T., Lopez, M.L., Graell, J & Puy, J. (2004). Assessment of relationships between sensory and instrumental quality of controlled atmosphere stored 'Fuji' apples for multivariate analysis. *Journal of Food Science*, 69, 368-375.

Jaeger, S.R., Lund, C.M., Lau, K. & Harker, F.R. (2003). In search of the "ideal' pear (*Pyrus* spp.): Results of a multidisciplinary exploration. *Journal of Food Science*, 68, 1108-1117.

Kader, A.A. (1989). Mode of action of oxygen and carbon dioxide on postharvest physiology of 'Bartlett' pears. *Acta Horticulturae*, 88, 161-167.

Ke, D., van Gorsel, H. & Kader, A.A. (1990). Physiological and quality responses of 'Bartlett' pears to reduced $O_2$ and enhanced $CO_2$ levels and storage temperature. *Journal of American Society of Horticultural Science*, 115, 453-439.

Ke, D. & Kader, A.A. (1992). External and internal factors influence fruit tolerance to low-oxygen atmospheres. *Journal of American Society of Horticultural Science*, 117, 913–918.

Lafer, G. (2011). Effect of different CA storage conditions on storability of 'Uta' pear. *Acta Horticulturae*, 909, 757-760.

Little, C.R. & Holmes, R.J. (2000). Storage technology for apples and pears: A guide to production, postharvest treatment and storage of pome fruit in Australia (pp. 420-515). Knoxfield: Highway Press Pty Ltd.

Mitcham, E.J. & Mitchell, F.G. (2002). Postharvest handling systems: Pome fruits. In Kader A.A. (Ed.), *Postharvest technology of horticultural crops* (3rd ed., pp. 333-344). University of California Publication 3311.

Pasquirello, M.S., Rega, P., Migliozzi, T., Capuano, L.R., Scotchini, M. & Petriccione, M. (2013). Effect of cold storage and shelf life on physiological and quality traits of early ripening pear cultivars. *Scientia Horticulturae*, 162, 341-350.

Pathare, P.B., Opara, U.L. & Al-Said, F.A. (2013). Colour measurement and analysis in Fresh and processed Foods: A review. *Food and Bioprocess Technology*, 6, 36-60.

Raffo, A., Kelderer, M., Paoletti, F. & Zanella A. (2009). Impact of innovative controlled atmosphere storage technologies and postharvest treatments on volatile compound production in cv. 'Pinova' apples. *Journal of Agricultural and Food Chemistry*, 57, 915-923.

Shewfelt, R.L. (2009). Measuring quality and maturity. In Florkowski W.J., Shewfelt, R.L., Brueckner, B., Prussia, S.E. & Lurie, S. (Eds.) *Postharvest and handling: A systems approach* (pp. 100-108). Elsevier Academic Press.

Sugar, D. & Basile S.R. (2014). Induction of ripening capacity in 'Packham's Triumph' and 'Gebhard Red 'D Anjou' pears by temperature and ethylene conditioning. *Postharvest Biology and Technology*, 9, 84-89.

Taiz, L. & Zeiger. E. (2010). Plant physiology (5th ed., p. 429). Sunderland Massachusets U.S.A.: Sinauer Associates, Incorporated.

Van der Merwe, J.A., Combrink, J.C. & Calitz F.J. (2003). Effect of controlled atmosphere storage after low initial low oxygen stress treatment on superficial scald development on South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae*, 600, 261-265.

Van der Merwe, J.A. (2013). Chroma meter technology provides accuracy. Postharvest Innovation Symposium Proceedings 19-21 November 2013, (unpublished poster).

Wang, Z. & Dilley, D.R. (2000). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology*, 18, 201-213.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, J.E. & Mattheis, J.P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology*, 53, 43-50.

Wills, R.B.H., McGlasson, W.B., Graham, D., Lee, T.H. & Hall, E.G. (1989). *Postharvest: An introduction to the physiology and handling of fruit and vegetables* ($5^{th}$ edn. pp. 17-38). University of New South Wales Press.

Zanella, A., Cazzanelli, P., Panarese, A., Coser, M., Cecchinel, M. & Rossi, O. (2005). Fruit fluorescence response to low oxygen stress: Modern storage technologies compared to 1-MCP treatment of apple. *Acta Horticulturae*, 682, 1535-1542.

Zanella, A., Cazzanelli, P. & Rossi, O. (2008). Dynamic controlled atmosphere (DCA) storage by means of chlorophyll fluorescence response for firmness retention in apple. *Acta Horticulturae*, 796, 77-82.

Table 1. $O_2$ and $CO_2$ concentrations associated with storage treatments of 'Packham's Triumph' pears during or before storage at -0.5 °C

| Storage treatment | Gas composition (%) | |
|---|---|---|
| | % $O_2$ | % $CO_2$ |
| DCA | 0.5 | 1 |
| CA | 1.5 | 2.5 |
| RA (control) | 20.95 | 0-1 |
| ILOS+CA | 0.5 | 2.5 |
| ILOS+RA | 0.5 | 0-1 |

Note: DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, For ILOS treatments, the gas composition refers only to the initial low gas stress condition and not the subsequent CA or RA applied.

Table 2. Summary of low oxygen storage treatments and duration applied to simulate typical South African shipping and handling periods for 'Packham's Triumph' pears.

| Storage treatment | Storage period (months) | Breakdown of storage period | | |
|---|---|---|---|---|
| | | Preconditioning (days) | Treatment | Shipment & handling simulation (weeks) |
| DCA | 2 | None | 10d* | 8w** |
| | 3 | | 6w * | 6w** |
| | 5 | | 16w * | 6w** |
| | 7 | | 24w* | 6w** |
| CA | 2 | None | 10d* | 8w** |
| | 3 | | 6w* | 6w** |
| | 5 | | 16w* | 6w** |
| | 7 | | 24w* | 6w** |
| Control (RA) | 2 | None | | 8w** |
| | 3 | | | 12w** |
| | 5 | | | 22w** |
| | 7 | | | 30w** |
| ILOS+CA | 2 | 10d* | | 8w** |
| | 3 | 10d* | 6w* | 6w** |
| | 5 | 10d* | 16w* | 6w** |
| | 7 | 10d* | 24w* | 6w** |
| ILOS+RA | 2 | 10d* | | 8w** |
| | 3 | 10d* | | 12w** |
| | 5 | 10d* | | 22w** |
| | 7 | 10d* | | 30w** |

Note: ILOS is a preconditioning once off 10d treatment, * Treatment period, ** Shipment and handling period, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Treatment periods were followed by 0, 3 and 7 days of shelf life at 20 °C.

Table 3. Effect of different storage treatments on skin colour of ‘Packham's Triumph’ pears after designated post storage intervals and after retailer’s shelf life conditions during the 2012 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| Storage period (months) | Shelf life (days) | Score | Colour | Score | Colour | Score | Colour | Score | Colour | Score | Colour |
| 2 | 0 | 3.13 m-p | GYY | 2.97 o-u | GY | 3.08 m-q | GYY | 2.99 n-t | GY | 3.04 n-t | GYY |
| | 7 | 4.04 a | FY | 3.93 ab | GYY | 3.67 cg | GYY | 3.96 ab | GYY | 3.84 a-d | GYY |
| 3 | 0 | 3.21 k-n | GYY | 3.05 n-s | GYY | 2.85 r-u | GY | 3.04 n-t | GYY | 3.05 n-s | GYY |
| | 7 | 3.85 a-d | GYY | 3.80 b-f | GYY | 3.68 c-g | GYY | 3,81 b-e | GYY | 3.89 a-c | GYY |
| 5 | 0 | 3.19 m-l | GYY | 2.76 u-x | GY | 2.52 y | GY | 3,04 n-t | GYY | 3.00 q-u | GYY |
| | 7 | 3.68 c-g | GYY | 3.82 b-e | GYY | 3.43 h-k | GYY | 3.58 f-i | GYY | 3.77 b-f | GYY |
| 7 | 0 | 3.41 h-l | GYY | 3.07 m-r | GYY | 2.83 s-v | GY | 2.91 p-t | GY | 3.28 j-m | GYY |
| | 7 | 3.70 c-g | GYY | 3.63 d-h | GYY | 3.60 e- i | GYY | 3.75 b-f | GYY | 3.69 c-g | GYY |
| *Significance level Pr>F* | | | | | | | | | | | |
| *Treatment* | *<0.0001* | | | | | | | | | | |
| *Period* | *<0.0001* | | | | | | | | | | |
| *Days* | *<0.0001* | | | | | | | | | | |
| *T X P* | *0.0402* | | | | | | | | | | |
| *T X D* | *0.0024* | | | | | | | | | | |
| *P X D* | *0.0005* | | | | | | | | | | |
| *T X P X D* | *0.0464* | | | | | | | | | | |
| *LSD $_{(5\%)}$* | *0.22* | | | | | | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life. Colour: GGY = green green yellow, GY = green yellow (acceptable) and GYY = green yellow yellow (acceptable), FY = full yellow (unacceptable). Means followed by different letters are significantly different at *$p<0.05$.GGY =1-1.99, GY=2-2.99, GYY = 3-3.99 and FY = 4-.4.99.*

Table 4. Effect of different storage treatments on skin colour of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2013 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| Storage period (months) | Shelf life (days) | Score | Colour | Score | Colour | Score | Colour | Score | Colour | Score | Colour |
| 2 | 0 | 2.71 j-q | GY | 2.77 h-q | GY | 2.71 j-q | GY | 2.87 d-l | GY | 2.79 f-p | GY |
| | 3 | 2.82 f-p | GY | 2.78 f-p | GY | 2.75 h-q | GY | 2.73 i-q | GY | 2.77 h-p | GY |
| | 7 | 2.90 d-k | GY | 2.94 c-j | GY | 2.82 f-o | GY | 2.90 d-k | GY | 2.79 f-p | GY |
| 3 | 0 | 3.10 b-e | GYY | 2.58 o-q | GY | 2.60 o-q | GY | 2.65 l-q | GY | 2.75 h-q | GY |
| | 3 | 2.65 l-q | GY | 2.74 i-q | GY | 2.77 h-p | GY | 2.62 n-q | GY | 2.82 f-o | GY |
| | 7 | 3.08 c-e | GYY | 2.96 c-i | GY | 2.73 j-q | GY | 2.89 d-k | GY | 2.99 c-h | GY |
| 5 | 0 | 3.02 c-f | GYY | 2.66 k-q | GY | 2.58 pq | GY | 2.62 n-q | GY | 3.10 b-d | GYY |
| | 3 | 3.00 c-g | GYY | 2.64 m-q | GY | 2.62 n-q | GY | 2.53 q | GY | 3.16 bc | GYY |
| | 7 | 3.58 a | GYY | 2.86 e-m | GY | 2.99 c-h | GY | 2.99 c-g | GY | 3.33 b | GYY |
| Significance level Pr>F | | | | | | | | | | | |
| *Treatment* | *0.1233* | | | | | | | | | | |
| *Period* | *0.8076* | | | | | | | | | | |
| *Days* | *0.0003* | | | | | | | | | | |
| *T X D* | *0.5123* | | | | | | | | | | |
| *P X D* | *0.2892* | | | | | | | | | | |
| *T X P* | *0.1312* | | | | | | | | | | |
| *T X P X D* | *0.2346* | | | | | | | | | | |
| *LSD $_{(5\%)}$* | *0.23* | | | | | | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, colour: GGY = green green yellow, GY = green yellow (acceptable) and GYY = green yellow yellow (acceptable), FY = full yellow (unacceptable). Means followed by different letters are significantly different at $p<0.05$.

Table 5. Effect of different storage treatments on skin colour parameters of 'Packham's Triumph' pears after 7 months storage life (0 day) during the 2012 harvest season.

| | Skin colour parameters | | | | | |
|---|---|---|---|---|---|---|
| Treatment | L* | a* | b* | H | SCS | YI |
| ILOS+RA | 69.48 $^{a}$ | -10.28 $^{a}$ | 45.67 $^{a}$ | 102.83 $^{c}$ | 2.99 $^{a}$ | 93.92 $^{ab}$ |
| ILOS+CA | 66.71 $^{b}$ | -13.05 $^{b}$ | 44.50 $^{a}$ | 106.43 $^{b}$ | 2.63 $^{b}$ | 94.98 $^{a}$ |
| DCA | 66.07 $^{b}$ | -15.61 $^{b}$ | 43.09 $^{b}$ | 109.95 $^{a}$ | 2.36 $^{c}$ | 93.14 $^{ab}$ |
| CA | 67.00 $^{b}$ | -15.00 $^{c}$ | 43.19 $^{b}$ | 109.96 $^{a}$ | 2.46 $^{bc}$ | 92.11 $^{b}$ |
| RA | 70.19 $^{b}$ | -10.65 $^{a}$ | 45.17 $^{a}$ | 103.34 $^{c}$ | 2.98 $^{a}$ | 91.96 $^{b}$ |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |
| *LSD $_{(5\%)}$* | *1.75* | *1.32* | *1.21* | *1.80* | *0.20* | *2.52* |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life. Means followed by different letters are significantly different at $p<0.05$. *L*=lightness, a*=CIE red (+)/green (-) colour attribute, b*= CIE yellow (+)/blue (-) colour attribute, H=* $\tan^{-1}\left(\frac{b*}{a*}\right)$, *SCS= skin colour score and YL = yellowing index.*

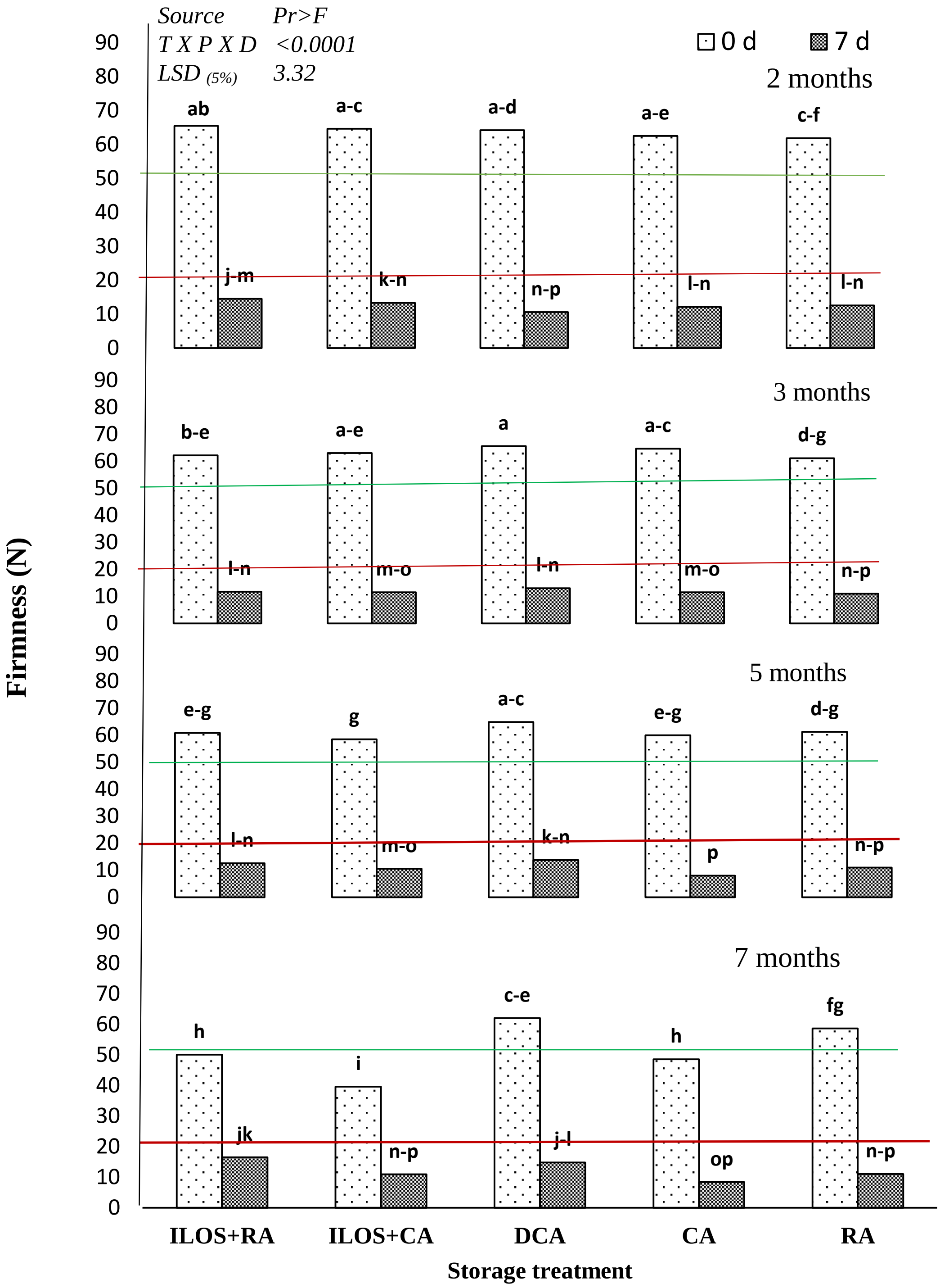


Figure 1. Effect of different storage treatments on firmness (N) of ‘Packham's Triumph’ pears after designated post storage intervals and after retailer’s shelf life conditions during the 2012 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life. Means followed by different letters are significantly different at $p<0.05$.The green line represents 50 N: minimum acceptable limit after storage (0 day) and red line represent 20 N: minimum acceptable limit at the end of shelf life (7 days).

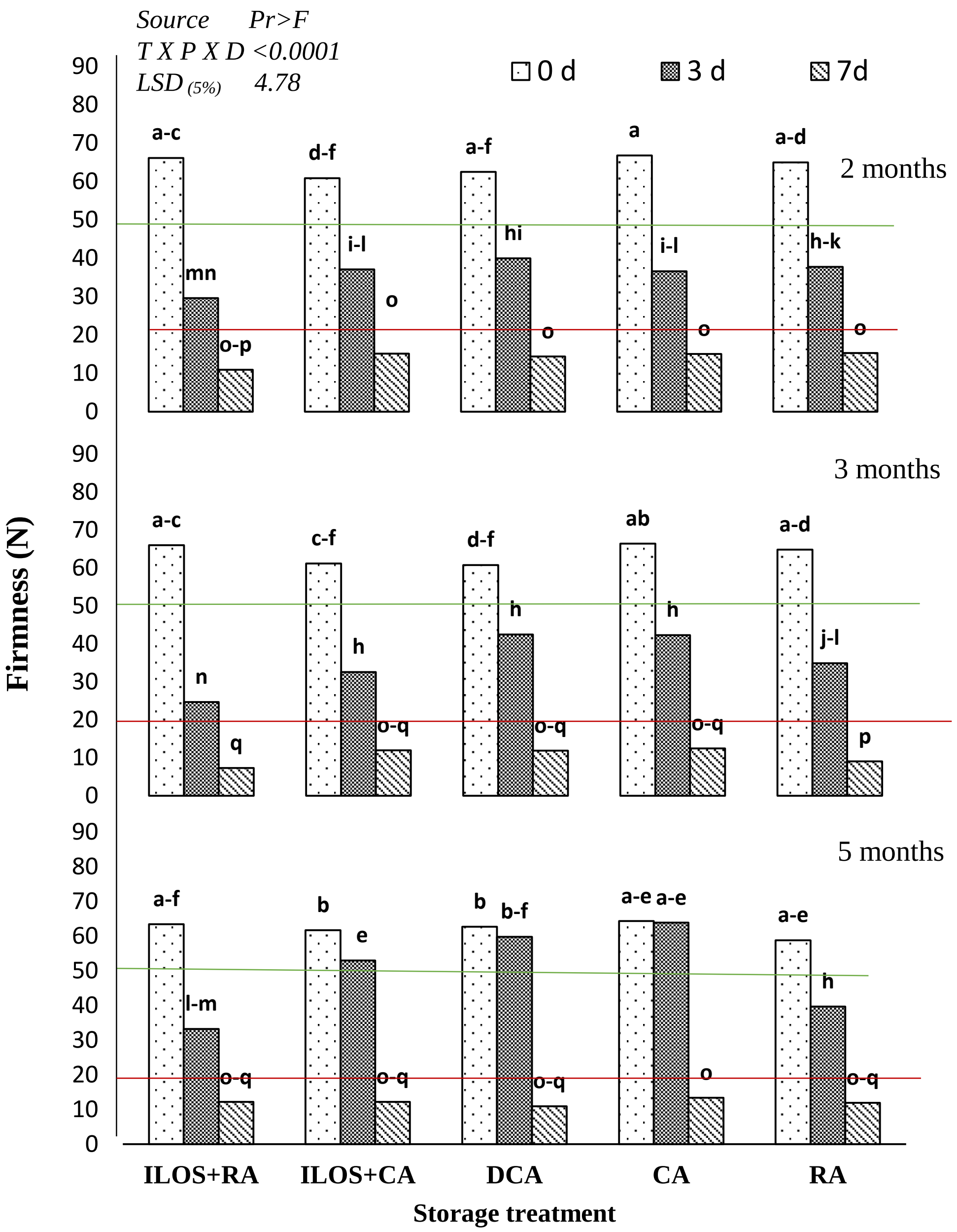


Figure 2. Effect of different storage treatments on firmness (N) of ‘Packham's Triumph’ pears after designated post storage intervals and after retailer’s shelf life conditions during the 2013 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life. Means followed by different letters are significantly different at $p<0.05$. The green line represents 50 N: minimum acceptable limit after storage (0 day) and red line represent 20 N: minimum acceptable limit at the end of shelf life (7 days).

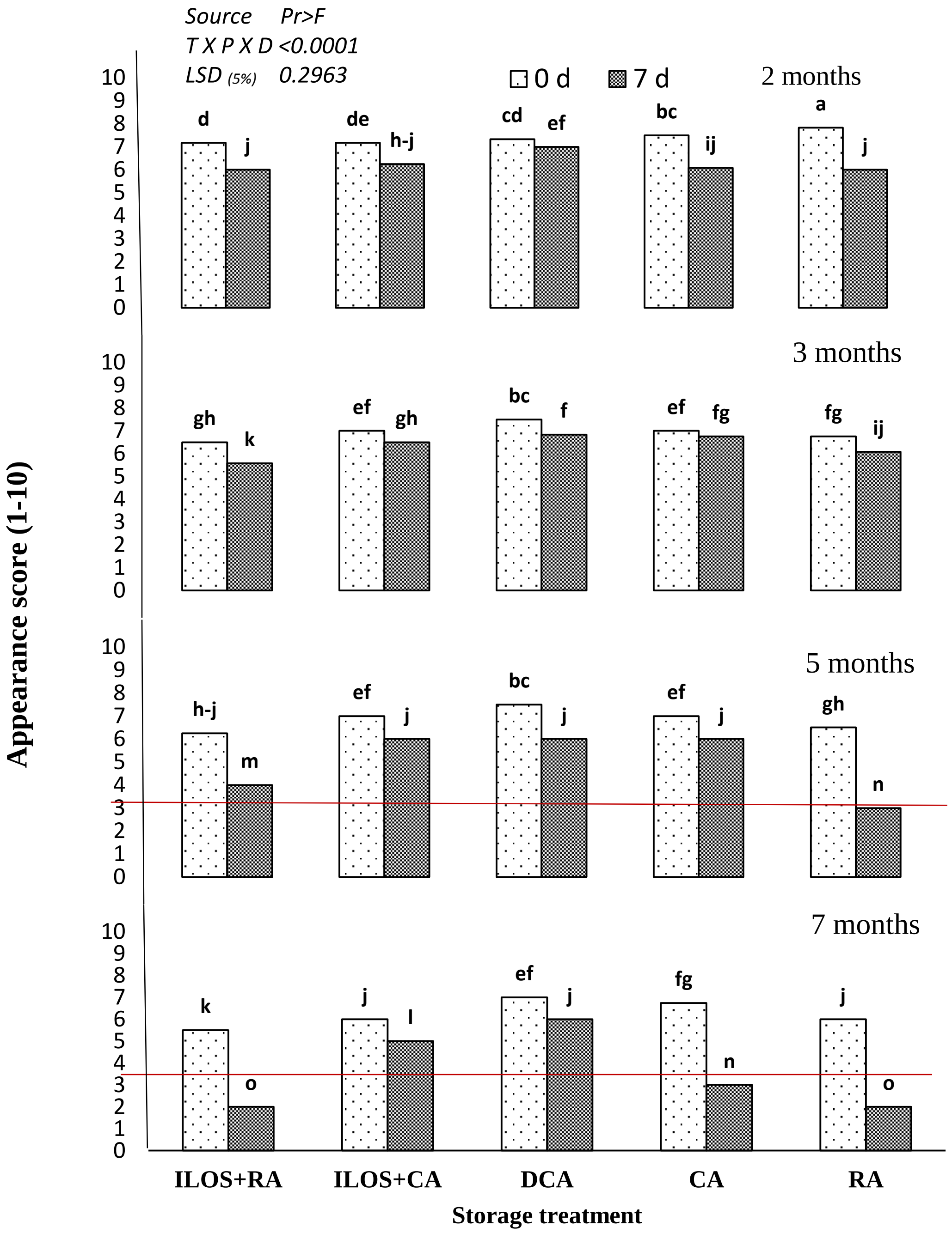


Figure 3. Effect of different storage treatments on appearance scores of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the during 2012 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA =regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, Appearance score (1-3 = poor appearance; 4-6= limited marketability and 7-10 = excellent appearance). Means followed by different letters are significantly different at $p<0.05$.Red line represents poor appearance.

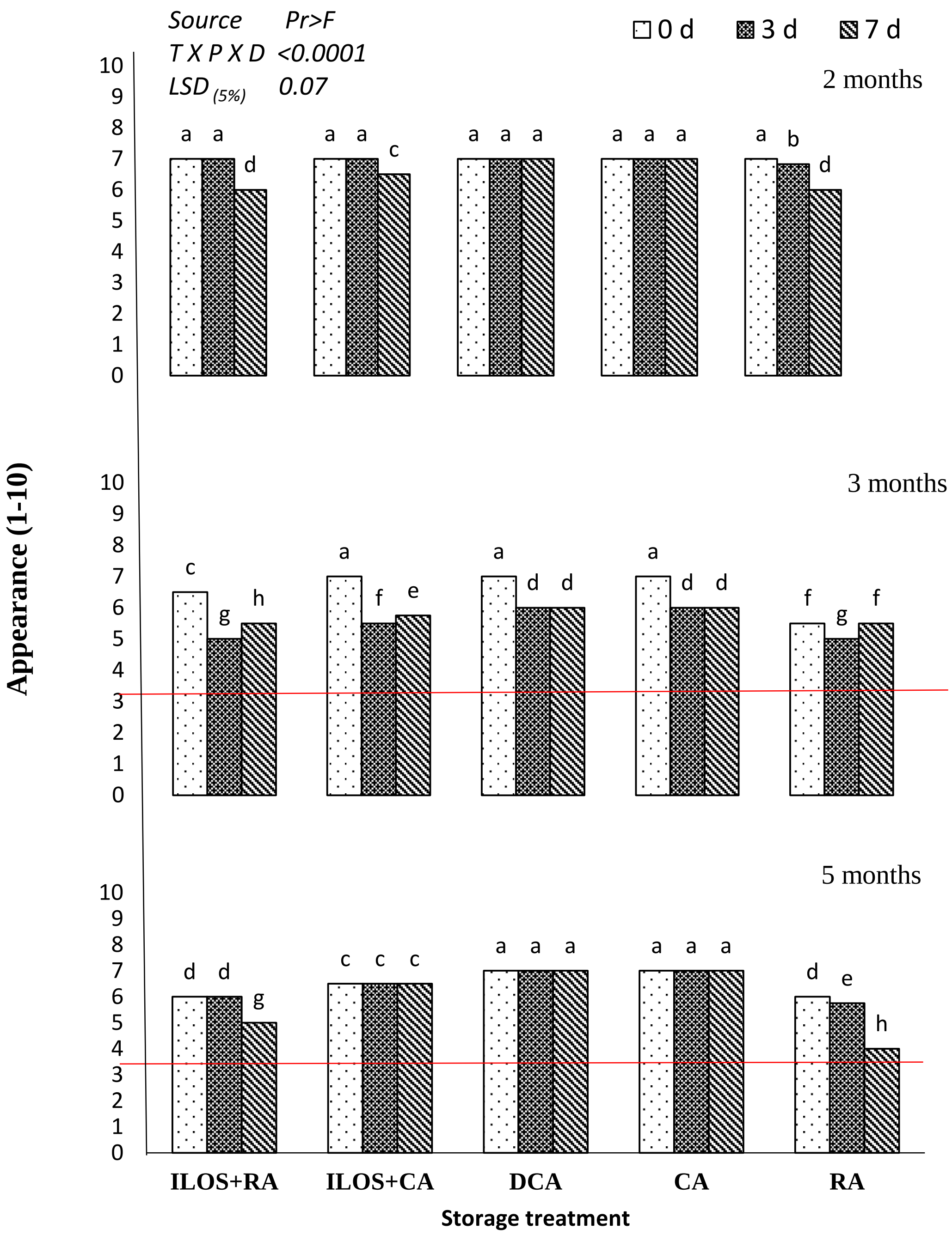


Figure 4. Effect of different storage treatments on appearance scores of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2013 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, Appearance score (1-3 = poor appearance; 4-6 = limited marketability and 7-10 = excellent appearance). Means followed by different letters are significantly different at $p<0.05$. Red line represents poor appearance.

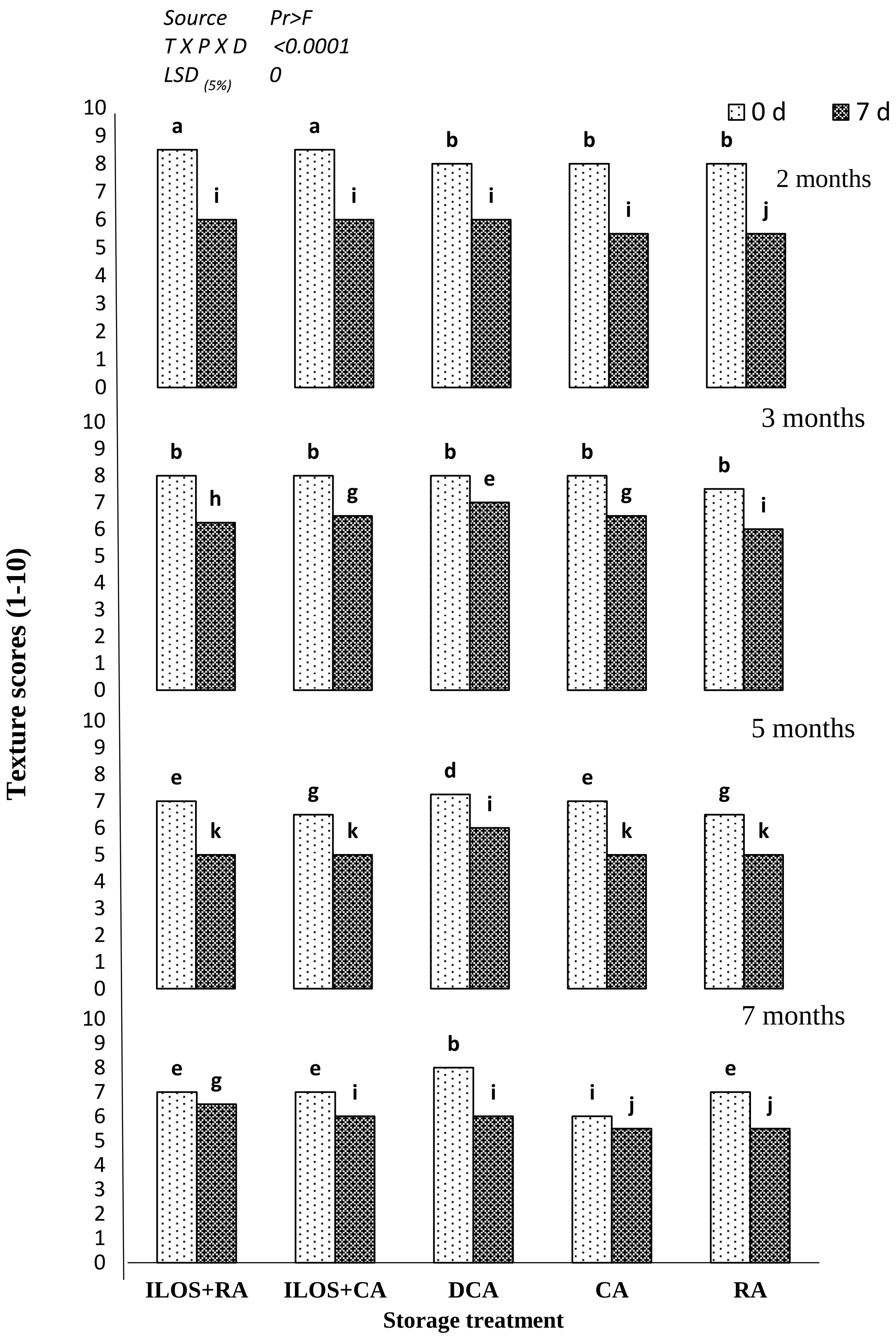


Figure 5. Effect of different storage treatments on texture scores of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

Note: ILOS is a preconditioning once off 10 days treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, 1-3 = poor texture, 4-6 = acceptable texture 7-10 = excellent texture. Means followed by different letters are significantly different at $p<0.05$.

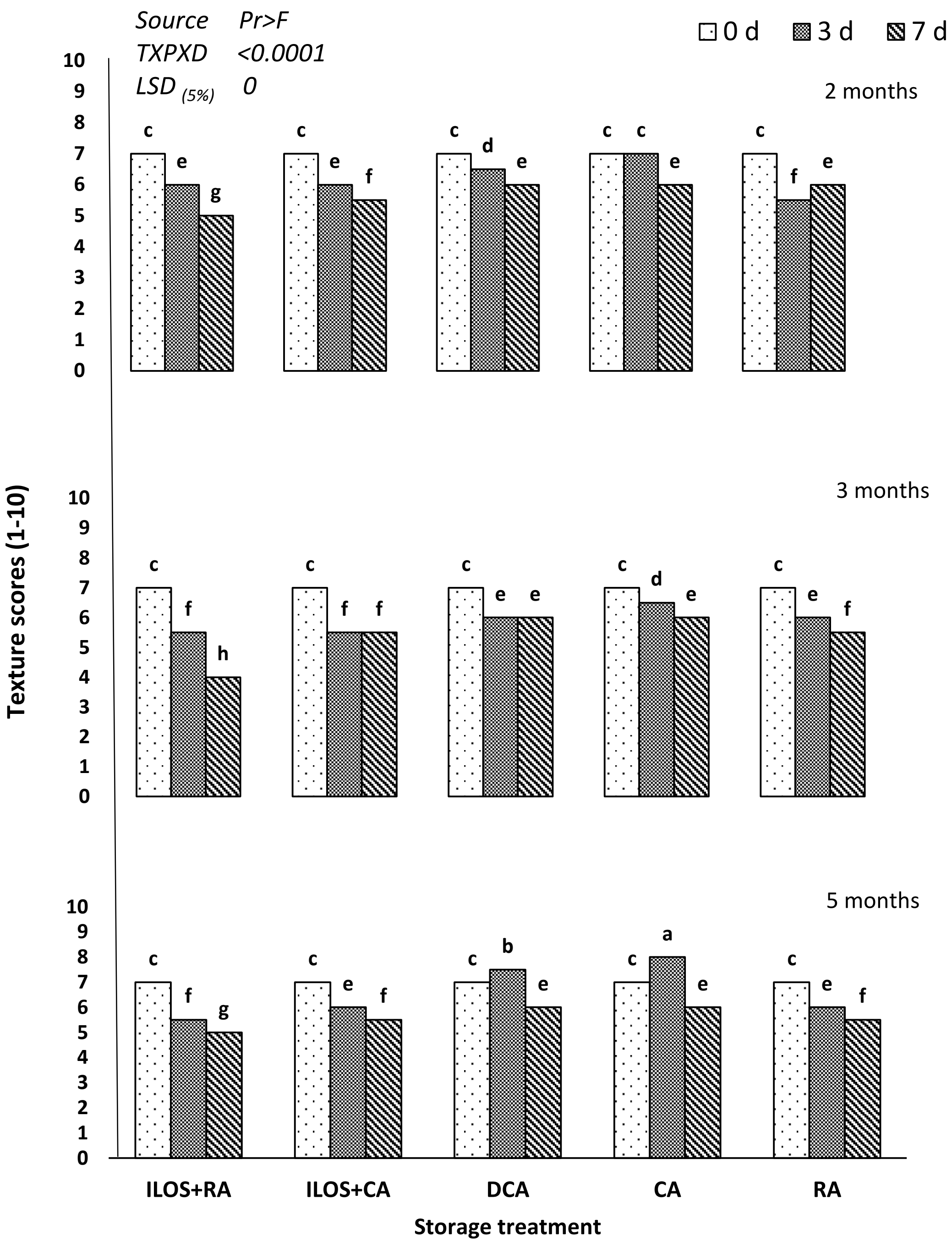


Figure 6. Effect of different storage treatments on texture scores of ‘Packham's Triumph’ pears after designated post storage intervals and after retailer’s shelf life conditions during the 2013 harvest season.

Note: ILOS is a preconditioning once off 10 days treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = Controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, 1-3 = poor texture, 4-6 = acceptable texture 7-10 = excellent texture. Means followed by different letters are significantly different at $p<0.05$.

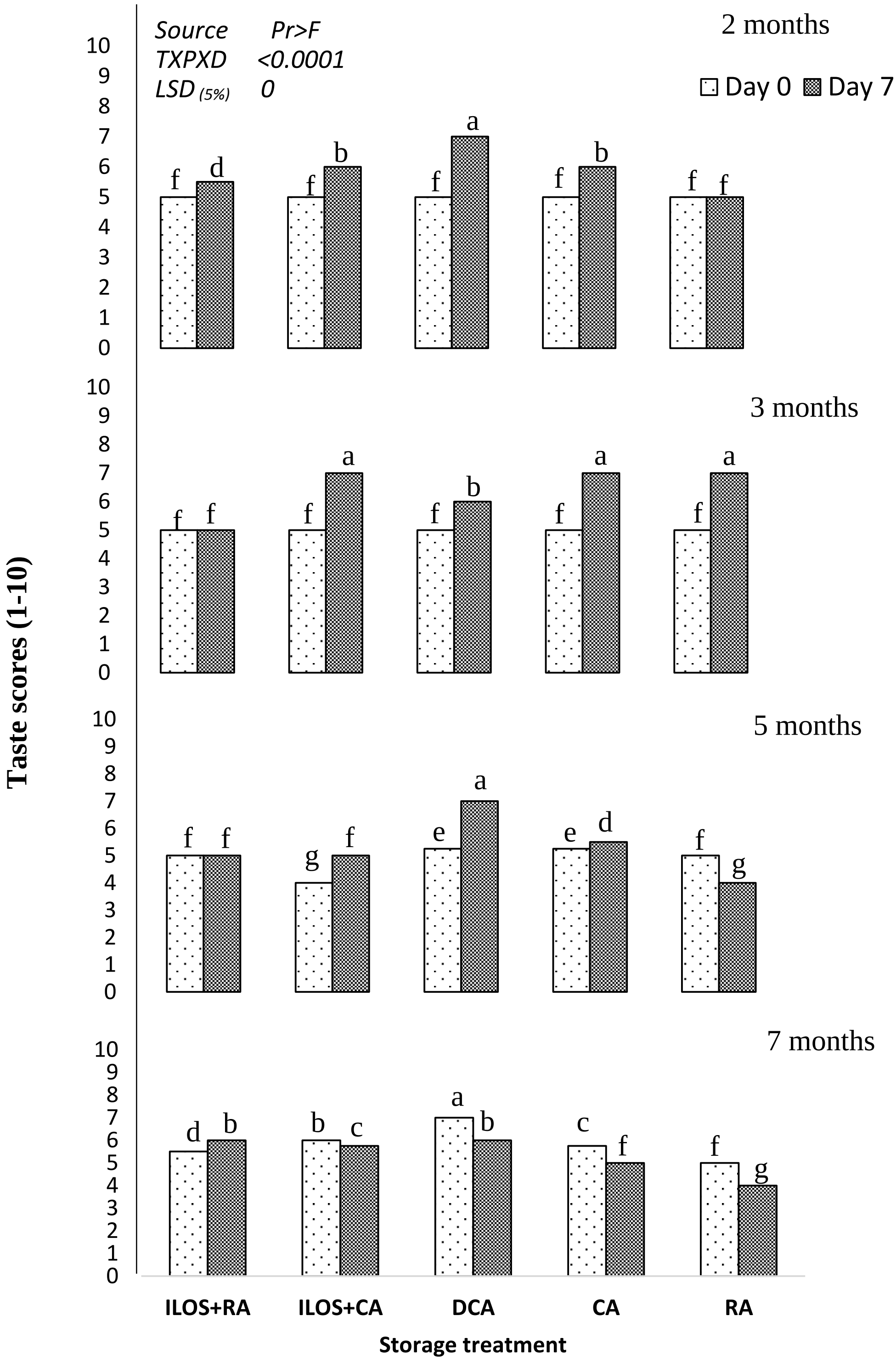


Figure 7. Effect of different storage treatments on the taste scores of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, taste: 1-3 = unacceptable taste, 4-6 = acceptable taste, 5-10 = excellent taste. Means with the same letter are not significantly different ($p>0.05$).

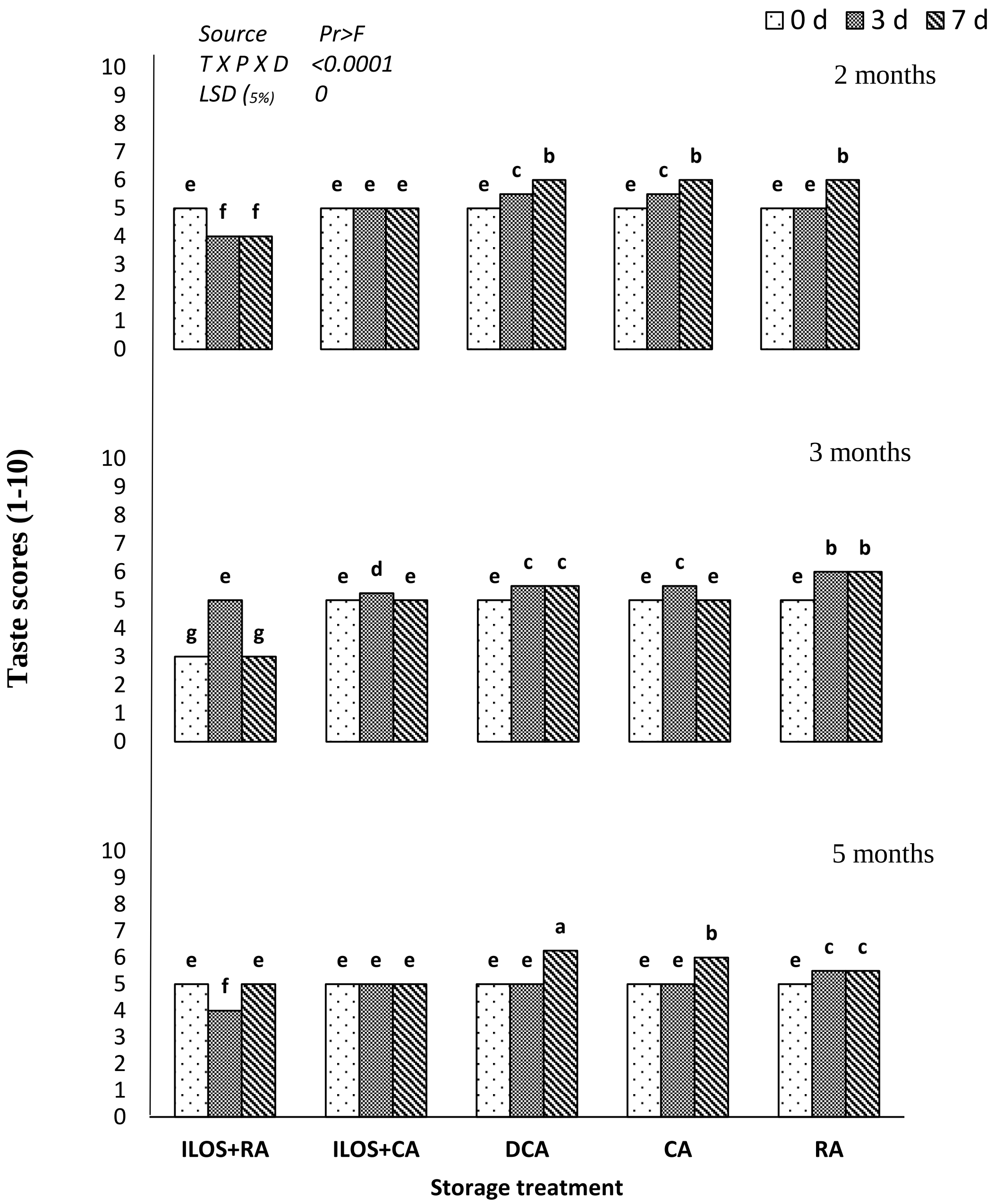


Figure 8. Effect of different storage treatments on the taste scores of 'Packham's Triumph' pears after designated post storage intervals and after retailer's shelf life conditions during the 2013 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA=regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = storage treatment x storage period x shelf life, taste: 1-3 = unacceptable taste, 4-6 = acceptable taste, 5-10 = excellent taste. Means followed by different letters are significantly different at $p<0.05$.

# PAPER 3: ELUCIDATING THE EFFECTS OF DYNAMIC CONTROLLED ATMOSPHERE AND INITIAL LOW OXYGEN STORAGE ON CONCENTRATION OF VOLATILES ASSOCIATED WITH SUPERFICIAL SCALD INCIDENCE IN ‘PACKHAM’S TRIUMPH’ (*PYRUS COMMUNIS L.*) PEAR

**Abstract**

‘Packham’s Triumph’ pears are susceptible to visible blemishing due to superficial scald which may develop during or after cold storage. Optimally harvested ‘Packham’s Triumph’ pears were stored for up to 7 months at -0.5 °C in dynamic controlled atmosphere (DCA), controlled atmosphere preceded by initial low oxygen stress (CA preceded by ILOS), controlled atmosphere (CA), regular atmosphere preceded by initial low oxygen stress (RA preceded by ILOS) and regular atmosphere or RA (control) treatments. Peel tissue samples for volatile analysis were taken at harvest, after 2, 3, 5 and 7 months after storage followed by 0, 3 and 7 days shelf life at 20 °C. Gas chromatography mass spectrometry (GC-MS) analysis was used to measure volatiles sesquiterpene 3-7-11-trimethyldodecatetraene (α-farnesene) and its oxidation by-product 6-methyl-5-hepten-2-one (MHO). This paper investigates how MHO and α-farnesene concentrations evolve during cold storage and how they relate to superficial scald incidence and severity in ‘Packham’s Triumph’ pears. MHO concentrations were significantly lower in scald suppressing treatments DCA, CA and CA preceded by ILOS and higher in RA and RA preceded by ILOS and the reverse was true for α-farnesene concentrations. The results suggest that DCA and ILOS inhibit incidence of superficial scald by keeping concentrations of MHO lower in ‘Packham’s Triumph’ pears. The α-farnesene and MHO displayed a precursor product relationship in this study confirming that MHO is an autoxidation product of α-farnesene. The levels of α-farnesene in the fruit peel of ‘Packham’s Triumph’ pears was negatively correlated to scald severity ($R^2$ = -0.90), whereas the concentrations of MHO in the fruit peel of ‘Packham’s Triumph’ pears are positively correlated with scald severity ($R^2$ = 0.93).

## 1. Introduction

‘Packham’s Triumph’ pears grown in South Africa are highly susceptible to a physiological storage disorder superficial scald after 2-3 months in cold storage. Contrary to other countries in the southern hemisphere utilising cold storage to extend the storage life of pears, South African marketers and are required to accommodate the shipping and handling periods to overseas markets. Approximately 48% of pears produced in South Africa are destined for export markets (PPPECB, 2014). Superficial scald is an important physiological disorder affecting some commercially important cultivars of apples and pears after or during cold storage. The symptoms of superficial scald on pome fruit are aggravated by transferring fruit from cold storage to higher ripening temperatures affecting the appearance of the fruit (Whitaker et al., 2009).

The sesquiterpene hydrocarbon 3-7-11-trimethyldodecatetraene (α-farnesene) occurs naturally in the coating of apples, pears and quinces in smaller quantities (Anet, 1969). This volatile compound, α-farnesene has been reported to play a significant role in the induction of superficial scald symptoms in 'Granny Smith' apples (Huelin and Coggiola, 1968; Huelin and Coggiola, 1970; Anet, 1969; Anet, 1972a). The earlier harvested 'Granny Smith' apples were found to have higher concentrations of α-farnersene than late harvested fruit (Huelin and Coggiola, 1968). The ketone 6-methyl-5-hepten-2-one (MHO) is the product of the oxidation of the α-farnesene (Anet, 1972b; Wang and Dilley, 2000a). Whitaker and Saftner (2000) showed that α-farnesene autoxidise to conjugated trienols which further oxidises to yield MHO in 'Granny Smith' apples. MHO is implicated in the incidence and severity of superficial scald disorder in 'Cortland apples' (Mir et al., 1999), however there is little information in literature of MHO involvement in superficial scald induction. Sensitivity of fruit to MHO may vary from season to season (Wang and Dilley, 2000a and 2000b).

Initial low oxygen stress (ILOS) inhibit superficial incidence by suppressing levels of MHO in 'Law Rome' and 'Granny Smith' apples (Wang and Dilley, 2000b), however there are no reports in literature on how ILOS affect MHO concentration in ''Packham's Triumph' pears. DCA treatment has been reported to inhibit superficial scald on 'd'Anjou' pears, however there is no reports in literature on how DCA inhibit superficial scald in terms of the biochemical markers MHO and α-farnesene in the 'Packham's Triumph' pears.

The aim of this paper was to investigate the biochemical mechanisms by which low oxygen treatments ILOS and DCA suppress the incidence of superficial scald in 'Packham's Triumph' pears. The specific objectives were to:

(1) Evaluate the effects of ILOS and DCA storage treatments on MHO and α-farnesene concentration of 'Packham's Triumph' pears , and
(2) Assess the association between these biomarkers and superficial scald incidence and severity on 'Packham's Triumph' pears.

## 2. Materials and methods

### 2.1. Fruit and tissue sampling

'Packham's Triumph' pears were harvested on 13-15th February during the 2012/2013 season at optimal maturity from Grabouw production area (34.1500°S, 19.0167°E) in the Western Cape Province, South Africa. Fruit for both seasons were sampled on arrival and transferred to the different storage treatments (RA preceded by ILOS; CA preceded by ILOS; DCA; CA and RA) in order to investigate how MHO and α-farnesene evolve during the respective storage periods at 2, 3, 5 and 7 months at -0.5 °C. Peel tissue (5 g) was removed from pears using a stainless steel grater from equatorial region of pears with 5 fruit from each replicate and immediately put in vials and weighed. The samples were prepared at the postharvest laboratory of Agricultural Research Council Infruitec-Nietvoorbij in Stellenbosch and immediately after preparation taken to Central Analytical Facility (CAF) at Stellenbosch

### 2.2. Storage treatments and periods

Fruit were stored for up to 7 months at -0.5 °C in 5 storage treatments at various $O_2$ and $CO_2$ combinations (Table 1).

#### *2.2.1. Regular atmosphere preceded by initial low oxygen stress (RA preceded by ILOS)*

In RA preceded by ILOS fruit were stressed with low oxygen at 0.7% for 10 days as a preconditioning treatment (Table 2), before being transferred into the RA room. For the 2 months storage period fruit were kept in ILOS for 10 days only, and were followed for 8 weeks in RA to simulate shipment and handling. 'Granny Smith' apples and 'Packham's Triumph' pears destined for the export market, are first stored under CA conditions between 8 and 12 weeks before being packed and exported under regular atmosphere as a general handling and shipment protocol in South Africa (Crouch, 2003). For 3 months storage period fruit were kept in ILOS for 10 days only and thereafter kept in RA for 12 weeks. For 5 months storage period fruit were kept in ILOS for 10 days and followed by 22 weeks in RA. For 7 months storage period fruit were kept in ILOS for 10 days only and thereafter followed by 30 weeks of RA to extend the marketing window.

#### *2.2.2. Controlled atmosphere preceded by initial low oxygen stress (CA preceded by ILOS)*

Fruit treated with CA preceded by ILOS were kept for 10 days in initial low oxygen stress before transfer into CA. For the two months storage period fruit are stored in ILOS for

10 days and transferred into CA for 8 weeks (Table 2). For three months storage period fruit were stored in ILOS for 10 days followed by 6 weeks in CA and thereafter transferred to RA for 6 weeks to simulate shipment and handling. After the 5 months storage period the fruit were stored in ILOS for 10 days and thereafter transferred to CA for 16 weeks followed by 6 weeks in RA to simulate shipment and handling. For 7 months storage period fruit were preconditioned with ILOS for 10 days and transferred into CA for 24 weeks followed by RA for 6 weeks to simulate shipment and handling (Table 2).

### *2.2.3. Dynamic controlled atmosphere (DCA)*

DCA treated fruit were kept for 10 days in DCA followed by 8 weeks in RA to simulate shipment and handling for the 2 months storage period (Table 2). For the 3, 5 and 7 months storage periods the DCA treated fruit were kept in the DCA room for 6, 16 and 24 weeks respectively, and then transferred into RA for 6 weeks to simulate shipment and handling. The DCA treatment facility was fitted with HarvestWatch™ (SAtlantic Inc. Halifax, N.S., Canada) fluorescence interactive response monitor sensor (FIRM) to monitor fruit response to low $O_2$ levels hourly. The selection of representative sample of 6 fruit for each FIRM sensor is crucial (Zanella et al., 2005), because the selected fruit represent the whole batch in the treatment room. The fruit were loaded into cold rooms and the desired fruit core temperature of -0.5 °C was achieved within 48 to 96 hours after harvest. The spiking for lower oxygen limit was set at 0.3% $O_2$ for both harvest seasons (2012 and 2013), and it was thereafter adjusted to 0.5% $O_2$ for the rest of the storage period as explained by DeLong et al. (2007). The DCA protocol and the pull down process should be completed within few days after receiving the fruit.

### *2.2.4. Controlled atmosphere (CA)*

CA fruit were kept for 10 days in CA followed by 8 weeks of RA for the 2 months storage period (Table 2). For the 3, 5 and 7 months storage period, fruit were kept in CA for 6, 16 and 24 weeks respectively and thereafter transferred to RA for 6 weeks to simulate shipment and handling.

### *2.2.5. Regular atmosphere (RA)*

RA was the control treatment for the trial. RA fruit were kept solely in RA for the duration of storage of 2, 3, 5 and 7 months (Table 2).

### 2.3 Superficial scald intensity (scald index)

The intensity of superficial scald evaluation was performed as described in Chapter 1, Section 2.3.1.

### 2.4 Gas chromatography-Mass spectrometry solid phase micro extraction (GC-MS SPME) analysis

Fruit peel (5 g) (5 fruit from each replicate) was taken and transferred to the 20 mL solid phase micro extraction method (SPME) vials (3 vials x 5 storage treatments = 15 vials) at each sampling interval. The trial consisted of 5 treatments as shown in (Table 2 of Research Chapter 1). Each replicate was a crate of 90-120 fruit, and only 5 fruit were taken from each replicate for volatile extraction. In total 15 fruit were used for each treatment for volatile analysis. The extraction and trapping of volatiles from the vial headspaces was done using the SPME method (Song et al., 1997; Caleb, 2013; Contreras and Beaudry, 2013). The analysis was performed using an Agilent GC model 6890 N (Agilent, Palo Alto, CA, USA) coupled with an Agilent mass spectrometer detector (MS), model 5975B Inert XOL EI/CI (Agilent, Palo Alto, CA, USA) and equipped with a CTC Analytics PAL auto sampler. Polydimethylsiloxane (PDMS) coated fibre (50/30 µm DVB/CAR/PDMS, Stableflex) was used for extraction and desorption of volatile compounds and thereafter carried out in the back injection port of the GC-MS (Agilent Technologies) in a split-less mode. The fibre was preconditioned for 2 min at 50 °C temperature and 250 rpm. Volatiles chromatographic separation was performed using a polar J&W DB-FFAP (60m 0.25 mm i.d., 0.5µm film thickness) capillary column. The oven temperature program was as follows: 40 °C held for 5 minutes, then ramped to a final temperature 230 °C at 10 °C/min and maintained for 6 min. The total run time for the method was 30.0 min. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min.

The injector operated in split less mode and was maintained at 24 °C throughout the analysis. Both the purge and flow and gas saver flow were activated at 50 mL/min for 5 min. The ion source and quadrapole temperatures were maintained at 230 °C and 150 °C respectively with the transfer line set at 250 °C. Targeted selective ion monitoring (SIM) was performed. The volatile compounds (MHO and α-farnesene) were identified using retention times and mass spectra in the National Institute for Standard and Technology Mass Spectral

Library. The farnesene and MHO results are reported as the $log_{10}$ of the GC peak area (abundance) (Whitaker and Saftner, 2000; Ju and Curry, 2002).

### 2.5 Statistical analysis and trial design

The trial design was a completely randomised three way factorial with storage treatment, storage duration and shelf life as sources of variance (5 treatments x 4 storage periods x 3 shelf life). Analysis of variance (ANOVA) was performed on all measurements using SAS enterprise Guide 5.1. Separate ANOVA was also performed for each type of storage and shelf life combinations. Student‘s Least Significant Difference was used at a 5% significance level for means comparisons. Pearson correlations were also performed to investigate relationships between volatiles and superficial scald incidence.

## 3. Results and discussion

### 3.1. Effects of low oxygen treatments on α-farnesene in the fruit peel of 'Packham's Triumph' pears

The α-farnesene concentrations in the peels of fruit stored at DCA, CA and CA preceded ILOS treatments increased with shelf life and were higher at 7 days but were lower at 0 day after 2 months in storage (Figure 1). At the same storage period the α-farnesene concentrations in the fruit peel of storage treatments RA preceded by ILOS and RA decreased with increased in shelf life period at 7 days and highest at 0 day. The α-farnesene concentrations in fruit stored at RA preceded ILOS, CA preceded by ILOS, CA and RA decreased at 7 days, however the α-farnesene concentrations in DCA treated fruit was not significantly different at 0 and 7 days after 3 months in storage (Figure 1). The concentrations of α-farnesene in the fruit peel of 'Packham's Triumph' pears is negatively correlated to scald severity ($R^2$ = -0.90), the higher the α-farnesene concentrations on the fruit peel the lower the scald incidence.

DCA treated fruit had α-farnesene concentrations which were not significantly different at 0 day and after 7 days during the 5 months cold storage (Figure 1). The CA preceded by ILOS, RA preceded by ILOS, CA and RA treated fruit had higher levels α-farnesene levels at 0 day and lower levels after 7 days during the 5 months cold storage period. The α-farnesene concentrations of RA preceded by ILOS treated fruit were not significantly different at 0 day and after 7 days shelf life after 7 months (Figure 1). There was no significant difference in α-farnesene concentration among RA preceded by ILOS, CA preceded by ILOS, DCA, CA and RA after 7 months in cold storage. The α-farnesene concentrations increased significantly from 0 day to 7 days shelf life after 7 months storage period in CA preceded by ILOS, DCA, CA and RA fruit. The results in this study confirm that α-farnesene concentrations increase with storage period and then decrease at the end of storage period (Meigh and Filmer, 1969; Whitaker, 2004). Whitaker et al. (2009) reported that α-farnesene accumulated from 8-12 weeks of storage and then decreased on 'Bartlett' grown in Northern California and Washington in the United States of America.

### 3.2. Effects of low oxygen treatments on the MHO concentration in the fruit peel of 'Packham's Triumph' pears

On arrival of the pears the MHO was not detected in the fruit peel of 'Packham's Triumph' pears in during the 2013 harvest (data not shown). MHO production of fruit begins within the first 2 months when fruit are stored in air (Wang and Dilley, 2000b). The volatile α-farnesene was found to be present on the fruit prior to storage confirming that it is a natural occurring compound in the fruit peel. Huelin and Murray (1966) found that α-farnesene was naturally in the coating of 'Granny Smith' apples. Similar results were reported by Ju and Curry (2002) on scald susceptible 'Delicious' and 'Granny Smith', and scald resistant 'Fuji' and 'Gala' apple cultivars. These findings further support the theory that MHO is by-product of the autoxidation of α-farnesene and this process is perhaps triggered by cold storage hence it has been reported that superficial scald is expressed on the fruit as a form of chilling injury (Watkins et al., 1995).

There was no significant difference among treatments on MHO concentrations in the fruit peel after 2 and 3 months cold storage (Figure 2). MHO concentration on DCA treated fruit was the lowest and not significantly different from the concentrations in CA preceded by ILOS treated fruit after 5 months. The MHO concentration in the peel of DCA treated fruit was not significantly different from the levels after 0 and 7 days of 5 months cold storage. The MHO concentrations in CA preceded by ILOS and CA 'Packham's Triumph' pears decreased significantly with period in shelf life periods a similar trend being observed for RA fruit. However for RA preceded by ILOS there was no significant difference in MHO levels at 0 day and 7 days after 5 months. The MHO concentration was higher than that of DCA, CA and CA preceded by ILOS treated fruit.

Treatments that controlled superficial scald (DCA, CA and CA preceded by ILOS) had the lowest MHO concentrations and fruit with higher MHO concentrations had highest severity of superficial scald. Low oxygen treatments (DCA, CA and CA preceded by ILOS) controlled superficial scald by keeping the MHO concentrations in the fruit peel of ‘Packham’s Triumph’ pears lower. Similar observations were reported by (Pesis et al., 2014) on ‘Bartlett’ and ‘Spadona’ pears grown in Northern California, USA and Northern Israel respectively. The authors further reported that the application of low $O_2$ stress were effective in controlling superficial scald by reducing ethylene production and the formation of α-farnesene oxidation products leading to less accumulation of MHO.

RA treated ‘Packham’s Triumph’ pears had higher MHO concentrations compared to RA preceded by ILOS, CA and CA preceded by ILOS fruit after 7 months in storage at 0 day (Figure 2). MHO concentrations were not significantly different, however DCA treated fruit had the lowest MHO concentrations in the fruit peel (Figure 2). RA and RA preceded by ILOS treated fruit showed superficial scald after 7 months of cold storage at the end of storage life. RA treated ‘Packham’s Triumph’ pears had 5% of the fruit affected, while RA preceded by ILOS had only 2% of the fruit affected. The fruit peel of RA preceded by ILOS treated fruit had higher MHO concentrations than all other treatments after 7 days of shelf life preceded by 7 months of cold storage.

Although MHO levels on DCA treated fruit were not significantly different from those of CA preceded by ILOS and CA fruit after 7 months followed by 7 days, there was however no superficial scald observed on DCA treated fruit. This finding suggests that there might be other mechanism involved in the cause of superficial scald such as reactive oxygen species (ROS) (Sabban-Amin et al., 2011). DCA treated fruit might have better antioxidant pool therefore scavenging ROS better than CA and CA preceded by ILOS treated fruit and resulting with fruit with no superficial scald symptoms.

### 3.3. MHO and α-farnesene correlation and involvement with superficial scald severity during the 2012 season

DCA and ILOS treatments were effective in suppressing the incidence of superficial scald on ‘Packham’s Triumph’ pears (Table 4 in Chapter 1). The effectiveness of DCA treatment in this study confirms findings reported by DeLong et al. (2004) on several apple cultivars (‘Cortland’, ‘Delicious’, ‘Golden Delicious’, ‘Honeycrisp’, ‘Jonagold’ and ‘McIntosh’) and by Zanella and Strüz (2013) on ‘Granny Smith’ and ‘McIntosh’ apples cultivars and on ‘d’Anjou’ pears by Mattheis and Rudell (2011). DCA treatment though effective in suppressing superficial scald on ‘Packham’s Triumph’ pears, it however requires installation of the DCA chlorophyll fluorescence monitor HarvestWatch™ system (SAtlantic Inc., Halifax., N.S., Canada) (DeLong et al., 2004) in the CA rooms to monitor fruit response.

For both seasons (2012 and 2013) the fruit spiked at 0.3 and kept at 0.5 % $O_2$ for the rest of the storage period for the DCA treatment. Similar reports of effectiveness of CA preceded by ILOS were observed on 'Granny Smith' apples for up to 6 months (Truter et al., 1994; van der Merwe et al., 2003), however, Lau (1997) reported contradictory results when ILOS treatment was tested on 'Starkimson Delicious' apples. CA preceded by ILOS treatment will further benefit South African growers as it can utilise existing CA rooms without any additional costs as long as the rooms remain airtight.

During the 2012 season at 5 months cold storage followed by 7 days shelf life, DCA and CA preceded by ILOS treatments were not significantly ($p$ = 0.05) different, and had the highest α-farnesene concentrations (Figure 3). There was no scald on DCA and CA preceded by ILOS treated fruit though they had the highest concentration of α-farnesene in the peel of the fruit. The peel of CA treated fruit had significantly lower concentrations of α-farnesene compared to DCA and CA preceded by ILOS, and there was negligible scald severity of 0.003 on CA treated fruit after 5 months followed by 7 days shelf life at 20 °C.

RA preceded by ILOS and RA treated fruit were not significantly different from each other and had the lowest α-farnesene concentrations after 5 months storage followed by 7 days shelf life at 20 °C. RA preceded by ILOS and RA stored fruit had significantly higher MHO levels after 5 months followed by 7 days and the highest incidence of superficial scald. DCA and CA preceded ILOS treated fruit had the lowest MHO concentrations in the peel and no superficial scald after 5 months of cold storage followed by 7 days shelf life at 20 °C (Figure 3).

The results in this study suggest that the mode of action of DCA and CA preceded by ILOS is by suppressing autoxidation of α-farnesene to its toxic by-product MHO. Similar results have been reported by several researchers (Chen et al., 1993; Wang and Dilley, 1999; Isidoro and Almeida, 2006). Superficial scald incidence is positively correlated to higher MHO levels in the fruit peel (Mir et al., 1999) during ripening temperature at 22 °C. The concentrations of α-farnesene decreased with shelf life period in all treatments, whereas the levels of MHO increases with shelf life period at 20 °C, the longer the shelf life period, the higher the MHO concentrations in fruit treated with DCA and CA preceded by ILOS.

At 7 days after 5 months storage, RA stored fruit had the highest MHO concentrations and had superficial scald incidence (Figure 3). It was also observed that at the same storage period fruit under CA at 7 days shelf life had the highest α-farnesene concentrations. DCA and CA preceded by ILOS treatments suppressed incidence of superficial scald (Research Paper 1 Table 4), and had the significantly lowest concentrations of MHO in the fruit peel (Figure 4). The α-farnesene and its autoxidation product MHO have a precursor product relationship (Figure 4 and Figure 5), when α-farnesene concentration is higher the MHO is lower. Same observations were reported by Whitaker et al. (2009) on 'Bartlett' pears grown in California and Washington in the United States. Severity of superficial scald may vary from season to season (von Mollendorff, 1996). During the 2012 season the severity index for superficial scald was higher than that on the 2013 season on 'Packham's Triumph' pears (Figure 3 and Figure 4).

The precursor product relationship of α-farnesene and MHO has been reported by several researchers (Wang and Dilley, 2000a; Whitaker et al., 2009). Huelin and Coggiola (1970) suggested that treatments that control superficial scald delay the oxidation of α-farnesene to its toxic end products; DCA and CA preceded by ILOS treatments seem to suppress the oxidation of α-farnesene. RA preceded by ILOS and RA treated fruit had the highest MHO concentrations and the highest incidence of superficial scald physiological disorder.

After 7 months in storage the incidence of superficial scald started at 0 day (Figure 5) on RA and RA preceded by ILOS treated by fruit. These findings indicate that superficial scald symptoms may appear even during cold storage and extended periods. Although the MHO concentrations were significantly higher on RA preceded by ILOS treated fruit than on RA fruit, the fruit from both treatments showed the presence of superficial scald. The MHO concentration on scalded RA fruit were not significantly different from MHO on scald controlling treatments (DCA, CA and CA preceded by ILOS). The results suggest that mechanisms other than higher MHO concentration are involved in suppressing superficial scald by DCA, CA and CA preceded by ILOS low oxygen treatments. Sabban-Amin suggested that reactive oxygen species (ROS) might play a role in damaging the fruit peel of pome fruit resulting in superficial scald (Sabban-Amin et al., 2011).

The α-farnesene concentrations were not significantly different at 0 day after 7 months in storage irrespective of the applied treatment (CA preceded by ILOS, CA, RA preceded by ILOS, RA and DCA) (Figure 5). The results suggest that α-farnesene might be involved as a precursor for MHO than being directly involved in causing superficial scald on pears. Pesis et al. (2014) showed that low oxygen pre-treatments controlled superficial scald by reducing ethylene and α-farnesene production resulting in less accumulation of MHO.

## 4. Conclusions

In this study the volatiles MHO and α-farnesene were implicated in the incidence of superficial scald in 'Packham's Triumph' pears. CA storage of fruit preceded by ILOS and DCA treatments controlled superficial scald by suppressing autoxidation of α-farnesene to MHO. Superficial scald symptoms appeared on the 'Packham's Triumph' pears when the α-farnesene concentrations were lower, and MHO concentrations were higher. Storing fruit in DCA and CA preceded ILOS effectively control the incidence of superficial scald on 'Packham's Triumph' pears by reducing MHO accumulation in the fruit peel. There was a strong negative correlation between superficial scald incidence and α-farnesene concentration in the fruit peel ($R^2$ = -0.90). The correlation between superficial scald incidence and MHO in the fruit peel of 'Packham's Triumph' pears was strongly positive ($R^2$ = 0.93). DCA, CA and CA preceded by ILOS offers an alternative to the use of DPA on 'Packham's Triumph' pears to suppress superficial scald postharvest disorder by suppressing autoxidation of α-farnesene to MHO in the fruit peel.

## 5. References

Anet, E.F.J.L. (1969). Autoxidation of α-farnesene. *Australian Journal of Chemistry*, 22, 2403-2410.

Anet, E.F.J.L. (1972a). Superficial scald a functional disorder of stored apples .VIII. Volatile products from the autoxidation of α-farnesene. *Journal of the Science of Food and Agriculture*, 23, 605-608.

Anet, E.F.J.L. (1972b). Superficial scald a functional disorder of apples. IX. Effect of maturity and ventilation. *Journal of the Science of Food and Agriculture,* 23, 763-769.

Caleb, O.J. (2013). *Modified atmosphere packaging of pomegranate arils*. PhD thesis Stellenbosch University. http://scholar.sun.ac.za.

Chen, P.M., Varga R.J. & Xiao, Y.Q. (1993). Inhibition of α-farnesene biosynthesis and its oxidation in the peel tissue of 'd'Anjou' pears by low $O_2$/elevated $CO_2$ atmospheres. *Postharvest Biology and Technology*, 3, 215-223.

Contreras, C. & Beaudry, R. (2013). Lipoxygenase-associated apple volatiles associated with aroma perception during ripening. *Postharvest Biology and Technology*, 82, 28-38.

DeLong, J.M., Prange, R.K., Leyte J.C. & Harrison, P.A. (2004). A new technology that determines low-oxygen thresholds in controlled atmosphere stored apples. *HortTechnology*, 14, 263-266.

Huelin, F.E. & Coggiola, I.M. (1968). Superficial scald, a functional disorder of stored apples. IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of a-farnesene in the fruit. *Journal of the Science of Food and Agriculture*, 19, 298-301.

Huelin, F.E. & Coggiola, I.M. (1970). Superficial scald, a functional disorder of stored apples. VII. - Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration and oxidation of α-farnesene in the fruit. *Journal of the Science of Food and Agriculture*, 21, 584-589.

Huelin, F.E. & Murray, K.E. (1966). α-farnesene in the natural coating of apples. *Nature* 210, 1260-1261.

Isidoro, N. & Almeida, D.P.F. (2006). α-farnesene, conjugated trienols and superficial scald in 'Rocha' pear as affected by 1–methylcyclopropane and diphenylamine. *Postharvest Biology and Technology*, 42, 49-56.

Ju, Z. & Curry, A.E. (2002). Effects of 6-methyl-5-hepten-2-one vapour on peel browning of 'Delicious' and 'Granny Smith' apples: open and closed system. *Postharvest Biology and Technology*, 25, 265-272.

Lau, O.L. (1997). Initial low oxygen stress offers no scald control benefits to 'Starkimson Delicious' apples in 0.7 kPa $O_2$ storage. *HortScience*, 32, 1239-1241.

Mattheis, J.P. & Rudell D. (2011). Responses of 'd'Anjou' pear (*Pyrus communis L*.) fruit to storage at low oxygen set points determined by monitoring fruit chlorophyll fluorescence. *Postharvest Biology and Technology*, 60, 125-129.

Meigh, D.F. & Filmer A.A.E. (1969). Natural skin coating of the apple and its influence on scald in storage. III. α-farnesene. *Journal of the Science of Food and Agriculture*, 20, 140-143.

Mir, N., Perez, R. & Beaudry, R.M. (1999). A post storage burst of 6-methyl-5-hepten-2-one (MHO) may be related to superficial scald development in 'Cortland' apples. *Journal of American Horticultural Science*, 124, 173-176.

PPECB, (2014). Annual Report 2013/2014. The Perishable Products Export Control Board. www.ppecb.com.

Pesis, E, Feygenberg, O., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J. & Ben-Arie, R. (2014). Low oxygen pre-storage treatment is effective in reducing chilling injuries of deciduous fruit. *International Journal of Postharvest Technology and Innovation*, 4, 23-32.

Sabban-Amin, R. Feygenberg, O., Belausov, E. & Pesis, E. (2011). Low oxygen and 1-MCP pre-treatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. *Postharvest Biology and Technology*, 62, 295-304.

Song, J., Gardner, B.D., Holland, J.F. & Beaudry, R.M. (1997). Rapid analysis of volatile flavour compounds in apple fruit using GC/Time of flight mass spectrometry. *Journal of Agricultural and Food Chemistry*, 45, 1801-1807.

Truter, A.B., Combrink, J.C. & Burger, S.A. (1994). Control of superficial scald in 'Granny Smith' apples by ultra-low and stress levels of oxygen as an alternative to diphenylamine. *Journal of Horticultural Science,* 69, 581-587.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae,* 600, 261-265.

Von Mollendorff, L.J. (1996). Development of physiological postharvest disorders. In Combrink, J.C. (Ed.), *Integrated management of post-harvest quality* (pp. 39-42). ARC INFRUITEC: Paarl printing, Paarl.

Wang, Z. & Dilley, D.R. (2000a). Hypobaric storage removes scald related volatiles during the low temperature induction of superficial scald of apples. *Postharvest Biology and Technology*, 18, 191-199.

Wang, Z. & Dilley, D.R. (2000b). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology*, 18, 201-213.

Wang, Z. & Dilley, D.R. (2001). Initial low oxygen stress controls superficial scald of apples without using postharvest chemical treatments. *Acta Horticulturae*, 553, 261-266.

Watkins, C.B., Bramlage, W.J. & Cregoe, B.A. (1995). Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *Journal of American Society of Horticultural Science*, 120, 88-94.

Whitaker, B.D. & Saftner, R.A. (2000). Temperature dependant autoxidation of conjugated trienols from apple peel yields 6-methyl-5-hepten-2-one, a volatile implicated in induction of superficial scald. *Journal of Agricultural and Food Chemistry*, 48, 2014-2043.

Whitaker, B.D. (2004). Oxidative stress and superficial scald of apple fruit. *HortScience*, 39, 933-937.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, J.E. & Mattheis, J.P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology*, 53, 43-50.

Zanella A. & Strüz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10th of EU's apples producing area. *Acta Horticulturae*, 1012, 419-426.

Table 1. $O_2$ and $CO_2$ concentrations associated with storage treatments of 'Packham's Triumph' pears during or before storage at -0.5 °C

| Storage treatment | Gas composition (%) | |
|---|---|---|
| | % $O_2$ | % $CO_2$ |
| DCA | 0.5 | 1 |
| CA | 1.5 | 2.5 |
| RA (control) | 20.95 | 0-1 |
| ILOS+CA | 0.5 | 2.5 |
| ILOS+RA | 0.5 | 0-1 |

Note: DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, For ILOS treatments, the gas composition refers only to the initial low gas stress condition and not the subsequent CA or RA applied.

Table 2. Summary of low oxygen storage treatments and duration applied to simulate typical South African shipping and handling periods for 'Packham's Triumph' pears.

| Storage treatment | Storage period (months) | Breakdown of storage period: Preconditioning (days) | Treatment | Shipment & handling simulation (weeks) |
|---|---|---|---|---|
| DCA | 2 | None | 10d* | 8w** |
| | 3 | | 6w * | 6w** |
| | 5 | | 16w * | 6w** |
| | 7 | | 24w* | 6w** |
| CA | 2 | None | 10d* | 8w** |
| | 3 | | 6w* | 6w** |
| | 5 | | 16w* | 6w** |
| | 7 | | 24w* | 6w** |
| Control (RA) | 2 | None | | 8w** |
| | 3 | | | 12w** |
| | 5 | | | 22w** |
| | 7 | | | 30w** |
| ILOS+CA | 2 | 10d* | | 8w** |
| | 3 | 10d* | 6w* | 6w** |
| | 5 | 10d* | 16w* | 6w** |
| | 7 | 10d* | 24w* | 6w** |
| ILOS+RA | 2 | 10d* | | 8w** |
| | 3 | 10d* | | 12w** |
| | 5 | 10d* | | 22w** |
| | 7 | 10d* | | 30w** |

Note: ILOS is a preconditioning once off 10d treatment, * Treatment period, ** Shipment and handling period, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Treatment periods were followed by 0, 3 and 7 days of shelf life at 20 °C.

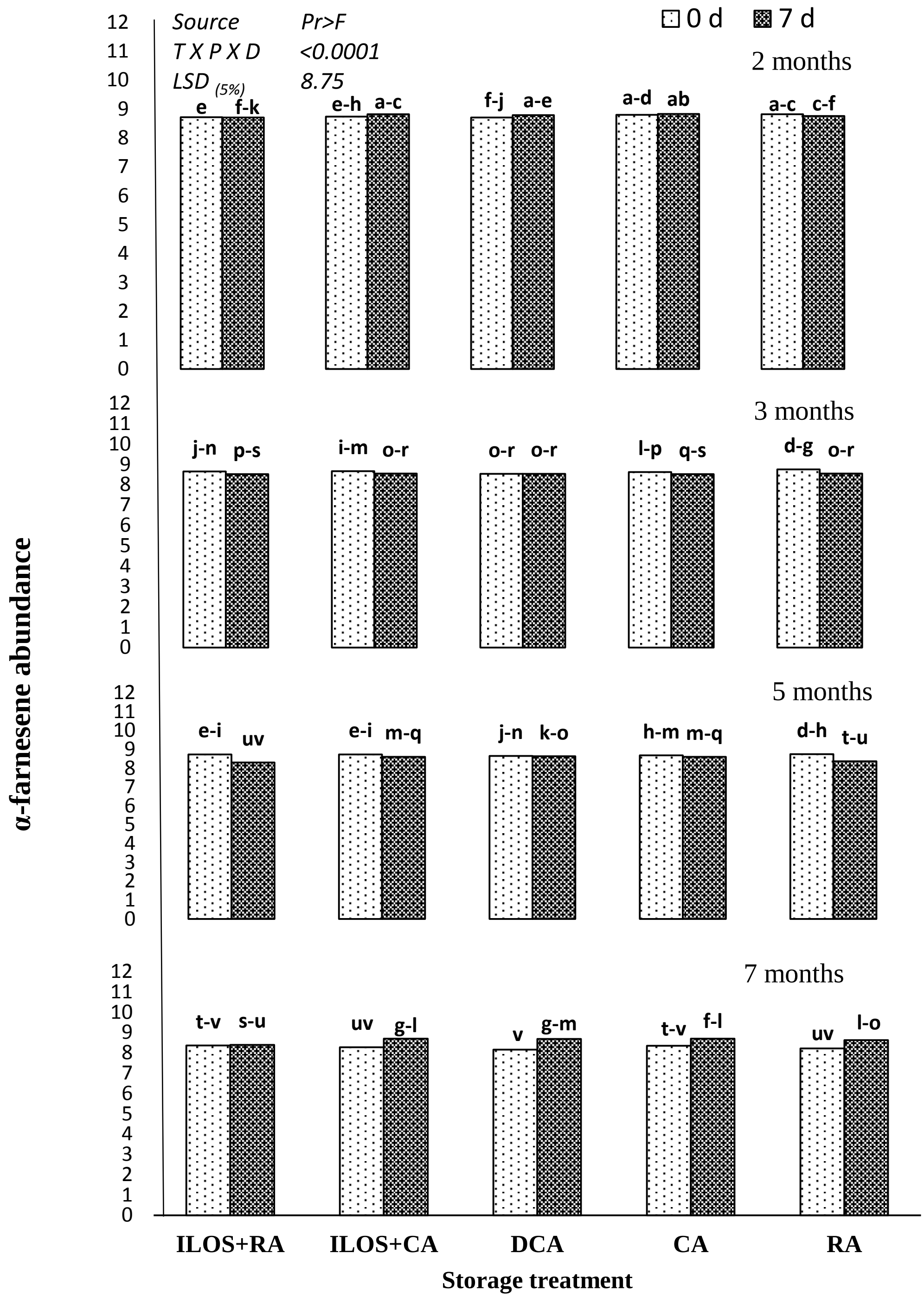


Figure 1. Effect of different storage treatments on abundance of α-farnesene in 'Packham's Triumph' pears peel after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with different letters are significantly different at $p<0.05$.T X P X D = Storage treatment x storage period x shelf life. Means with the different letters are significantly different at $p<0.05$. Abundance of α - farnesene is the $\log_{10}$ (peak area).

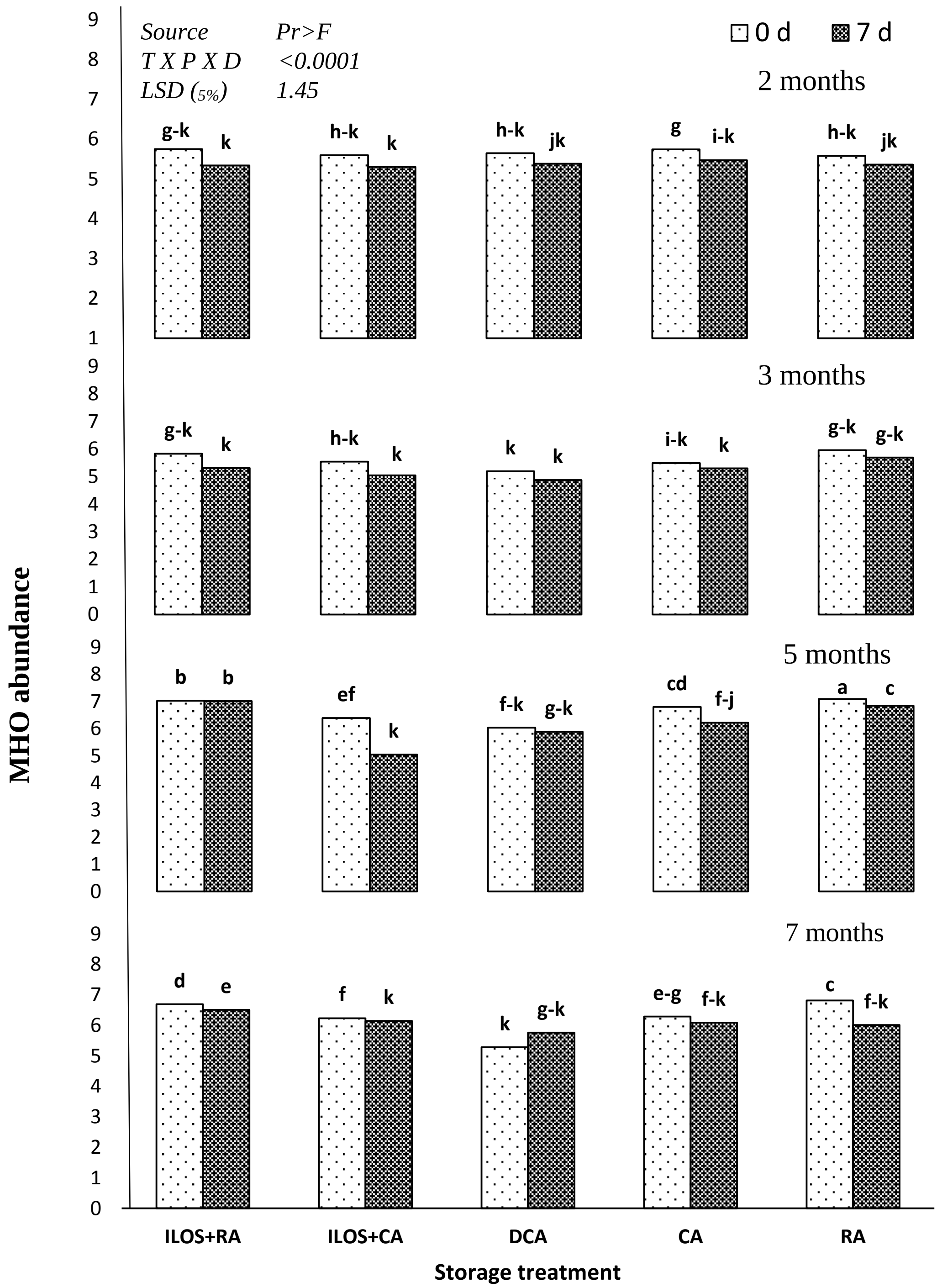


Figure 2. Effect of different storage treatments abundance of 6-methyl-5-hepten-2-one (MHO) on ‘Packham's Triumph’ pears peel after designated post storage intervals and after retailer’s shelf life conditions during the 2012 harvest season.

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with different letters are significantly different at $p<0.05$. T X P X D = storage treatment x storage period x shelf life. Means with the different letters are significantly different at $p<0.05$. Abundance of MHO is the $\log_{10}$ (peak area).

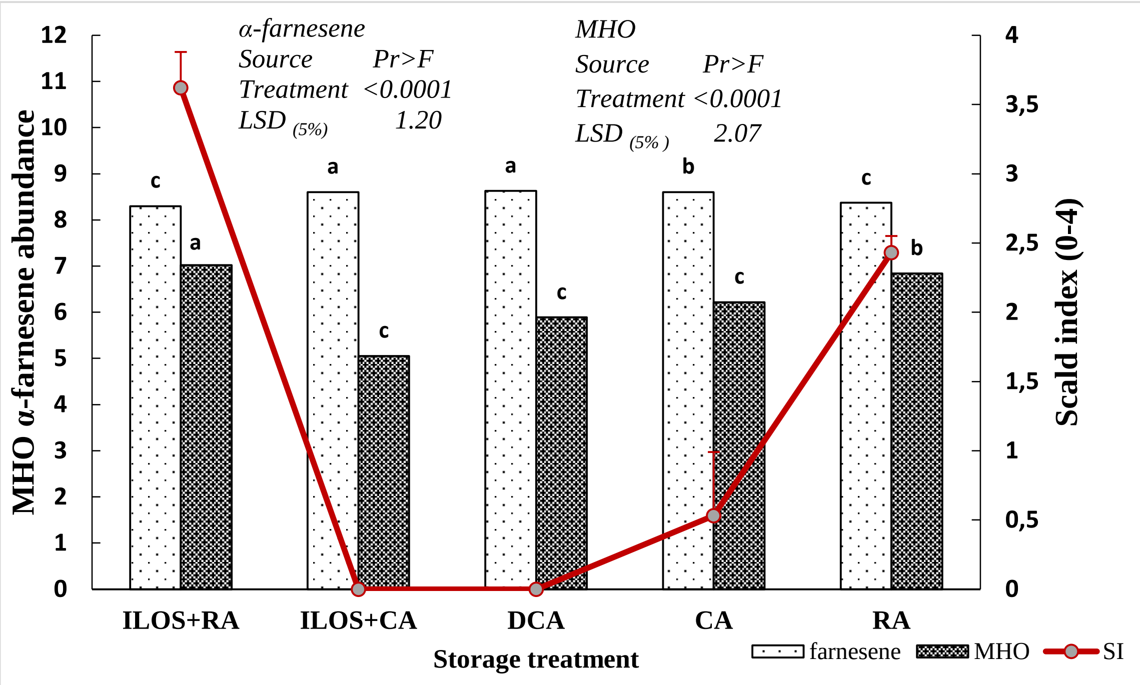


Figure 3. Effect of α-farnesene and 6-methyl-5-hepten-2-one (MHO) on scald index (SI) of the fruit peel of 'Packham's Triumph' pears stored in different storage treatments for 5 months of cold storage followed by 7 days in shelf life during the 2012 harvest season.

Note: ILOS treatment is once-off preconditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with the different letters are significantly different at $p<0.05$. Abundance of MHO and farnesene is the $\log_{10}$ (peak area).

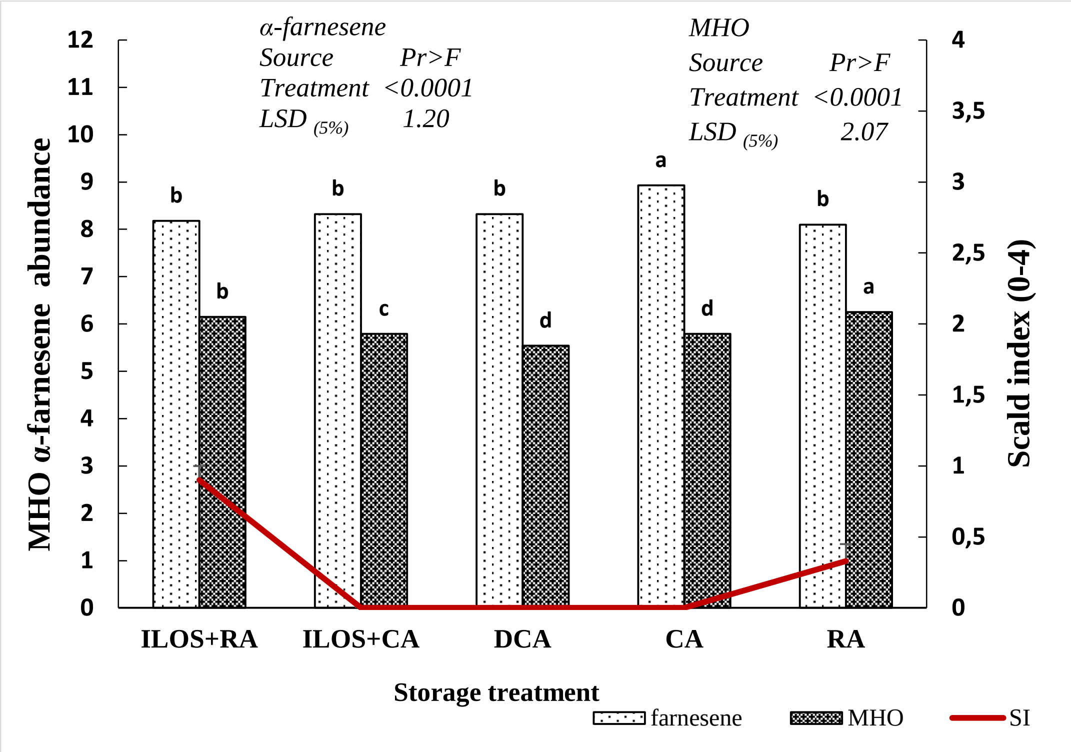


Figure 4. Effect of α-farnesene and 6-methyl-5-hepten-2-one (MHO) on scald index (SI) of the fruit peel of 'Packham's Triumph' pears stored in different storage treatments for 5 months of cold storage followed by 7 days in shelf life during the 2013 harvest season.

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with the different letters are significantly different at $p<0.05$. Abundance of MHO and α-farnesene is the $\log_{10}$ (peak area).

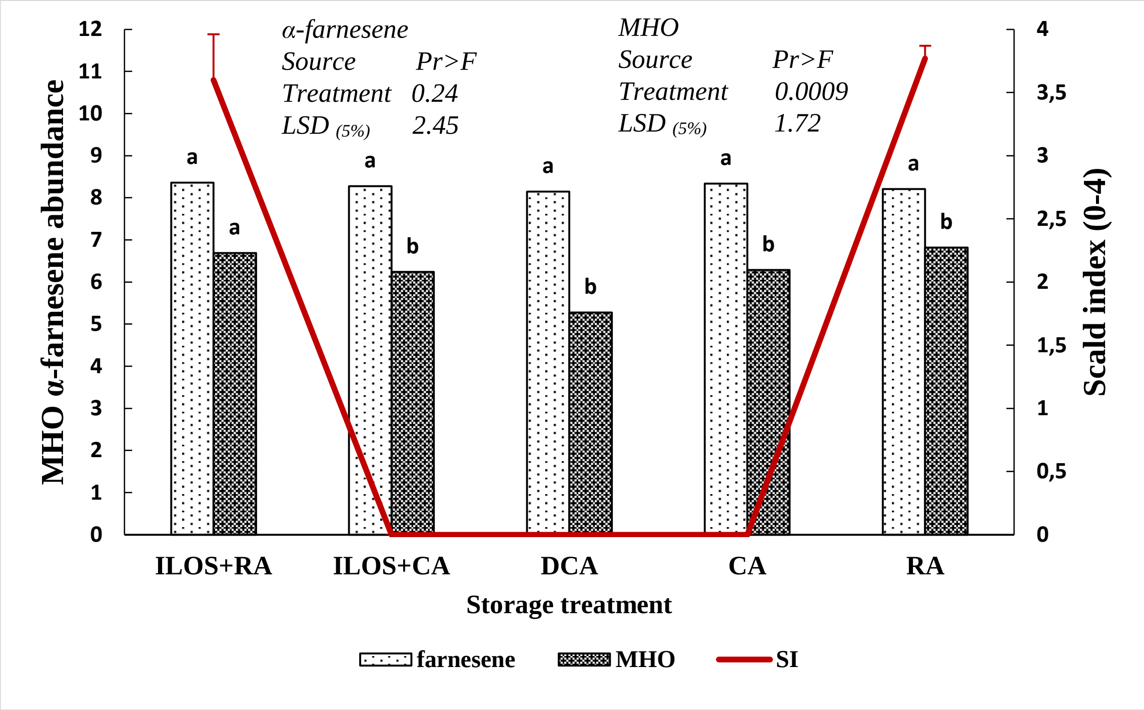


Figure 5. The effect of α-farnesene and 6-methyl-5-hepten-2one (MHO) on scald index (SI) of the fruit peel of 'Packham's Triumph' pears stored in different storage treatments for 7 months followed by 0 day in shelf life during the 2012 harvest season.

Note: ILOS is a once off pre-conditioning 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with the different letters are significantly different at $p<0.05$. Abundance of MHO and α-farnesene is the $\log_{10}$ (peak area).

# PAPER 4: DYNAMIC CONTROLLED ATMOSPHERE AND INITIAL LOW OXYGEN STRESS AS ALTERNATIVE TECHNOLOGIES FOR CONTROL OF SUPERFICIAL SCALD ON 'GRANNY SMITH' (*MALUS DOMESTICA BORKH.*) APPLES.

**Abstract**

Superficial scald incidence is a major problem in 'Granny Smith' apples due to maximum residue levels (MRL's) for the historically used synthetic antioxidant diphenylamine (DPA), making it impossible to use it commercially for most export markets. This paper therefore focuses on non-chemical techniques of superficial scald suppression dynamic controlled atmosphere (DCA) and initial low oxygen stress (ILOS). DCA effectively controlled superficial scald on 'Granny Smith' apples for up to 7 months in cold storage and offers an alternative to the use of DPA in long term storage. Superficial scald symptoms seem to appear after transfer of fruit from cold storage (0 °C) to warmer temperature (20 °C). After 7 months the symptoms of superficial scald appeared during cold storage. Optimally harvested 'Granny Smith' apples showed better resistance to superficial scald than pre-optimally harvested fruit. CA preceded by ILOS treatments was not effective on controlling superficial scald on 'Granny Smith' apples. DCA and CA treated 'Granny Smith' apples were firmer than CA preceded by ILOS, RA preceded by ILOS and RA fruit after 7 months. DCA, CA preceded by ILOS and CA treated fruit retained skin colour better than RA preceded by ILOS fruit, although not significantly different from RA stored fruit after 7 months. 'Granny Smith' apples generally maintain internal quality (TSS and TTA) over extended storage periods, there was no significant difference irrespective of treatment applied. DCA, CA and CA preceded by ILOS treatments maintained texture, appearance and taste better than RA preceded by ILOS and control (RA) treatments on 'Granny Smith' apples after long term cold storage of 7 months.

## 1. Introduction

The major market for South African grown apples is the European Union (EU), Asia and Africa requiring long term storage and transportation to these markets without compromising quality of the fruit. The export volume of fresh apples is estimated at 339 321 tons (42.7% of the total production) with the value of R4 768 856 (PPECB, 2014), with 'Granny Smith' apple being the most exported. 'Granny Smith' apple has longer storage potential; however it is highly susceptible to storage physiological disorder superficial scald

(Calvo et al., 2002). Superficial scald is an important physiological disorder effecting apple and pear cultivars globally after cold storage. Superficial scald is expressed as a chilling injury (Watkins et al., 1995) and affects the peel but not the underlying flesh (Wills et al., 1989) making the fruit unsuitable for fresh produce market.

Consumers prefer fruits and vegetables with no chemical pesticides, and this has resulted with reviews of legislation during postharvest handling (Thompson, 2003). South Africa like most developing countries is under pressure to adhere to set maximum residue limit (MRL) to avoid loss of competitiveness in the global market. For decades the synthetic antioxidant diphenylamine (DPA) has been used as a drench to prolong the storage life of South African grown apples, however its use has been restricted from 5 ppm to 0.1 ppm MRL effective from April 2014 (Commission Regulation (EU) No. 772/2013 of 8 August 2013). It is crucial that non-chemical technologies be investigated on locally grown fruit as alternatives to the use of the DPA to control superficial scald and prolong storage and shelf life of 'Granny Smith' apple and maintain eating quality.

Dynamic controlled atmosphere (DCA) technology is already implemented in most apple and pear growing countries including United States and South Tyrol in Italy (Zanella and Strüz, 2013). South African pome fruit industry faces a unique challenge as there is a need to incorporate handling and transportation time when developing and testing storage technologies as the industry is export orientated. A shipment and handling simulation period of 4-6 weeks has been incorporated in the trial. Technologies that abide with legislation on MRL's are required to extend the storage life of pome fruit. DCA technology has shown potential on scald control of 'Granny Smith' and 'Red Delicious' apple cultivars grown and stored in South Tyrol, Italy (Zanella and Strüz, 2013) and on 'Cortland', 'Golden Delicious', 'Honey Crisp', 'Jonagold', 'McIntosh' and 'Delicious' apples (DeLong et al., 2004). Various cultivars respond differently to gas combinations (Wills et al., 1989).

Controlled atmosphere preceded by initial low oxygen stress (ILOS+CA) has shown positive results on superficial scald control on 'Granny Smith', 'Law Rome, 'Idared' and 'Red Delicious' apples (Wang and Dilley, 2000; Little and Holmes, 2000; Calvo et al., 2002; van der Merwe et al., 2003). The main advantage of ILOS is the inhibition of superficial scald and is achieved without the use of synthetic antioxidants (Little and Holmes, 2000) and no added costs for installation of software as is the case with the use of DCA. It is crucial that pull down to desired low oxygen level be achieved in the first five to ten days otherwise delays may render the treatment ineffective (Little and Holmes, 2000).

Controlled atmosphere technologies: such as DCA, CA preceded by ILOS, CA, RA preceded by ILOS were investigated to determine their efficacy to control superficial scald and maintain quality of ‘Granny Smith’ apples during long term storage to offer an alternative to the use of DPA for the South African growers and exporters to safe guard the industry. DPA was however not utilised in this trial to avoid cross contamination in the storage rooms (Robatscher et al., 2012). Due to limited information on the effects on controlled atmosphere technologies on the keeping quality of ‘Granny Smith’ apples, this study will further investigate the effects of the treatments on internal and sensory quality of the fruit during and after long term storage.

The objectives of this study were to:

(1) Assess the effectiveness of ILOS, CA and DCA treatments on control of superficial scald physiological disorder on ‘Granny Smith’ apples after long term storage.

(2) Assess the effectiveness of initial low oxygen stress (ILOS), controlled atmosphere (CA) and dynamic controlled atmosphere (DCA) to maintain quality parameters: firmness, skin colour, total soluble solids (TSS), titratable acidity (TTA) and sugar to acid (TSS/TTA) ratio of ‘Granny Smith’ apples after long term cold storage.

(3) Assess the effectiveness of ILOS, CA and DCA on sensory quality parameters (taste, texture and appearance) of ‘Granny Smith’ apples after long term storage.

## 2. Materials and methods

### 2.1. Fruit supply

‘Granny Smith’ apples were harvested at pre-optimal and optimal maturities on 28$^{th}$ March and on 06$^{th}$ of April of 2012 and 2013 harvest seasons, respectively (Table 1). The fruit were obtained from Grabouw production area (34.1500°S, 19.0167°E), Western Cape, South Africa, transported in bulk bins to the postharvest laboratory at Agricultural Research Council (ARC) Infruitec-Nietvoorbij in Stellenbosch, and sorted immediately on arrival to remove ones with external defects such as sunburn, mechanical damage and decay. After sorting, fruit were put into crates which represented a replicate each, and were put into treatment rooms at 0 °C. Each treatment was in triplicate and each replicate consisted of 90-120 fruit.

## 2.2. Storage treatments

Fruit were stored for up to 7 months at 0 °C, and 5 storage treatments were applied (CA preceded by ILOS, RA preceded by ILOS, dynamic controlled atmosphere, controlled atmosphere and regular atmosphere) at various $O_2$ and $CO_2$ combinations (Table 2).

### *2.2.1. Regular atmosphere preceded by initial low oxygen stress (RA preceded by ILOS)*

In RA preceded by ILOS fruit were stressed with low oxygen at 0.7% for 10 days as a pre-conditioning treatment (Table 3), before transfer into the RA room. For 2 months storage period fruit were kept in ILOS for 10 days only and for 8 weeks in RA to simulate shipment and handling. For 3 months storage period fruit were kept in ILOS for 10 days only and thereafter kept in RA for 12 weeks. For 5 months storage period fruit were kept in ILOS for 10 days and followed by 22 weeks in RA. For 7 months storage period fruit were kept in ILOS for 10 days only and thereafter followed by 30 weeks of RA to extend marketing window.

### *2.2.2. Controlled atmosphere preceded by initial low oxygen stress (CA preceded by ILOS)*

Fruit treated with CA preceded by ILOS were kept for 10 days in initial low oxygen stress before transfer into CA. For 2 months storage period fruit are stored in ILOS for 10 days and transferred into CA for 8 weeks (Table 3). For 3 months storage period fruit were stored in ILOS for 10 days in ILOS followed by 6 weeks in CA and thereafter transferred to RA room to simulate shipment and handling. After the 5 months storage period, fruit were stored in ILOS for 10 days and thereafter transferred to CA for 16 weeks followed by 6 weeks in RA to simulate shipment and handling. For 7 months storage period fruit were preconditioned with ILOS for 10 days and transferred into CA for 24 weeks followed by RA to simulate shipment and handling (Table 3).

### *2.2.3. Dynamic controlled atmosphere (DCA)*

DCA treated fruit were kept for 10 days in DCA followed by 8 weeks in RA to simulate shipment and handling for the 2 months storage period (Table 3). For 3, 5 and 7 months storage period the DCA treated fruit were kept in DCA room for 6, 16 and 24 weeks respectively and then transferred into RA for 6 weeks to simulate shipment and handling.

DCA treatment room was fitted with HarvestWatch™ (SAtlantic Inc. Halifax, N.S., Canada) fluorescence interactive response monitor sensor (FIRM) to monitor fruit response to low $O_2$ levels hourly. The selection of representative sample of 6 fruit for each FIRM sensor is crucial (Zanella et al., 2005), because the selected fruit represent the whole batch in the treatment room. The fruit were loaded into cold rooms and the desired fruit core temperature of 0°C was achieved within 48 to 96 hours after harvest. The spiking for lower oxygen limit was set at 0.3% $O_2$ for both harvest seasons (2012 and 2013), and it was thereafter adjusted to 0.5% $O_2$ for the rest of the storage period as explained by DeLong et al. (2007).

*2.2.4. Controlled atmosphere (CA)*

CA fruit were kept for 10 days in CA followed by 8 weeks of RA for the 2 months storage period (Table 3). For the 3, 5 and 7 months storage period, fruit were kept in CA for 6, 16 and 24 weeks respectively and thereafter transferred to RA for 6 weeks to simulate shipment and handling.

*2.2.5. Regular atmosphere (RA)*

RA was the control treatment for the trial. For RA fruit were kept solely in RA for the duration of storage of 2, 3, 5 and 7 months (Table 3).

2.3. Physiological disorders

*2.3.1. Superficial scald intensity (Scald index)*

Fruit affected with superficial scald physiological disorder were counted for each replicate (90-120) and the number of fruit affected was recorded as percentage. Each fruit was inspected visually and the percentage area of the fruit surface affected (intensity) with the disorder was recorded. All the fruit in each replicate were evaluated for superficial scald disorder. The intensity of the disorder was evaluated and graded according to an index used by Magwaza et al. (2012) on rind breakdown of 'Nules Clementine' mandarins based on the percentage surface area of the fruit affected. Grade 0 = no scald, Grade 1 = Slight scald (0-25%), Grade 2 = Moderate scald (26-50%), Grade 3 = Severe scald (51-75%) and Grade 4 = Extreme scald (76-100%).

### *2.3.2. Decay incidence*

The incidence of decay was evaluated for each replicate during each evaluation at shelf life conditions at 0, 7 and 14 days after 2, 3, 5 and 7 months. The number of decayed fruit was recorded as a percentage of the replicate total.

## *2.4. Physico-chemical properties*

### *2.4.1. Firmness (N)*

A total of 10 fruit were used to evaluate firmness for each replicate. From each of the five treatments, of 30 fruit were used to evaluate firmness of 'Granny Smith' apples. Each treatment was replicated three times and each treatment consisted of 90-120 fruit. Measurements for firmness were taken with a mounted penetrometer (GUSS fruit texture analyser, South Africa), using a plunger of 11.1 mm in diameter. Each fruit was peeled from opposite sides and the plunger pressed on to the peeled flesh and the firmness reading recorded. For each replicate, 10 fruit is used and 20 readings recorded (two readings per fruit).

### *2.4.2. Skin colour*

A total of 10 fruit were used to evaluate skin ground colour for each replicate, thus 30 fruit for each treatment. Skin ground colour using (Chroma Meter CR 400/410 Konica Minolta Sensing Inc. Japan) and each treatment was in triplicate. For each replicate 10 measurements were taken as a replicate consisted of 10 fruit. The skin colour index was calculated using the following equation:

Skin colour index = $\frac{L*.a*.b*}{3} - 2.8 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$ (1)

(Van der Merwe, 2013).

L* = CIE lightness coordinate, a* = CIE red (+)/green (-) colour attribute, b* = CIE yellow (+)/blue (-) colour attribute. The skin colour scores were indexed as follows: Skin colour score 0-0.99 = full green (G), Skin colour score 1-1.99 = green green yellow (GGY), Skin colour score 2-2.99 = green yellow (GY), Skin colour score 3-3.99 = green yellow yellow (GYY) and, skin colour score 4-5.00 = full yellow (Y), based on the indexing used by Little and Holmes (2000).

*2.4.3. Total soluble solids (TSS) (°Brix)*

Fruit segments (20g of each fruit) were cut transversely from each of the 10 fruit used per replicate. The fruit were processed for juice using a domestic juicer (Mellerware Liquafresh juice extractor III). The juice was then squeezed into a beaker. The juice was mixed and allowed to stand for five minutes, and approximately 5 mL was taken using a syringe for TSS measurements. A drop of the juice was placed onto the calibrated refractometer (Palette PR 32 α (˚Brix 32) ATAGO Co. LTD, Japan) and the reading recorded.

*2.4.4. Titratable acidity (TTA) (% malic acid)*

A total number of 10 fruit was used for TTA measurements from each replicate for each treatment (five treatments) applied. Each replicate had 90-120 fruit and 10 fruit were used for TTA measurements, resulting in 30 fruit for each applied treatment being used. Fruit segments (20 g from each fruit) were cut from each of the 10 fruit from each replicate and blended, and 10 mL of the representative juice sample titrated with a 0.1N sodium hydroxide solution to a pH of 8.2 using titrator (Crison Titromatic, Crison Instruments E 08238 Allela, Barcelona Spain). The volume of sodium hydroxide added was recorded and the acid content calculated.

*2.4.5. Sugar to acid (TSS/TTA) ratio*

The sugar to acid ratio was measured using the following formula:

$$\text{TSS/TTA ratio} = \frac{\text{Total soluble solids}}{\text{Titratable acidity}} \qquad (2)$$

2.5. Sensory quality attributes

A trained panel of highly experienced five members were assessing sensory attributes at the Agricultural Research Council (ARC) postharvest laboratory, Infruitec-Nietvoorbij, Stellenbosch South Africa. The panel was comprised of three females and two males with more than five years' experience in conducting sensory evaluations on 'Granny Smith' apples grown in South Africa. Panel members are employed by the ARC postharvest laboratory. All participating judges are every-day apple consumers. One of the panel members had more than 20 years' experience in apple postharvest assessment. Three of the panel members had five years apple postharvest assessment.

Thirty apples per treatment (storage treatment × storage period × shelf life period) were used for sensory analysis. A total number of 10 randomly selected fruit from each replicate (thus 30 fruit from each treatment) were placed on white trays and evaluated, and appearance score (1-10), texture score (1-10) and taste scores (1-10) and recorded. Appearance of the fruit was evaluated first, and thereafter fruit were cut and evaluated tor taste and texture. Codes were used for treatments applied to eliminate bias from the judges. Mineral water was used as palate cleansers between samples.

#### *2.5.1. External appearance*

External appearance of 'Granny Smith' apples was scored based on freedom from defects, decay, mechanical damage, scuff marks, uniformity of colour and physiological disorder incidence. The score of 1-3 represented poor appearance, a score of 4-6 represented limited marketability, whereas a score of 7-10 was the good appearance.

#### *2.5.2. Texture*

After evaluating the fruit for external appearance the fruit were cut and evaluated for texture. Texture of the 'Granny Smith' apples was scored based on crunchiness, crispiness of the first bite. Texture scores were from 1-10. The texture scores from 1-3 represented poor texture, texture scores of 4-6 represented good texture, whereas the scales of 7-10 represented excellent texture.

#### *5.2.1. Taste*

After evaluating the fruit for texture the fruit used were tasted by panel members and scored from 1-10. The taste score of 1-3 represented unacceptable taste, and scores of 4–6 represented acceptable taste, whereas scores of 7-10 represented excellent taste. Taste was scored based on pear flavour development and the presence of off-flavours. Unacceptable taste was mostly due to the presence of alcoholic taste in the fruit leading to off flavours or the presence of bland flavour due to lack of apple flavour development.

### 2.6. Statistical analysis and trial design

The trial design was a completely randomised 3–way factorial with treatment (storage protocol), storage period (at 0 °C), and retailer's shelf life (ripening at 20 °C) period as sources of variation. 'Granny Smith' apples were subjected to five storage treatments namely:

controlled atmosphere preceded by initial low oxygen stress (ILOS+CA), regular atmosphere preceded by initial low oxygen stress (ILOS+RA), dynamic controlled atmosphere (DCA), controlled atmosphere (CA) and regular atmosphere (RA) as a control (Table 2). The fruit were stored for various storage periods at 0 °C (Table 3). Each storage interval was followed by retailer's shelf life at 0, 7 and 14 days at 20 °C at which fruit were evaluated.

The treatments were in triplicate with 90–120 fruit per replicate. For each replicate of 90-120 fruit were put in a crate, 10 fruit were evaluated for external appearance, texture and taste, thus 30 fruit for each treatment. Categorical data (superficial scald and decay incidence) were arcsin transformed prior to statistical analysis. Data was subjected to Analysis of variance (ANOVA) using General linear model (GLM) (SAS Enterprise Guide 5.1) with treatments; storage period and shelf life as sources of variation. Separation of means ($p=0.05$) were performed using the Least significance difference (LSD) method. Pearson correlations were performed using SAS enterprise guide 5.1. Pearson correlations were used to quantify the relationships among assessed parameters.

## 3. Results and Discussion

### 3.1. Physiological disorders

#### *3.1.1. Superficial scald incidence (%) and intensity (scald index)*

There was no superficial scald on pre-optimal 'Granny Smith' apples treated with RA preceded by ILOS, CA preceded by ILOS, DCA and CA after 2 months cold storage (Table 4). The onset of superficial scald on pre-optimal 'Granny Smith' apples for 2012 season was after 7 days shelf life preceded by 2 months storage period on RA treated fruit with 0.77% of fruit affected. The onset of superficial scald on optimal 'Granny Smith' fruit was on RA preceded by ILOS treatment and the scald index of the affected fruit was 0.04 after 2 motnhs in storage followed by 14 days shelf life (Table 4), and there was no superficial scald on RA, CA preceded by ILOS, CA and DCA treated fruit.

Pre-optimally harvested fruit developed superficial scald after 2 months followed by 7 days shelf life whereas optimally harvested fruit developed scald after 2 months followed by 14 days shelf life. Ju and Curry (2002) reported onset of superficial scald in regular atmosphere (RA) stored fruit at 4 months storage in 'Granny Smith' and 'Delicious' apples. Pre-optimally harvested fruit are more prone to the disorder than optimally harvested fruit.

Erkan and Pekmezci (2004) found that later harvested ‘Granny Smith’ apples grown in Turkey had the highest intensity of superficial scald than pre-optimally harvested fruit.

The onset of superficial scald on RA preceded by ILOS treated pre-optimal harvested fruit was after 3 months of cold storage followed by 7 days shelf life during the 2012 season (Table 4). At the end of storage life, 0 day of 5 months cold storage period there was no superficial scald on all treatments, but after 7 and 14 days in shelf life the RA preceded by ILOS and RA fruit were affected but CA preceded by ILOS, DCA and CA fruit were not affected.

At 0 day after 5 months cold storage, RA preceded by ILOS and RA treated fruit were affected with superficial scald at 84 and 91% respectively (Table 4). After 7 and 14 days shelf life of 5 months cold storage period RA preceded by ILOS, CA preceded by ILOS, CA and RA fruit were affected with superficial scald but DCA treated fruit still inhibited symptoms of superficial scald. Ju and Curry (2002) observed that CA treated fruit developed scald after 5 months in storage, however it should be noted that in this study the fruit were kept in CA for 16 weeks (4 months) followed by 6 weeks in RA, to simulate shipment and handling required for export markets, whereas in other countries only CA is applied as a storage treatment.

At 0 day after 7 months cold storage period, RA preceded by ILOS and RA treated fruit were affected and the percentage of fruit affected was 10% higher than that of the 5 months storage period at 0 day (Table 4). At 0 day after 7 months cold storage, CA preceded by ILOS, CA and DCA fruit showed no symptoms of superficial scald. After 7 and 14 days of shelf life after 7 months period RA preceded by ILOS, RA, CA preceded by ILOS and CA had superficial scald and DCA was the only treatment that still inhibited superficial scald on pre-optimal ‘Granny Smith’ apples.

After extended periods in cold storage the symptoms of superficial scald may appear whilst fruit are still in cold storage (Lotze et al., 2003; Lurie and Watkins, 2012). RA and RA preceded by ILOS treated fruit had superficial scald after 5 and 7 months storage periods during the 2012 season on pre-optimal ‘Granny Smith’ apples. The development of superficial scald has been reported by several researchers that it may be induced cold storage but may become visible only after the transfer of fruit to warmer temperatures (Ingle, 2001;

Lotze et al., 2003; Mitcham and Elkins, 2007; Lurie and Watkins, 2012), however in this study fruit developed symptoms of superficial scald whilst still in cold storage.

The incidence and intensity of superficial scald increases with days in shelf life on ‘Granny Smith’ apples. In RA preceded by ILOS treated fruit after 2 months during the 2012 season there was no scald (index = 0) was observed at 0 day, however after 7 days shelf life the index was 1.82. The same trend of increase in index from 0 day to 7 days was observed in 2013 season (Table 5). The scald index was 0 on 0 day after 3 months of cold storage during the 2012 season, but increased to 1.02 after 7 days shelf life at 20 °C on RA preceded by ILOS treated fruit. Similar trends of increase of scald intensity with increased period in shelf life was observed by Ju and Curry (2002) on ‘Delicious’ and ‘Granny Smith’ apples and by Erkan and Pekmezci (2004) on ‘Granny Smith’ apples. The incidence of superficial scald started at 0 day of 5 months, on RA and RA preceded by ILOS treated optimal and pre-optimal harvested ‘Granny Smith’ apples during the 2013 season (Table 5).

DCA treatment suppressed the incidence of superficial scald up to 7 months followed by 14 days of shelf life at 20 °C during the 2012 season on pre-optimal and optimally matures ‘Granny Smith’ apples. The positive results of DCA technology to supress superficial scald has been reported by Zanella and Strüz (2013) on ‘Red Delicious’ apples grown in South Tyrol, Italy and by DeLong et al. (2004) on ‘Cortland’, ‘Delicious’, ‘McIntosh’, ‘Golden Delicious’, ‘Jonagold’ and ‘Honeycrisp’ apples.

Although there was no scald on DCA fruit, CA preceded by ILOS and control (RA) fruit had superficial scald severity index of 0.40 and 1.04 respectively after 7 months in cold storage in 2012 harvest season. After 7 months in cold storage ILOS treated ‘Granny Smith’ apples showed symptoms of superficial scald, and thereby render ILOS an ineffective treatment for superficial scald for long term storage. During the 2013 harvest season CA preceded by ILOS treated fruit showed symptoms of superficial scald after 14 days of shelf life. Contradicting reports by van der Merwe et al., 2003 and Combrink et al. (1994) indicated that ILOS is effective on controlling superficial scald on South African grown ‘Granny Smith’ and ‘Top Red’ apples. Pesis et al. (2014) reported that initial low oxygen stress is effective in controlling superficial scald on ‘Granny Smith’ apples grown in Northern Israel for six months cold storage. However it should be noted that in South Africa the 7 months period includes 6 weeks of RA to simulate shipment and handling protocol as observed during export to overseas markets, whereas in other countries fruit are stored in low oxygen treatments throughout. The storage protocol for storing apples and pears require that fruit at least 6 weeks of RA be included in the protocol to simulate the shipping and handling period by sea freight (Crouch, 2003).

*3.1.2. Decay incidence*

Decay in stored fruit affects external appearance negatively and eating quality of the fruit. Decay can result in major losses in long term CA storage (Lennox et al., 2004). Susceptibility of fruit to decay incidence increases with the onset of ripening during shelf life conditions (Kader, 1989). There was no significant difference of decay incidence in 'Granny Smith' apples among treatments (DCA, CA, CA preceded by ILOS, RA preceded by ILOS and RA), and the average decay incidence was below 2% in all treatments at all storage periods (data not shown).

3.2. Physico-chemical properties

*3.2.1. Firmness (N) retention of 'Granny Smith' apples after storage*

During the 2012 season there was no significant interaction among the main effects T X P X D *Pr>F= 0.7285* at 0.05 significance level, however there was interaction between treatment applied during storage and storage period *Pr>F=<0.0001* at 0.05 significance level.

DCA and CA treated 'Granny Smith' apples retained firmness better than RA and RA preceded by ILOS fruit after 2 months in cold storage during the 2012 growing season (Table 6). There was no significant difference in firmness retention of DCA, CA and CA preceded by ILOS treated 'Granny Smith' apples. RA and CA preceded by ILOS treated fruit were not significantly different in firmness retention but were firmer than RA preceded by ILOS treated 'Granny Smith' apples. The benefit of DCA on firmness retention has been reported by Zanella et al. (2008) on several apples cultivars. DCA, CA and CA preceded by ILOS treated fruit retained firmness better than RA and RA preceded by ILOS treated fruit during the 2012 growing season after 3 months in cold storage (Table 6). RA preceded by ILOS and RA treated fruit were not significantly different in firmness retention after 3 months in cold storage.

DCA, CA and CA preceded by ILOS treated 'Granny Smith' apples were significantly firmer than the control (RA) fruit. CA treated fruit were significantly firmer that

DCA fruit after 5 months in cold storage (Table 6). DCA treated fruit were significantly firmer than CA preceded by ILOS treated fruit. RA 'Granny Smith' apples were firmer than RA preceded by ILOS fruit. These findings suggest that ILOS once-off treatment offers no benefit of firmness retention on 'Granny Smith' apples. Zanella et al. (2008) showed that DCA retains firmness better than ultra-low oxygen treatment followed by CA.

After 7 months in storage DCA and CA treated fruit retained firmness better than RA preceded by ILOS and RA stored fruit and were not significantly different from each other. RA preceded by ILOS fruit was significantly firmer than RA fruit at the end of 7 months period. DCA, CA preceded by ILOS and CA fruit was firmer after 5 months and 7 months storage than periods 2 and 3 months. RA preceded by ILOS and RA fruit lost firmness with increased period in storage (Table 6).

*3.2.2. Skin colour retention of 'Granny Smith' apple after storage*

The fruit colour scores of 'Granny Smith' apples was between 1-1.99 (GGY) based on skin colour indexing and below acceptable levels after 2 months in storage at 0 day and after 7 days shelf life (Table 7). The fruit were still too green. After 14 days of shelf life CA preceded by ILOS and RA fruit colour scores were above acceptable level at GYY and the fruit were too yellow. The colour scores of 'Granny Smith' apples were within acceptable scores of 2-2.99 on RA preceded by ILOS, CA preceded by ILOS, DCA, CA and RA treated fruit, except however at 14 days for RA preceded by ILOS and RA colour score was GYY (too yellow and above acceptable limit) after the 3 and 5 months storage periods. The colour score was GYY and above acceptable level of 2-2.99 on RA preceded by ILOS treated fruit after 7 months followed by 14 days of shelf life (Table 7).

*3.2.3. TSS (°Brix) content of 'Granny Smith' apples after storage*

Statistical analysis showed that there was significant interaction among main effects (storage treatment, storage period and shelf life) $Pr>F$ = 0.4209 during the 2012 harvest season on the TSS of optimally mature 'Granny Smith' apples (Table 8). TSS is a useful measure for fruit flavour and in some countries such as Australia it is mainly used to determine the minimum acceptable eating quality of some apples cultivars Jonathan and Red Delicious (Little and Holmes, 2000). The minimum acceptable limit for TSS at harvest is 11.0 and 11.5 at wholesale market for 'Granny Smith' apples (Little and Holmes, 2000). There were no significant changes in TSS levels in all treatments after 2 and 3 months of cold storage (Table 8). After 7 months in storage at 0 day, RA preceded by ILOS and CA preceded by ILOS treated fruit had the highest TSS level, whereas DCA, CA and RA treated fruit had the lowest (Table 8). The changes in the TSS content of 'Granny Smith' apples during storage were subtle therefore making it difficult to distinguish any clear patterns. Kvikliene et al (2006) observed the similar results on 'Auskis' apples.

*3.2.4. TTA (% malic acid) content of 'Granny Smith' apples after storage*

There was no significant interaction among main effects (storage treatment, storage period and retailer's shelf conditions) on the TTA of optimally mature 'Granny Smith' apples during the 2012 harvest season $Pr>F$ = 0.0994 (Table 8). There were no significant changes in TTA content of optimally harvested 'Granny Smith' apples at the end of storage life at 0 day after 2 months of cold storage, and after 7 and 14 days on RA preceded by ILOS and CA preceded by ILOS fruit (Table 8). The TTA content was significantly higher on CA treated fruit at 0 day, but decreased with shelf life at 7 days; however there were no significant changes between 7 and 14 days shelf life. There were no significant changes in TTA content of DCA and RA treated fruit at 0 and after 7 days shelf life, however the TTA decreased from 7 days and was lowest after 14 days.

The TTA level was highest at 0 day (after removal from cold storage), and decreased with 7 days shelf, and subsequently decreased after 14 days in shelf life in RA preceded by ILOS treated fruit after 2 months in cold storage (Table 8). The decrease of TTA during fruit maturation has been reported on pomegranate by Fawole (2013). There were no significant differences in TTA content at 0 day, after 7 days and at 14 days on RA preceded by ILOS fruit after 3 months in cold storage (Table 8). DCA treated 'Granny Smith' apples had higher TTA at 0 day, but decreased significantly after 7 days and 14 days, and the same trend was observed on CA and RA treated fruit. There were no significant changes on RA preceded by ILOS treated fruit at 0 day and 7 days shelf life but decreased significantly after 14 days in shelf life. There was no significant difference between 0 and 7 days shelf life however there was a significant decline after 14 days shelf life on CA preceded by ILOS treated 'Granny Smith' apples. There were no significant changes in TTA content in DCA treated fruit at 0 day, after 7 and 14 days in shelf life after 5 months cold storage (Table 8). The TTA content of 'Granny Smith' apples decreased significantly with shelf life, it was highest at the beginning of shelf life and lowest at the end of shelf life at 14 days in all treatments after 7 months of storage (Table 8).

*3.2.5. TSS/TTA ratio of 'Granny Smith' apples after storage*

There was significant interaction among main effects (storage treatment, storage period and shelf life) *Pr>F* = 0.0215 during the 2012 harvest season (Table 9). There were no significant changes in TSS/TTA levels after 2, 3 and 5 months in storage irrespective of treatment applied during the 2012 season (Table 9). After 7 months in storage TSS/TTA ratio increased after 7 and 14 days shelf life. DCA treated fruit had the highest TSS/TTA ratio in 'Granny Smith' apples at 14 days after 7 months in cold storage, however it was not significantly different from the control treated fruit. The better retention of TSS/TTA ratio indicate that this treatment maintain flavour and internal quality of fruit. The end of flavour life results from losses in sugars, acids, and aroma volatiles (especially esters) (Kader, 2008).

3.3. Sensory quality attributes

*3.3.1. External appearance of optimally mature 'Granny Smith' apples after cold storage*

External appearance is one for the important quality parameters used by consumers at the point of sale such as supermarket to inform their choice (Jaeger et al., 2003). There was significant interaction among the main effects (storage treatment, storage period and shelf life conditions) during the 2012 harvest season on optimally mature 'Granny Smith' apples (Table 10). Generally amongst all treatments and storage periods there was a decline of appearance scores from 0 day, 7 to 14 days of shelf life during the 2012 harvest season. After 14 days shelf life, during the 3 months storage period the appearance scores were poor and unacceptable (scores of 1-3) in RA preceded by ILOS and RA treated fruit due to the presence of superficial scald on the 'Granny Smith' apples (Table 5 and Table 10). Greasiness was also observed no RA and RA preceded by ILOS stored 'Granny Smith' apples and this has also affected the scores as greasiness makes the fruit undesirable to most consumers after 7 and 14 days in shelf life, after 3 months in cold storage. Superficial scald affects the appearance of fruit and reduce its marketability at the fresh produce market (Little and Holmes, 2000).

After 7 months in storage, at 0 day; 'Granny Smith' apples had the poor unacceptable appearance scores of 2 and 3 on RA preceded by ILOS and RA fruit respectively due to the presence of superficial scald during storage (Table 5 and Table 10). The appearance scores for 7 and 14 days remained very low and unacceptable after 7 months in storage on RA preceded by ILOS and RA treated fruit. DCA, CA and CA preceded by ILOS maintained better scores of 7-10 during the 7 months period at 0 day and after 7 days in shelf life on 'Granny Smith' apples (Table 10). However after 14 days in shelf life the DCA, CA and CA preceded by ILOS treated fruit had the limited marketability scores of 6 and 5.75 respectively. DCA, CA and CA preceded by ILOS treatments maintained better appearance scores. Similar finding were reported by Echeverria et al. (2004) on 'Fuji' apples.

*3.3.2. Texture scores of optimally mature 'Granny Smith' apples after cold storage*

There was significant interaction of the main effects of texture scores of 'Granny Smith' apples harvested at optimal maturity during the 2012 harvest season $Pr>F$ = <0.0001 (Table 11). The texture scores of optimally harvested 'Granny Smith' apples decreased from 0 day to 7 days, and form 7 to 14 days in shelf life, irrespective of treatment applied during storage during the 2012 harvest season (Table 11). All storage treatments: CA preceded by ILOS, RA preceded by ILOS, DCA, CA and RA treatments had excellent texture scores of 7.5, 7.5, 8, 8, and 8 respectively at 0 day after 2 months in cold storage. Texture scores for RA treated fruit were significantly higher than that of RA preceded by ILOS treated fruit and CA preceded by ILOS treated fruit, although not significantly different from scores of fruit treated with DCA and CA at 0 day of 2 months storage period.

After 3 months in cold storage the scores were significantly higher on DCA, CA and CA preceded by ILOS treated fruit at a score of 7 which is graded as excellent texture, and the texture score was higher than that of RA and RA preceded by ILOS treated fruit at 5.75 and 6 respectively (acceptable texture) (Table 11). After 14 days in shelf life DCA, CA preceded by ILOS treated fruit had significantly higher scores than CA, RA preceded by ILOS and RA stored fruit, however the texture level for the treatments was still acceptable.

After 5 months storage period DCA and CA preceded by ILOS treated fruit had significantly higher texture score of 7 (excellent texture) than RA, RA preceded by ILOS and CA preceded by ILOS fruit with a score of 6 (good texture) at 0 day (Table 11). After 14 days of the 5 months storage period, DCA treated fruit had significantly higher texture scores than other treatments and maintained texture scores of excellent quality at 7. CA and CA preceded by ILOS treated fruit maintained texture scores of acceptable texture at 5.50 and 6 respectively. CA preceded by ILOS treated fruit was significantly firmer than CA treated fruit after 14 days shelf life of the 5 months storage period. RA and RA preceded by ILOS treated fruit had the lowest scores of poor texture at 2.00 and 3.00 respectively; however the RA preceded by ILOS treated fruit were statistically significantly firmer than RA treated fruit during the 14 days of the 5 months storage period (Table 11).

At 0 day of the 7 months storage period, DCA treated ‘Packham’s Triumph’ pears were significantly firmer than fruit treated with other treatments (CA, CA preceded by ILOS, RA preceded by ILOS and RA), and had a score of excellent texture (7) (Table 11). CA and CA preceded by ILOS treated fruit had scores of acceptable texture and were not significantly different statistically. The texture score for RA preceded by ILOS treated fruit was significantly higher than the score for RA treated fruit; however the scores were of acceptable texture. After 7 days in shelf life, the texture scores for control fruit were significantly lower and fell to the class of poor texture; however the texture scores for RA preceded by ILOS, CA preceded by ILOS, CA and DCA were still of acceptable texture.

After 14 days shelf life and 7 months cold storage, RA and RA preceded by ILOS treated fruit had texture scores of 2 which are graded as of poor texture. The fruit stored in CA, CA preceded by ILOS and ILOS had significantly higher texture scores after 14 days of the 7 months cold storage period and the scores were still of acceptable texture. The DCA, CA and CA preceded by ILOS treatments maintained texture scores at acceptable levels for human consumption after long term storage and shelf life. Echeverrial et al. (2004) reported similar findings on ‘Fuji’ apples. According to Chen and Opara (2013) texture is one of the key quality attributes used in the fresh and processed food industry to assess product quality and acceptability.

#### *3.3.3. Taste scores of optimally mature ‘Granny Smith’ apples after cold storage*

There was significant interaction of the main effects of texture scores of ‘Granny Smith’ apples harvested at optimal maturity during the 2012 harvest season $Pr>F$ = <0.0001 (Table 11). Taste score increased from 0 day to 7 days after 2 months in cold storage irrespective of storage treatment applied. The similar trend was observed on DCA, CA preceded by ILOS, RA and RA preceded by ILOS treated fruit from 7 to 14 days shelf life. At 2 months storage period the fruit had acceptable taste scores of 4-6 irrespective of storage treatment applied except for CA preceded by ILOS after 7 days in shelf life.

After 3 months in storage the taste scores for ILOS treated fruit both CA preceded by ILOS and RA preceded by ILOS had the same taste scores after 0 day and after 7 and 14 days shelf life, and the scores were of acceptable taste scores of 4-6 (Table 11). The taste scores for DCA and CA treated fruit after 3 months in storage increased from 0 (6.0) to 7 (6.5) days, and after 7 days the scores were of excellent taste. However after 14 days shelf life the DCA fruit had the lower score of 6 which rates as acceptable but the CA fruit had the excellent score of 6.25.

After 5 months in cold storage the taste scores of the 'Granny Smith' apples were not significantly different irrespective of treatment applied during storage, and the score was 6 which are acceptable taste score (Table 11). However, after 7 days in shelf life the taste scores fell to unacceptable taste RA and RA preceded by ILOS fruit, though it remained acceptable on DCA, CA and CA preceded by ILOS fruit. Similar trend was observed after 14 days shelf life. The trends observed during the 5 months storage period were observed on the 7 months storage period.

## 4. Conclusions

DCA treatment effectively controlled superficial scald on pre-optimal and optimally mature 'Granny Smith' apples for up to 7 months. Pre-optimally harvested 'Granny Smith' apples are more prone to superficial scald than optimally mature fruit. Incidence and severity of superficial scald may vary from season to season. DCA offers a promising alternative to the use of DPA for South African exporters. CA preceded by ILOS and CA treatments effectively controlled superficial scald on 'Granny Smith' apples for up to 3 months only. Superficial scald increases from 0 to 7 days at shelf life conditions. When storage period is extended (7 months) symptoms of superficial scald appeared during cold storage in RA and RA preceded by ILOS treated fruit. CA and CA preceded by ILOS treatments offered no control of superficial scald on 'Granny Smith' apples.

DCA and CA retained firmness better than ILOS treated fruit. ILOS followed by CA does not retain firmness better than CA on 'Granny Smith' apples. 'Granny Smith' apples stored in RA were firmer than RA preceded by ILOS fruit. 'Granny Smith' apples retain firmness during storage and none of the treatment had the firmness retention falling below 50 N which is the minimum acceptable limit after storage (Little and Holmes, 2000). In conclusion DCA treatment offers an alternative non-chemical control to superficial scald for South African growers and exporters of 'Granny Smith' apples. The end of storage life is mostly identified using presence of physiological disorders by marketers, retailers and consumers and will not accept wastage in excess of 5% to 10% (Little and Holmes, 2000).

‘Granny Smith’ apples affected with superficial scald had the lowest appearance score due to visible blemishes on the fruit peel. DCA treated fruit had no superficial scald and had the highest score at the end of storage and at the end of shelf life. Taste scores increased with increased shelf life, the score increased significantly in all treatments from 0 to 7 days, due to intensification of flavours thereby increasing taste scores. Texture decreased with time in shelf life; however DCA, CA and CA preceded by ILOS maintained texture scores better than RA preceded by ILOS and RA treatments. Overall DCA, CA and CA preceded by ILOS treatment maintained sensory quality attributes of ‘Granny Smith’ apples (appearance, texture and taste) better than RA preceded by ILOS and RA after long term storage. Similar findings of maintenance of sensory quality attributes by low oxygen storage technologies were reported by Echeverria et al. (2004) on ‘Fuji’ apples.

## 5. References


Calvo, G., Salvador, M.E. & Sanchez, E. (2002). Control of superficial scald in 'Beurre'd Anjou' pears with low oxygen levels. *Acta Horticulturae*, 596, 879-882.

Combrink, J.C., Benic, L.M., Lotze, E. & Truter, A.B. (1994). Integrated management of postharvest fruit quality. *Acta Horticulturae*, 368, 657-666.

Commission Regulation (EU) No. 772/2013 of 8 August 2013. *Official Journal of European Union.*

Chen, L. & Opara, U.L. (2013). Texture measurement approaches in fresh and processed foods-A review. *Food Research International*, 51, 823–835.

Crouch, I. (2003). 1-Methylcyclopropene (SmartFresh™) as an alternative to modified atmosphere and controlled atmosphere storage of apples and pears. *Acta Horticulturae*, 600, 433-436.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2004). A new technology that determines low-oxygen thresholds in controlled atmosphere stored apples. *HortTechnology*, 14, 262-266.

DeLong, J.M., Prange, R.K., Leyte, J.C. & Harrison, P.A. (2007). Chlorophyll fluorescence based low-$O_2$ CA storage of organic 'Cortland' and 'Delicious' apples. *Acta Horticulturae*, 737, 31-37.

Echeverria, G., Lara, I., Fuentes, T., Lopez, M.L., Graell, J & Puy, J. (2004). Assessment of relationships between sensory and instrumental quality of controlled atmosphere stored 'Fuji' apples for multivariate analysis. *Journal of Food Science*, 69, 368-375.

Erkan, M. & Pekmezci, M. (2004). Harvest date influences superficial scald development in 'Granny Smith' apples during long term storage. *Turkish Journal of Agriculture*, 28, 397-403.

Fawole, A.M. (2013). Maturity indexing, pharmacological properties and postharvest performance of pomegranate fruit grown in South Africa. PhD thesis_Stellenbosch University. http://scholar.sun.ac.za.

Ingle, M. (2001). Physiology and biochemistry of superficial scald of apples and pears. *Horticultural Reviews*, 27, 228-261.

Jaeger, S.R., Lund, C.M., Lau, K. & Harker, F.R. (2003). In search of the 'Ideal' pear (*Pyrus* spp.): Results of a multidisciplinary exploration. *Journal of Food Science,* 68, 1108-1117.

Ju, Z. & Curry, E.A. (2002). Effects of 6-methyl-5-hepten-2-one vapour on peel browning of 'Delicious' and 'Granny Smith' apples: open vs. closed system. *Postharvest Biology and Technology*, 25, 265-272.

Kader, A.A. (1989). Mode of action of oxygen and carbon dioxide on postharvest physiology of 'Bartlett' pears. *Acta Horticulturae*, 88, 161-167.

Kader, A.A. (2008). Perspective flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, 88, 1863-1868.

Kvikliene, N., Kviklys, D. & Viskelis, P. (2006). Changes in fruit ripening and storage in the apple cultivar 'Auskis'. *Journal of Fruit and Ornamental Plant Research*, 14, 195-202.

Lennox, C.L., Spotts, R.A. & Booysen, M.A. (2004). Incidence of decay of 'D Anjou' pear and control with a thiabendazole drench. *Plant Disease*, 88, 474-478.

Little, C.R. & Holmes, R.J. (2000). Controlled atmosphere storage. In *Storage technology for apples and pears: A guide to production, postharvest treatment and storage of pome fruit in Australia* (pp. 420-515). Knoxfield: Highway Press Pty Ltd.

Lotze, E., van der Merwe, K. & Rogalska, S. (2003). *Manual identification of post-harvest disorders of pome and stone fruit*. ARC Infruitec-Nietvoorbij.

Lurie, S. & Watkins, B. (2012). Superficial scald etiology and control. *Postharvest Biology and Technology*, 65, 44-60.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J., Nieuwoudt, H., Mouazen, AM., Saeys, W. & Nicolai, B.M. (2012). Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. *Postharvest Biology and Technology*, 74, 1-10.

Mitcham, E.J. & Elkins, R.B. (2007). *Pear production and handling manual* (p. 159). University of California Agriculture and Natural Resources Publication 3483.

PPECB, 2014. Annual Report 2013/2014. The Perishable Products Export Control Board retrieved on the 22nd of October 2015. www.ppecb.com.

Robatscher, P., Eisenstecken, D., Sacco, F., Pohl, H., Berger, J., Zanella, A. & Oberhuber, M. (2012). Diphenylamine residues in apples caused by contamination in fruit storage facilities. *Journal of Agricultural and Food Chemistry,* 60, 2205-2211.

Thompson, A.K. (2003). *Disease control in fruit and vegetables harvesting, handling and storage* (2nd ed., pp. 71-85). Kent: Blackwell Publishing.

Van der Merwe, J.A. (1996). Maturity standards for deciduous fruit. In Combrink, J.C (Eds.) *Integrated management of postharvest quality* (pp. 25-32). ARC Infruitec-Nietvoorbij: Paarl Printing.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae,* 600, 261-265.

Van der Merwe, J.A. (2013). Chroma meter technology provides accuracy. Postharvest Innovation Symposium Proceedings 19-21 November 2013, (unpublished poster).

Wang, Z. & Dilley, D.R. (2000). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology*, 18, 201-213.

Watkins, C.B., Bramlage W.J. & Cregoe, B.A. (1995). Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *Journal of American Society for Horticultural Science,* 120, 88-94.

Wills, R.B.H., McGlasson, W.B., Graham, D., Lee, T.H. & Hall, E.G. (1989). *Postharvest: An introduction to the physiology and handling of fruit and vegetables* (5th edn, p. 78). University of New South Wales Press.

Zanella, A., Cazzanelli, P., Panarese, A., Coser, M., Cecchinel, M. & Rossi, O. (2005). Fruit fluorescence response to low oxygen stress: Modern storage technologies compared to 1-MCP treatment of apple. *Acta Horticulturae*, 682, 1535-1542.

Zanella, A., Cazzanelli, P. & Rossi, O. (2008). Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apple. *Acta Horticulturae*, 796, 77-88.

Zanella, A. & Strüz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10$^{th}$ of EU's apples producing area. *Acta Horticulturae,* 1012, 419-426.

Table 1. Maturity indices for ‘Granny Smith’ apples harvested for the growing seasons of 2012 according to the standard maturity indices used in South Africa (van der Merwe, 1996).

| Maturity indices | Fruit maturity | | |
|---|---|---|---|
| | Standard | Pre-optimal | Optimal |
| Firmness (N) | 73.5 | 79.05 | 81.03 |
| Skin colour | 1.9 | 1.32 | 1.38 |
| TSS (Brix) | 11.7 | 10.67 | 11.8 |
| TTA (% malic acid) | 0.71 | 1.56 | 1.5 |
| TSS/TTA ratio | 16.48 | 6.84 | 7.86 |

Table 2. $O_2$ and $CO_2$ concentrations associated with storage treatment during or before storage of ‘Granny Smith’ apples.

| Storage treatment | Gas composition (%) | |
|---|---|---|
| | % $O_2$ | % $CO_2$ |
| DCA | 0.5 | 1 |
| CA | 1.5 | 2.5 |
| RA (control) | 20.95 | 0-1 |
| ILOS+CA | 0.5 | 2.5 |
| ILOS+RA | 0.5 | 0-1 |

Note: DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, For ILOS treatments, the gas composition refers only to the initial low gas stress condition and not the subsequent CA or RA applied.

Table 3. Breakdown of storage periods (months) for 'Granny Smith' apples on applied treatments during storage into pre-conditioning (days), treatment and simulated shipment and handling (weeks) periods.

| Storage treatment | Storage period (months) | Breakdown of storage period | | |
|---|---|---|---|---|
| | | Preconditioning (days) | Treatment (weeks) (-0.5 °C) | Shipment & handling simulation (RA) (20 °C) |
| DCA | 2 | None | 10d* | 8w** |
| | 3 | | 6w * | 6w** |
| | 5 | | 16w * | 6w** |
| | 7 | | 24w* | 6w** |
| CA | 2 | None | 10d* | 8w** |
| | 3 | | 6w* | 6w** |
| | 5 | | 16w* | 6w** |
| | 7 | | 24w* | 6w** |
| Control (RA) | 2 | None | | 8w** |
| | 3 | | | 12w** |
| | 5 | | | 22w** |
| | 7 | | | 30w** |
| ILOS+CA | 2 | 10d* | 8w* | |
| | 3 | 10d* | 6w* | 6w** |
| | 5 | 10d* | 16w* | 6w** |
| | 7 | 10d* | 24w* | 6w** |
| ILOS+RA | 2 | 10d* | | 8w** |
| | 3 | 10d* | | 12w** |
| | 5 | 10d* | | 22w** |
| | 7 | 10d* | | 30w** |

Note: ILOS is a preconditioning once off 10d treatment, * Treatment period, ** Shipment and handling period, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 4. Effect of different storage treatments on scald severity index (SI) of pre-optimal and optimally mature 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.77±0.06 | 0 |
| | 14 | 0 | 0.04±0.03 | 0 | 0 | 0 | 0 | 0 | 0 | 1.27±0.25 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 1.02±0.04 | 0.71±0.10 | 0 | 0 | 0 | 0 | 0 | 0 | 1.82±0.36 | 1.03±0.08 |
| | 14 | 1.19±0.14 | 1.31±0.09 | 0 | 0 | 0 | 0 | 0 | 0 | 2.19±0.30 | 1.70±0.61 |
| 5 | 0 | 1.04±0.39 | 1.15±0.33 | 0 | 0 | 0 | 0 | 0 | 0 | 1.99±0.22 | 0.56±0.18 |
| | 7 | 2.99±0.14 | 3.62±0.26 | 0.03±0.04 | 0.75±0.11 | 0 | 0 | 0.39±0.12 | 0.81±0.05 | 2.91±0.23 | 2.43±0.12 |
| | 14 | 3.16±0.11 | 3.81±0.29 | 8.71±0.84 | 2.29±0.04 | 0 | 0 | 0.18±0.09 | 1.62±0.08 | 3.15±0.15 | 3.26±0.35 |
| 7 | 0 | 2.90±0.16 | 3.60±0.37 | 0 | 0.05±0.04 | 0 | 0 | 0 | 1.00±0.00 | 3.38±0.46 | 3.77±0.10 |
| | 7 | 3.70±0.07 | 3.87±0.11 | 5.45±3.87 | 0.83±0.20 | 0 | 0 | 0.32±0.07 | 1.13±0.34 | 3.88±0.05 | 3.34±0.16 |
| | 14 | 3.92±0.07 | 3.93±0.03 | 10.49±2.92 | 0.40±0.22 | 0 | 0 | 1.41±0.30 | 1.04±0.52 | 3.39±0.15 | 2.95±0.36 |

*Significance level Pr>F*

| | Pre-optimal | Optimal |
|---|---|---|
| *Treatment* | *<0.0001* | *<0.0001* |
| *Period* | *<0.0001* | *<0.0001* |
| *Days* | *0.0770* | *<0.0001* |
| *T X P X D* | *<0.0001* | *<0.0001* |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. SI = scald index (0 = no scald; 4 = extreme scald). T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 5. Effect of different storage treatments on scald severity index (SI) of pre-optimal and optimally mature 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2013 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Storage period (months) | Shelf life (days) | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| | | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt | Pre-opt | Opt |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.92±0.57 | 0.99±0.71 |
| | 14 | 0.02±0.01 | 0.01±0.01 | 0.08±0.04 | 0 | 0 | 0 | 0.03±0.03 | 0.020.03 | 1.46±0.63 | 1.43±0.79 |
| 5 | 0 | 0.85±0.09 | 0.99±0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 1.62±0.53 | 1.17±0.06 |
| | 7 | 2.85±0.08 | 3.42±0.26 | 0.14±0.07 | 0.94±0.08 | 0 | 0 | 0.44±0.14 | 0.68±0.12 | 3.83±0.12 | 2.89±0.74 |
| | 14 | 2.74±0.45 | 3.76±0.07 | 0.21±0.04 | 1.18±0.31 | 0 | 0 | 0.57±0.18 | 0.99±0.02 | 4.00±0.00 | 3.87±0.04 |
| *Significance level Pr>F* | | | | | | | | | | | |
| | Pre-optimal | Optimal | | | | | | | | | |
| *Treatment* | *<0.0001* | *<0.0001* | | | | | | | | | |
| *Period* | *<0.0001* | *<0.0001* | | | | | | | | | |
| *Days* | *<0.0001* | *<0.0001* | | | | | | | | | |
| *TXPXD* | *<0.0001* | *<0.0001* | | | | | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. SI = scald index (0 = no scald; 4 = extreme scald). T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 6. Effect of different storage treatments on firmness (N) retention of pre-optimally harvested ‘Granny Smith’ apples after designated post storage and after 7 days shelf life conditions during the 2012 harvest season.

| | Storage treatment | | | | |
|---|---|---|---|---|---|
| Storage period (months) | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 75.41 $^{f}$ | 79.98 $^{c\text{-}e}$ | 80.86 b-d | 80.82 b-d | 78.28 e |
| 3 | 75.44 $^{f}$ | 80.58 $^{b\text{-}e}$ | 81.99 b-d | 82.42 bc | 74.87 f |
| 5 | 65.03 $^{g}$ | 79.92 de | 82.75 b | 85.56 a | 61.35 h |
| 7 | 60.42 $^{h}$ | 80.65 b-e | 87.44 a | 85.52 a | 56.40 i |
| *Significance level Pr>F* | | | | | |
| *Treatment* | *<0.0001* | | | | |
| *Period* | *<0.0001* | | | | |
| *T X P* | *<0.0001* | | | | |
| *T X D* | *<0.0001* | | | | |
| *P X D* | *<0.0001* | | | | |
| *T X P X D* | *0.7285* | | | | |
| *LSD* $_{(5\%)}$ | *1.97* | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by Initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD’s test).

Table 7. Effect of different storage treatments on skin colour of optimally harvested 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| | | Score | Colour | Score | Colour | Score | Colour | Score | Colour | Score | Colour |
| 2 | 0 | 1.63 $^{xy}$ | GGY | 1.58 $^{z}$ | GGY | 1.45 z | GGY | 1.45 $^{z}$ | GGY | 1.44 $^{z}$ | GGY |
| | 7 | 1.79 $^{wx}$ | GGY | 1.82 $^{w}$ | GGY | 1.71 $^{w\text{-}y}$ | GGY | 1.64 $^{xy}$ | GGY | 1.58 $^{yz}$ | GGY |
| | 14 | 2.93 $^{d\text{-}f}$ | GY | 3.10 $^{bc}$ | GYY | 2.91 $^{ef}$ | GY | 2.83 $^{f\text{-}i}$ | GY | 3.02 $^{c\text{-}e}$ | GYY |
| 3 | 0 | 2.69 $^{i\text{-}n}$ | GY | 2.64 $^{l\text{-}o}$ | GY | 2.48 $^{p\text{-}s}$ | GY | 2.43 $^{q\text{-}t}$ | GY | 2.47 $^{p\text{-}t}$ | GY |
| | 7 | 2.82 $^{f\text{-}k}$ | GY | 2.71 $^{h\text{-}n}$ | GY | 2.66 $^{k\text{-}m}$ | GY | 2.43 $^{q\text{-}t}$ | GY | 2.42 $^{q\text{-}t}$ | GY |
| | 14 | 2.79 $^{f\text{-}k}$ | GY | 2.80 $^{f\text{-}k}$ | GY | 2.66 $^{k\text{-}m}$ | GY | 2.85 $^{f\text{-}i}$ | GY | 2.8 $^{f\text{-}l}$ | GY |
| 5 | 0 | 2.41 $^{r\text{-}v}$ | GY | 2.33 $^{s\text{-}u}$ | GY | 2.17 $^{uv}$ | GY | 2.12 $^{v}$ | GY | 2.20 $^{uv}$ | GY |
| | 7 | 2.73 $^{g\text{-}m}$ | GY | 2.39 $^{s\text{-}t}$ | GY | 2.31 $^{u\text{-}t}$ | GY | 2.40 $^{q\text{-}t}$ | GY | 2.55 $^{n\text{-}q}$ | GY |
| | 14 | 3.19 $^{ab}$ | GYY | 2.87 $^{e\text{-}g}$ | GY | 2.78 $^{f\text{-}k}$ | GY | 2.83 $^{f\text{-}i}$ | GY | 3.09 $^{b\text{-}d}$ | GYY |
| 7 | 0 | 2.60 $^{m\text{-}q}$ | GY | 2.32 $^{s\text{-}u}$ | GY | 2.12 $^{v}$ | GY | 2.19 $^{v}$ | GY | 2.49 $^{o\text{-}r}$ | GY |
| | 7 | 2.91 $^{ef}$ | GY | 2.44 $^{q\text{-}t}$ | GY | 2.38 $^{r\text{-}t}$ | GY | 2.40 $^{q\text{-}t}$ | GY | 2.42 $^{q\text{-}t}$ | GY |
| | 14 | 3.27 $^{a}$ | GYY | 2.79 $^{f\text{-}k}$ | GY | 2.85 $^{f\text{-}i}$ | GY | 2.67 $^{j\text{-}n}$ | GY | 2.86 $^{e\text{-}h}$ | GY |

| *Significance level Pr>F* | |
|---|---|
| *Treatment* | *<0.0001* |
| *Period* | *<0.0001* |
| *Shelf life* | *<0.0001* |
| *T X P* | *<0.0001* |
| *T X D* | *<0.0001* |
| *P X D* | *0.4674* |
| *T X P X D* | *0.0003* |
| *LSD $_{(5\%)}$* | *0.1626* |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. GGY=green green yellow (unacceptable), GY=green yellow (acceptable) and GYY=green yellow yellow (unacceptable). T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 8. Effect of different storage treatments on total soluble solids (°Brix) and titratable acidity (% malic acid) of optimally mature 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| Storage period (months) | Shelf life (days) | TSS | TTA | TSS | TTA | TSS | TTA | TSS | TTA | TSS | TTA |
| 2 | 0 | 14.17 $^{b-d}$ | 1.30 $^{c-j}$ | 13.73 $^{d-j}$ | 1.44 $^{a-d}$ | 13.77 $^{d-i}$ | 1.30 $^{c-j}$ | 13.37 $^{h-q}$ | 1.53 $^{a}$ | 13.20 $^{j-t}$ | 1.28 $^{e-n}$ |
| | 7 | 13.73 $^{d-j}$ | 1.35 $^{c-h}$ | 14.63 $^{ab}$ | 1.30 $^{c-j}$ | 13.87 $^{d-h}$ | 1.24 $^{g-p}$ | 13.47 $^{f-p}$ | 1.17 $^{j-r}$ | 13.30 $^{h-q}$ | 1.21 $^{h-p}$ |
| | 14 | 13.50 $^{f-o}$ | 1.26 $^{e-n}$ | 13.67 $^{e-l}$ | 1.34 $^{c-h}$ | 13.13 $^{k-t}$ | 1.16 $^{l-r}$ | 12.90 $^{p-u}$ | 1.13 $^{o-t}$ | 12.97 $^{o-u}$ | 1.12 $^{o-u}$ |
| 3 | 0 | 13.97 $^{d-g}$ | 1.48 $^{a-c}$ | 14.67 $^{ab}$ | 1.43 $^{a-e}$ | 13.87 $^{d-h}$ | 1.27 $^{e-n}$ | 13.23 $^{i-q}$ | 1.32 $^{c-h}$ | 13.37 $^{h-q}$ | 1.25 $^{g-p}$ |
| | 7 | 13.60 $^{e-m}$ | 1.20 $^{h-q}$ | 15.13 $^{a}$ | 1.34 $^{c-h}$ | 14.17 $^{b-d}$ | 1.18 $^{i-r}$ | 13.43 $^{g-q}$ | 1.13 $^{o-t}$ | 13.33 $^{h-r}$ | 1.14 $^{m-t}$ |
| | 14 | 13.53 $^{f-o}$ | 1.50 $^{ab}$ | 14.03 $^{c-f}$ | 1.43 $^{a-e}$ | 13.60 $^{e-l}$ | 1.27 $^{e-n}$ | 13.13 $^{k-q}$ | 1.28 $^{e-n}$ | 13.30 $^{h-q}$ | 1.33 $^{c-h}$ |
| 5 | 0 | 13.70 $^{d-k}$ | 1.14 $^{m-t}$ | 14.47 $^{bc}$ | 1.39 $^{a-g}$ | 13.73 $^{d-i}$ | 1.11 $^{p-v}$ | 13.33 $^{h-r}$ | 1.14 $^{m-t}$ | 12.97 $^{o-u}$ | 1.16 $^{k-q}$ |
| | 7 | 13.60 $^{e-m}$ | 1.29 $^{e-n}$ | 13.03 $^{m-t}$ | 1.24 $^{g-p}$ | 13.57 $^{f-n}$ | 0.98 $^{u-w}$ | 13.23 $^{i-q}$ | 1.04 $^{r-w}$ | 12.87 $^{q-u}$ | 0.99 $^{s-x}$ |
| | 14 | 13.67 $^{e-l}$ | 0.96 $^{u-w}$ | 13.80 $^{d-i}$ | 1.04 $^{r-w}$ | 13.60 $^{e-m}$ | 0.94 $^{wx}$ | 13.00 $^{l-t}$ | 0.99 $^{s-x}$ | 12.77 $^{r-u}$ | 0.85 $^{xy}$ |
| 7 | 0 | 13.80 $^{d-i}$ | 1.22 $^{h-p}$ | 14.27 $^{b-d}$ | 1.35 $^{b-h}$ | 13.83 $^{k-t}$ | 1.13 $^{m-t}$ | 13.23 $^{i-q}$ | 1.41 $^{a-f}$ | 12.70 $^{tu}$ | 0.96 $^{u-w}$ |
| | 7 | 13.33 $^{h-r}$ | 0.96 $^{u-w}$ | 12.73 $^{s-u}$ | 1.25 $^{g-p}$ | 14.27 $^{b-d}$ | 0.95 $^{wx}$ | 13.77 $^{d-j}$ | 0.95 $^{wx}$ | 12.90 $^{p-u}$ | 0.84 $^{xy}$ |
| | 14 | 13.43 $^{g-q}$ | 0.88 $^{xy}$ | 12.77 $^{r-u}$ | 1.03 $^{r-w}$ | 13.77 $^{d-j}$ | 0.95 $^{wx}$ | 13.10 $^{n-t}$ | 0.90 $^{w-y}$ | 12.40 $^{u}$ | 0.75 $^{y}$ |

| *Significance level Pr>F* | TSS | TTA |
|---|---|---|
| *Treatment* | *<0.0001* | *<0.0001* |
| *Period* | *<0.0001* | *<0.0001* |
| *Shelf life(days)* | *<0.0001* | *<0.0001* |
| *T X P* | *<0.0001* | *<0.0001* |
| *T X D* | *0.0019* | *<0.0001* |
| *P X D* | *0.0761* | *0.0651* |
| *T X P X D* | *0.4209* | *0.0994* |
| *LSD 5%* | *0.5695* | *0.1577* |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 9. Effect of different storage treatments on sugar to acid ratios of optimally mature 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment ILOS+RA | ILOS+CA | DCA | CA | RA |
|---|---|---|---|---|---|---|
| 2 | 0 | 10.95 $^{k-w}$ | 9.51 $^{u-y}$ | 10.59 $^{o-x}$ | 9.07 $^{yz}$ | 10.32 $^{p-w}$ |
| | 7 | 10.18 $^{r-y}$ | 11.00 $^{j-v}$ | 11.19 $^{j-v}$ | 11.48 $^{j-r}$ | 11.02 $^{j-v}$ |
| | 14 | 10.78 $^{n-x}$ | 10.25 $^{p-x}$ | 11.40 $^{l-t}$ | 11.44 $^{i-r}$ | 11.60 $^{i-q}$ |
| 3 | 0 | 9.42 $^{xy}$ | 10.27 $^{p-x}$ | 10.95 $^{l-x}$ | 10.05 $^{s-y}$ | 10.71 $^{i-q}$ |
| | 7 | 11.34 $^{i-s}$ | 11.32 $^{i-t}$ | 12.06 $^{g-n}$ | 11.86 $^{i-o}$ | 11.70 $^{i-q}$ |
| | 14 | 9.03 $^{yz}$ | 9.85 $^{u-x}$ | 10.73 $^{n-x}$ | 10.27 $^{p-y}$ | 9.99 $^{s-x}$ |
| 5 | 0 | 12.27 $^{g-m}$ | 10.41 $^{o-x}$ | 12.37 $^{g-k}$ | 11.67 $^{i-q}$ | 11.22 $^{j-v}$ |
| | 7 | 10.84 $^{l-x}$ | 10.55 $^{o-x}$ | 13.86 $^{c-f}$ | 12.74 $^{f-i}$ | 13.06 $^{e-h}$ |
| | 14 | 14.23 $^{b-e}$ | 13.23 $^{e-g}$ | 14.44 $^{b-d}$ | 13.18 $^{d-g}$ | 15.08 $^{a-c}$ |
| 7 | 0 | 11.31 $^{i-u}$ | 10.80 $^{m-x}$ | 12.28 $^{g-k}$ | 9.76 $^{v-y}$ | 13.28 $^{d-g}$ |
| | 7 | 13.89 $^{c-f}$ | 9.85 $^{u-y}$ | 15.09 $^{a-c}$ | 14.54 $^{b-d}$ | 13.06 $^{e-h}$ |
| | 14 | 15.04 $^{b-d}$ | 12.46 $^{f-j}$ | 16.17 $^{a}$ | 14.63 $^{b-d}$ | 16.47 $^{a}$ |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | *<0.0001* | | | | | |
| *Period* | *<0.0001* | | | | | |
| *Shelf life (d)* | *<0.0001* | | | | | |
| *T X P* | *<0.0001* | | | | | |
| *T X D* | *0.0579* | | | | | |
| *P X D* | *<0.0001* | | | | | |
| *T X P X D* | *0.0215* | | | | | |
| *LSD 5%* | *1.4682* | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

Table 10. Effect of different storage treatments on appearance scores of optimally mature ‘Granny Smith’ apples after designated post storage intervals and after retailer’s shelf life conditions during the 2012 harvest season.

| Storage period (months) | Shelf life (days) | Storage treatment | | | | |
|---|---|---|---|---|---|---|
| | | ILOS+RA | ILOS+CA | DCA | CA | RA |
| 2 | 0 | 8.00 $^{a}$ | 8.00 $^{a}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ |
| | 7 | 7.00 $^{b}$ | 7.00 $^{b}$ | 6.50 $^{c}$ | 6.50 $^{c}$ | 6.50 $^{c}$ |
| | 14 | 6.33 $^{d}$ | 5.75 $^{g}$ | 5.75 $^{g}$ | 7.00 $^{b}$ | 5.50 $^{h}$ |
| 3 | 0 | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ |
| | 7 | 4.50 $^{i}$ | 6.50 $^{c}$ | 6.00 $^{e}$ | 6.50 $^{c}$ | 4.00 $^{j}$ |
| | 14 | 2.00 $^{l}$ | 5.50 $^{h}$ | 5.92 $^{f}$ | 5.92 $^{f}$ | 2.00 $^{l}$ |
| 5 | 0 | 5.50 $^{h}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 5.50 $^{h}$ |
| | 7 | 2.00 $^{h}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 2.00 $^{l}$ |
| | 14 | 1.00 $^{m}$ | 5.50 $^{h}$ | 7.00 $^{b}$ | 6.00 $^{e}$ | 2.00 $^{l}$ |
| 7 | 0 | 2.00 $^{l}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 3.00 $^{k}$ |
| | 7 | 1.00 $^{m}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 7.00 $^{b}$ | 2.00 $^{l}$ |
| | 14 | 1.00 $^{m}$ | 5.75 $^{g}$ | 6.00 $^{e}$ | 5.75 $^{g}$ | 1.00$^{m}$ |
| *Significance level Pr>F* | | | | | | |
| *Treatment* | *<0.0001* | | | | | |
| *Period* | *<0.0001* | | | | | |
| *Shelf life (days)* | *<0.0001* | | | | | |
| *T X P* | *<0.0001* | | | | | |
| *T X D* | *<0.0001* | | | | | |
| *P X D* | *<0.0001* | | | | | |
| *T X P X D* | *<0.0001* | | | | | |
| *LSD $_{(5\%)}$* | *0.0708* | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. T X P X D = Treatment x storage period x shelf life period. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD’s test).

Table 11. Effect of different storage treatments on taste and texture scores of optimally mature 'Granny Smith' apples after designated post storage intervals and after retailer's shelf life conditions during the 2012 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| Storage period (months) | Shelf life (days) | Taste | Texture | Taste | Texture | Taste | Texture | Taste | Texture | Taste | Texture |
| 2 | 0 | 5.00 $^{f}$ | 7.50 $^{b}$ | 5.00 $^{f}$ | 7.50 $^{b}$ | 5.00 $^{f}$ | 8.00 $^{a}$ | 5.00 $^{f}$ | 8.00 $^{a}$ | 5.00 $^{f}$ | 8.00 $^{a}$ |
| | 7 | 6.00 $^{c}$ | 6.75 $^{d}$ | 6.25 $^{b}$ | 6.50 $^{e}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 5.50 $^{e}$ | 7.00 $^{c}$ | 5.50 $^{e}$ | 7.00 $^{c}$ |
| | 14 | 5.00 $^{f}$ | 6.00 $^{f}$ | 5.50 $^{e}$ | 5.50 $^{g}$ | 5.50 $^{e}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 6.00 $^{f}$ | 4.00 $^{g}$ | 5.00 $^{h}$ |
| 3 | 0 | 6.00 $^{c}$ | 6.50 $^{e}$ | 5.00 $^{f}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 5.75 $^{d}$ | 6.00 $^{f}$ |
| | 7 | 6.00 $^{c}$ | 5.50 $^{g}$ | 5.00 $^{f}$ | 6.00 $^{f}$ | 6.50 $^{a}$ | 6.50 $^{e}$ | 6.50 $^{a}$ | 6.00 $^{f}$ | 6.00 $^{c}$ | 5.00 $^{h}$ |
| | 14 | 6.00 $^{c}$ | 5.00 $^{h}$ | 5.00 $^{f}$ | 6.00 $^{f}$ | 6.00 $^{c}$ | 6.00 $^{f}$ | 6.25 $^{b}$ | 5.00 $^{h}$ | 5.00 $^{f}$ | 5.00 $^{h}$ |
| 5 | 0 | 6.00 $^{c}$ | 6.00 $^{f}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 6.00 $^{f}$ | 6.00 $^{c}$ | 6.00 $^{f}$ |
| | 7 | 3.00 $^{h}$ | 3.00 $^{j}$ | 5.75 $^{d}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 6.00 $^{c}$ | 7.00 $^{c}$ | 2.00 i | 2.00 $^{k}$ |
| | 14 | 3.00 $^{h}$ | 3.00 $^{j}$ | 6.50 $^{d}$ | 6.00 $^{f}$ | 5.50 $^{e}$ | 7.00 $^{c}$ | 5.00 $^{f}$ | 5.50 $^{g}$ | 3.00 $^{h}$ | 2.00 $^{k}$ |
| 7 | 0 | 6.00 $^{c}$ | 5.00 $^{h}$ | 5.50 $^{e}$ | 6.00 $^{f}$ | 5.50 $^{e}$ | 7.00 $^{c}$ | 5.50 $^{e}$ | 6.00 $^{f}$ | 3.00 $^{h}$ | 4.00 $^{i}$ |
| | 7 | 4.00 $^{g}$ | 5.50 $^{g}$ | 6.00 $^{c}$ | 6.50 $^{e}$ | 6.00 $^{c}$ | 6.50 $^{e}$ | 5.50 $^{e}$ | 6.00 $^{f}$ | 2.00 $^{i}$ | 2.00 $^{k}$ |
| | 14 | 2.00 $^{l}$ | 2.00 $^{k}$ | 5.50 $^{e}$ | 5.00 $^{h}$ | 5.75 $^{d}$ | 6.00 $^{f}$ | 5.50 $^{e}$ | 5.50 $^{g}$ | 1.00 $^{j}$ | 2.00 $^{k}$ |

| *Significance level Pr>F* | Taste | Texture |
|---|---|---|
| *Treatment* | *<0.0001* | *<0.0001* |
| *Period* | *<0.0001* | *<0.0001* |
| *Shelf life (days)* | *<0.0001* | *<0.0001* |
| *T X P* | *<0.0001* | *<0.0001* |
| *T X D* | *<0.0001* | *<0.0001* |
| *P X D* | *<0.0001* | *<0.0001* |
| *T X P X D* | *<0.0001* | *<0.0001* |
| *LSD $_{(5\%)}$* | *0* | *0* |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by initial low oxygen stress, ILOS+CA = controlled atmosphere preceded by initial low oxygen stress, DCA = dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere. TXPXD = Treatment x storage period x shelf life. Means with the same storage period and days at 20 °C, followed by the same letter are not sifnificantly different at P<0.05 (LSD's test).

# PAPER 5: EFFECTS OF SCALD SUPPRESSION BY DYNAMIC CONTROLLED ATMOSPHERE AND INITIAL LOW OXYGEN STORAGE TECHNOLOGIES ON CONCENTRATION OF VOLATILES IN 'GRANNY SMITH' APPLES.


## Abstract

'Granny Smith' apples were stored for up to 7 months at 0 °C in initial low oxygen stress and controlled atmosphere technologies during which peel tissue samples for volatile analysis were taken after 2, 3, 5, and 7 months of cold storage. Gas chromatography-mass spectroscopy (GC-MS) was used to assess concentrations of .6-methyl-5-hepten-2-one (MHO) and α-farnesene. The concentration of α-farnesene in the fruit peel were negatively correlated to scald severity ($R^2$ = -0.85 in optimally harvested fruit and $R^2$ = -0.80 in pre-optimally harvested fruit), whereas the concentration of MHO in the fruit peel of 'Granny Smith' apples were positively correlated with scald severity ($R^2$ = 0.85 in optimally mature fruit and $R^2$ = 0.57 in pre-optimally mature fruit). These findings suggest that α-farnesene and its oxidation by-product MHO appear to be implicated in the incidence of superficial scald, and displayed a precursor product relationship.

DCA treatment appears to control superficial scald by suppressing concentrations of MHO and keep α-farnesene concentration high. The findings suggest that DCA inhibits incidence of superficial scald on 'Granny Smith' apples by suppressing autoxidation of α-farnesene to its by-product MHO, hence the MHO concentration was significantly lower in DCA treated fruit than in CA, CA preceded by ILOS, RA and RA preceded by ILOS treatments.


## 1. Introduction

'Granny Smith' is the second most planted apple cultivar in South Africa accounting for approximately 81% of the total apple production of the country (DAFF, 2014). South African 'Granny Smith' apples are stored for extended periods in cold storage making controlled atmosphere an integral part of the export and inland market chain. Export of apples to Europe, Asia and Africa is approximately 38% (171 578 tons), 34% (163 083 tons) and 27% (116 130 tons) respectively (DAFF, 2014). South African export of apples amounted to 42.9% of the world production ranking number 7 in the world. Approximately 5 725 432 tons of apples were produced in the Southern Hemisphere during 2013 and South Africa was the fourth largest producer (14.2% in 2011) of apples in the southern hemisphere after Brazil, Chile and Argentina (DAFF, 2014).

South Africa is the largest exporter of apples in the Southern Hemisphere (28%) followed by New Zealand and Argentina 18.3% and 8.5% respectively. South Africa has a good and strong reputation in well-known established international markets (DAFF, 2014). It is imperative to maintain standards in terms of compliance with required Maximum Residue Limits (MRL) for postharvest pesticides, especially that of diphenylamine (DPA). DPA is used on apples and pears to control superficial scald. Most markets have reduced the allowed MRL of DPA on apples to 0.1 ppm effective April 2014 forcing markets such as South Africa to seek viable non-chemical strategies for the control of superficial scald on its stored susceptible apple cultivars such as ‘Granny Smith’.

Superficial scald occurs as visible blemishing on the fruit peel which may develop during or after cold storage. ‘Granny Smith’ apples are thick skinned apples with a high level of natural wax and the lowest deterioration rate (firmness, skin colour and texture) during storage (Little and Holmes, 2000), however they are susceptible to superficial scald physiological disorder. It has been reported in literature and widely accepted that the volatiles sesquiterpene 3-7-11-trimethyldodecatetraene α-farnesene, and 6-methyl-5-hepten-2-one (MHO) are related to superficial scald development (Anet, 1969; Anet, 1972a; Anet, 1972b).

The sesquiterpene hydrocarbon 3-7-11-trimethyldodecatetraene (α-farnesene) occurs naturally in the coating of apples, pears and quinces in smaller quantities (Anet, 1969). The ketone 6-methyl-5-hepten-2-one (MHO) is the product of the oxidation of the α-farnesene (Anet, 1972a; Wang and Dilley, 2000). Whitaker and Saftner (2000) showed that α-farnesene autoxidise to conjugated trienols which further oxidises to yield MHO. MHO is implicated in the incidence and severity of superficial scald disorder in apples and pears. Sensitivity of fruit to MHO may vary from season to season (Wang and Dilley, 2000), same as sensitivity of fruit to scald development may vary from season to season. Dynamic controlled atmosphere (DCA) inhibits superficial scald incidence by suppressing levels of MHO, thus reducing the oxidation of α-farnesene to MHO in ‘Granny Smith’ apples (Wang and Dilley, 2000).

The aim of this paper was to investigate how α-farnesene and MHO are affected by DCA, CA preceded by ILOS, CA, RA preceded by ILOS and RA storage treatments in suppressing the incidence of superficial scald in ‘Granny Smith’ apples. The objectives of this paper were to investigate:

(1) The effects of ILOS and DCA treatments on MHO and α-farnesene of pre-optimal and optimally harvested 'Granny Smith' apples.

(2) Whether MHO and α-farnesene are implicated in superficial scald incidence and severity of pre-optimally and optimally harvested 'Granny Smith' apples.

## 2. Materials and methods

### 2.1. Fruit and tissue sampling, storage treatments and periods

'Granny Smith' apples were harvested at pre-optimal and optimal on the 28$^{th}$ of March and the 06$^{th}$ of April (2012 and 2013 seasons) maturities from Grabouw production area (34.1500°S, 19.0167°E) in the Western Cape Province, South Africa (Chapter 4 Section 2.1). Fruit were sampled on arrival and transferred to various storage treatments with varying $O_2$ and $CO_2$ combinations as in Chapter 4 section 2.2. Fruit were sampled and assessed at 0, 7 and 14 days shelf life periods at 20 °C following cold storage.

### 2.2. Superficial scald intensity (scald index)

The evaluation and intensity of superficial scald was performed as described in Chapter 4, section 2.3.1.

### 2.3. Gas chromatography-Mass Spectrometry solid phase micro-extraction (GC-MS SPME) analysis

The analysis of volatiles using GCMS SPME was performed on 'Granny Smith' apple peel tissue as described for the 'Packham's Triumph' pears in Chapter 3, Section 2.4.

### 2.4. Statistical analysis and trial design

The trial design was a completely randomised 3 way factorial with treatment (storage treatment, storage period and shelf life) as sources of variance. The treatments were replicated three times with 90-120 fruit per replicate. For analysis of volatiles 5 fruit were used per replicate, thus for each treatment 15 fruit were used. Analysis of variance (ANOVA) was performed on all measurements using SAS enterprise Guide 5.1. Separate ANOVA's were also performed for each storage and shelf life combinations. Student-Newman-Keuls

(SNK) was used at a 5% significance level for means comparisons. Pearson correlations were also performed.

## 3. Results and discussions

### 3.1. Effect of treatments on α-farnesene in the fruit peel of optimally harvested 'Granny Smith' apples

The α-farnesene concentrations in the peels of 'Granny Smith' apples stored of RA preceded by ILOS, CA preceded by ILOS and DCA treatments were not significantly different at 0 day, 7 days and at 14 days in optimally harvested 'Granny Smith' apples following 2 months in cold storage (Table 1). At the same storage period the α-farnesene concentrations in the fruit peel of CA and control treated fruit decreased with increased shelf life period at 7 days and lowest at 14 days. There was, however, no significant difference in abundance of α-farnesene at 0 day and after 7 days in shelf life of CA and control treated fruit. The α-farnesene levels in fruit stored at RA preceded by ILOS, CA preceded by ILOS, CA and control were not significantly different after 7 days and 14 days in optimally harvested 'Granny Smith' apples following 3 months of cold storage during the 2012 season (Table 1). The results in this study confirm that α-farnesene concentrations increase with storage period and then decrease at the end of storage period (Meigh and Filmer, 1969).

There was no significant difference in α-farnesene concentration in the fruit peel of CA preceded by ILOS, DCA, CA and RA preceded by ILOS treated fruit at 0 day, after 7 days and at 14 days following 5 months cold storage (Table 1). The α-farnesene concentration in RA preceded by ILOS treated fruit was higher at 0 day and lower after 7 days, however there was no significant difference between 7 and 14 days after 5 months in cold storage. The α-farnesene concentration decreases with shelf life storage (Bauchot et al., 1999). The α-farnesene concentrations in RA preceded by ILOS treated fruit were not significantly different between 0, 7 and 14 days following 7 months cold storage during the 2012 season (Table 1). The α-farnesene concentrations were higher at 14 days and lowest at 0 day in the fruit peel of CA preceded by ILOS, DCA, CA and RA treated fruit after 7 months of cold storage, contrary to patterns observed in earlier storage intervals (2. 3 and 5 months) (Table 1).

### 3.2. Effect of treatments on the MHO level in the fruit peel of optimally harvested 'Granny Smith' apples

On arrival of the 'Granny Smith' apples MHO was not detected in the fruit peel during the 2013 harvest (data not shown). The volatile α-farnesene was found to be present on the fruit prior to storage confirming that it is a natural occurring compound in the fruit peel. Huelin and Murray (1966) found that α-farnesene was naturally in the coating of apples. Similar results were reported by Ju and Curry (2002) on scald susceptible 'Delicious' and 'Granny Smith', and scald resistant 'Fuji' and 'Gala' apple cultivars. These findings further support the theory that MHO is by-product of the autoxidation of α-farnesene and this process is perhaps triggered by cold storage hence it has been reported that superficial scald is a chilling injury (Watkins et al, 1995). There was no significant difference in the concentration of MHO in 'Granny Smith apples treated with DCA, RA preceded by ILOS and CA preceded by ILOS after 2 months at 0 day, followed by 7 and 14 days shelf life (Table 1). At the same period CA and RA fruit had the lowest MHO concentrations at 14 days with RA having the lowest. There was no superficial scald on all the treatments after 2 months in cold storage during the 2012 season (Paper 4 Section 3.1.1).

There were no significant differences in the MHO concentration in the fruit peel of 'Granny Smith' apples for all storage treatments after 2 months storage at 0 day, followed by 7 and 14 days (Table 1). The same trend was observed at 3 months storage period; however the MHO concentrations were the lowest in DCA treated fruit in comparison to all other treatments (CA preceded by ILOS, CA, RA preceded by ILOS and RA). 'Granny Smith' apples treated with DCA showed the lowest concentration of MHO after 7 months in cold storage (Table 1), furthermore compared to other treatments DCA was the only treatment without incidence of superficial scald.

### 3.3. MHO and α-farnesene interaction and involvement with superficial scald severity in the fruit peel of pre-optimally harvested 'Granny Smith' apples

There was no significant difference in α-farnesene concentration in the fruit peel of pre-optimally harvested 'Granny Smith' apples after 7 months in storage (Figure 1) on DCA, CA and CA preceded by ILOS treated fruit. There was no significant difference in α-farnesene levels of RA and RA preceded by ILOS stored fruit and their α-farnesene concentrations were lower. The Pearson correlation was $R^2$ = -0.80 between α-farnesene concentrations in the pre-optimally harvested 'Granny Smith' peel and superficial scald severity index. Treatments which offered control of scald (DCA, CA and CA preceded by ILOS) or had lower superficial scald percentage had higher α-farnesene concentrations that those that had scald (RA preceded by ILOS and RA) which the lowest α-farnesene concentrations.

During the same storage period (7 months) RA stored 'Granny Smith' apples had higher MHO levels than CA preceded by ILOS fruit but not significantly different from DCA, CA and RA preceded by ILOS treated fruit. Pre-optimal harvested 'Granny Smith' apples treated with DCA had higher MHO concentration after 7 months in storage but no superficial scald incidence. This might be attributed to the fact that pre-optimally harvested fruit might be lacking in mechanism to scavenge free radicals resulting from autoxidation of α-farnesene to MHO. It has been reported that superficial scald is a form of chilling injury (Watkins et al., 1995) and therefore membranes in the fruit peel can be destabilised by chilling (Taiz and Zeiger, 2010). Reduced fluidity of membranes at low temperatures cause reduced function in protein components of the membranes and this result in inhibition of many biochemical processes leading to increase in production of reactive oxidation species (ROS). ROS when in excess causes oxidative damage to proteins, lipids, RNA and DNA. ROS generate oxidative stress and destroys cellular and metabolic functions leading to cell death.

Reactive oxygen species (ROS) are balanced by cellular anti-oxidative mechanisms that prevent cellular damage, however during cold storage fruit are exposed to low temperature and the oxidative stress lead to generation of highly reactive oxygen species (Taiz and Zeiger, 2010). The most common ROS in plant cells are superoxide ($O2^{\cdot -}$), singlet ($^1O_2$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^{\cdot}$) (Taiz and Zeiger, 2010). Common antioxidants in fruit peel include water soluble ascorbate (Vitamin C) and the lipid soluble α-tocopherol. Ascorbate plays an important role in detoxifying ROS generated by ozone exposure.

The Pearson correlation was $R^2 = 0.57$ between MHO levels and superficial scald severity index in the pre-optimally harvested 'Granny Smith' apples compared to $R^2 = 0.80$ of MHO concentration in optimally harvested 'Granny Smith' apples. These results suggest that other mechanism is involved in the incidence of superficial scald. Pre-optimally harvested fruit may lack the mechanism to suppress the symptoms of superficial scald due to reduced levels of antioxidants systems that helps with scavenging of free radicals. Naturally occurring anti-oxidants that inhibit oxidation of α-farnesene is lower in pre-optimally harvested fruit than in optimal fruit (von Mollendorff, 1996).

### 3.4. The effect of MHO and α-farnesene on superficial scald severity in the fruit peel of optimally harvested 'Granny Smith' apples

DCA treatment was effective in suppressing the incidence of superficial scald on 'Granny Smith' apples. The effectiveness of DCA treatment in this study confirms findings reported by DeLong et al., (2004) on several apple cultivars ('Cortland', 'Delicious', 'Golden Delicious', 'Honeycrisp', 'Jonagold' and 'McIntosh') and by Zanella and Strüz (2013) on 'Granny Smith' and 'McIntosh' apples cultivars and on 'd'Anjou' pears by Mattheis and Rudell (2011). DCA treatment though effective in suppressing superficial scald on 'Granny Smith' apples, however requires installation of the DCA chlorophyll fluorescence monitor HarvestWatch system (SAtlantic Inc., Halifax., N.S., Canada) (DeLong et al., 2004) in the CA rooms to monitor fruit response.

For both seasons the DCA stored fruit spiked at 0.3 and kept at 0.5 % $O_2$ for the rest of the storage period. CA preceded by ILOS and CA were only effective for up to 2 months followed by 7 days shelf life but developed scald after 14 days on 2013 season (paper 4). CA preceded by ILOS offered no positive results on the control of superficial scald on 'Granny Smith' apples confirming findings by Lau (1997) on 'Starkimson Delicious' apples. However these results are contradictory to findings by Truter et al. (1994) and van der Merwe et al. (2003) which reported control of superficial scald on 'Granny Smith' and 'Top Red' apples for up to 6 months.

Optimally harvested 'Granny Smith' apples, RA preceded by ILOS stored fruit had significantly higher MHO concentration compared to control fruit and the highest severity index of superficial scald after 7 months of cold storage followed by 14 days of shelf life (Figure 2). DCA treated fruit had the lowest MHO concentration in the peel and no superficial scald after 7 months cold storage period followed by 14 days of shelf life at 20 °C (Figure 1). The results in this study suggest that the mode of action of DCA is by suppressing autoxidation of α-farnesene to its toxic by-product MHO. Similar results have been reported by several researchers (Chen et al., 1993; Wang and Dilley, 1999; Isidoro and Almeida, 2006). Pearson correlation between superficial scald severity index and MHO concentrations was $R^2$ = 0.85. Superficial scald incidence is positively correlated to higher MHO concentration in the fruit peel (Mir et al., 1999) during ripening temperature at 22 °C.

The α-farnesene and its autoxidation by-product MHO have a precursor product relationship (Figure 1), when concentration of α-farnesene is higher MHO is lower. Same observations were reported by Whitaker et al., (2009) on ‘Bartlett’ pears grown in California and Washington in the United States. The precursor product relationship of α-farnesene and MHO has been reported by several researchers (Wang and Dilley, 2000; Whitaker et al., 2009). Huelin and Coggiola (1970) suggested that treatments that control superficial scald delay the oxidation of α-farnesene to its toxic end products and DCA seem to suppress the oxidation of α-farnesene. RA preceded by ILOS had the highest MHO concentration and had a similar high incidence of superficial scald physiological disorder.

RA treated ‘Granny Smith’ apples were significantly different from RA preceded by ILOS fruit which had the lowest α-farnesene concentrations after 14 days shelf life at 20 °C following 7 months of cold storage, and the scald index was 3.93 for RA preceded by ILOS and 2.95 for RA fruit (Figure 2). CA preceded by ILOS, DCA and CA treated fruit had the highest α-farnesene concentrations and the levels among treatments were not significantly different after 7 months in storage. There was strong negative correlation for concentrations of α-farnesene and scald index with the Pearson correlation of $R^2$ = -0.85.

## 4. Conclusions

For long term storage of ‘Granny Smith’ apples of pre-optimal and optimal maturities, DCA technology effectively controlled superficial scald up to 7 months. The volatiles MHO and α-farnesene were implicated in the incidence of superficial scald; there was a strong negative correlation between superficial scald and α-farnesene concentration whereas there was strong positive correlation between MHO concentration and superficial scald on ‘Granny Smith’ apples. Storing fruit with DCA controlled superficial scald by, perhaps, suppressing autoxidation of α-farnesene to MHO as evidenced by the appearance of superficial scald on the fruit corresponded with high concentration of α-farnesene and lower concentration of MHO. DCA offers an alternative to the use of DPA as a non-chemical technology to control of superficial scald disorder during long term cold storage on ‘Granny Smith’ apples.

## 5. References

Anet, E.F.J.L. (1969). Autoxidation of α-farnesene. *Australian Journal of Chemistry,* 22, 2403-2410.

Anet, E.F.J.L. (1972[a]). Superficial scald a functional disorder of stored apples. VIII. Volatile products from the autoxidation of α-farnesene. *Journal of the Science of Food and Agriculture*, 23, 605-608.

Anet, E.F.J.L. (1972[b]). Superficial scald a functional disorder of apples. IX. Effect of maturity and ventilation. *Journal of the Science of Food and Agriculture,* 23, 763-769.

Bauchot, A. D., Reid, S. J., Ross, G. S. & Burmeister, D. M. (1999). Induction of apple scald by anaerobiosis has similar characteristics to naturally occurring superficial scald in 'Granny Smith' apple fruit. *Postharvest Biology and Technology*, 16, 9-14.

Chen, P.M., Varga, R.J. & Xiao, Y.Q. (1993). Inhibition of α-farnesene biosynthesis and its oxidation in the peel tissue of 'd'Anjou' pears by low $O_2$/elevated $CO_2$ atmospheres. *Postharvest Biology and Technology,* 3, 215-223.

DAFF, (2014). A profile of the South African apple market value chain retrieved on the 22[nd] of October 2015. www.daff.gov.za.

DeLong, J.M., Prange, R.K., Leyte J.C. & Harrison, P.A. (2004). A new technology that determines low-oxygen thresholds in controlled atmosphere stored apples. *HortTechnology,* 14, 263-266.

Huelin, F.E. & Coggiola, I.M. (1970). Superficial scald, a functional disorder of stored apples. VII. Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration and oxidation of α-farnesene in the fruit. *Journal of the Science of Food and Agriculture*, 21, 584-589.

Huelin, F.E. & Murray, K.E. (1966). α-farnesene in the natural coating of apples. *Nature,* 210, 1260-1261.

Isidoro, N. & Almeida, P.F. (2006). α-farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. *Postharvest Biology and Technology*, 42, 49-56.

Ju, Z. & Curry, A.E. (2002). Effects of 6-methyl-5-hepten-2-one vapour on peel browning of 'Delicious' and 'Granny Smith' apples: open and closed system. *Postharvest Biology and Technology*, 25, 265-272.

Lau, O.L. (1997). Initial low oxygen stress offers no scald control benefits to 'Starkimson Delicious' apples in 0.7 kPa $O_2$ storage. *HortScience,* 32, 1239-1241.

Little, C.R. & Holmes, R.J. (2000). *Controlled atmosphere storage* in storage technology for apples and pears: A guide to production, postharvest treatment and storage of pome fruit in Australia (pp. 420-515). Knoxfield: Highway Press Pty Ltd.

Mattheis, J.P. & Rudell, D. (2011). Responses of 'd'Anjou' pear (*Pyrus communis* L.) fruit to storage at low oxygen set points determined by monitoring fruit chlorophyll fluorescence. *Postharvest Biology and Technology*, 60, 125-129.

Meigh, D.F. & Filmer, A.A.E. (1969). Natural skin coating of the apple and its influence on scald in storage. III. α-farnesene. *Journal of the Science of Food and Agriculture*, 20, 140-143.

Mir, N., Perez, R. & Beaudry, R.M. (1999). A post storage burst of 6-methyl-5-hepten-2-one (MHO) may be related to superficial scald development in 'Cortland' apples. *Journal of American Horticultural Science*, 124, 173-176.

Pesis, E, Feygenberg, O., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J. & Ben-Arie, R. (2014). Low oxygen pre-storage treatment is effective in reducing chilling injuries of deciduous fruit. *International Journal of Postharvest technology and Innovation,* 4, 23-32.

Taiz, L. & Zeiger, E. (2010). *Plant Physiology* ($5^{th}$ edn, pp. 764-777). Sunderland Massachusets U.S.A.: Sinauer Associates, Incorporated.

Truter, A.B., Combrink, J.C. & Burger, S.A. (1994). Control of superficial scald in 'Granny Smith' apples by ultra-low and stress levels of oxygen as an alternative to diphenylamine. *Journal of Horticultural Science,* 69, 581-587.

Van der Merwe, J.A, Combrink, J.C. & Calitz, F.J. (2003). Effect of controlled atmosphere storage after initial low oxygen stress treatment on superficial scald development on

South African grown 'Granny Smith' and 'Top Red' apples. *Acta Horticulturae,* 600, 261-265.

Von Mollendorff, L.J. (1996). Development of physiological post-harvest disorders. In Combrink J.C. (Ed.) *Integrated management of postharvest quality* (p.41). ARC INFRUITEC: Paarl Printing, Paarl.

Wang, Z. & Dilley, D.R. (1999). Controls superficial scald of apples by low oxygen atmospheres. *HortScience,* 34, 1145-1151.

Wang, Z. & Dilley, D.R. (2000). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology,* 18, 201-213.

Watkins, C.B., Bramlage, W.J. & Cregoe, B.A (1995). Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *Journal of American Society of Horticultural Science,* 120, 88-94.

Whitaker, B.D. & Saftner, R.A. (2000). Temperature dependant autoxidation of conjugated trienols from apple peel yields 6-methyl-5-hepten-2-one, a volatile implicated in induction of superficial scald. *Journal of Agriculture, Food and Chemistry,* 48, 2014-2043.

Whitaker, B.D., Villalobos-Acuña, M., Mitcham, J.E. & Mattheis, J.P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology,* 53, 43-50.

Zanella, A. & Strüz, S. (2013). Replacing DPA postharvest treatment by strategical application of novel storage technologies controls scald in 1/10$^{th}$ of EU's apples producing area. *Acta Horticulturae,* 1012, 419-426.

Table 1. Effect of different storage treatments on the α-farnesene abundance in the fruit peel of optimally harvested 'Granny Smith' apples after designated storage periods and after retailer's shelf life conditions during the 2012 harvest season.

| | | Storage treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ILOS+RA | | ILOS+CA | | DCA | | CA | | RA | |
| Storage period (months) | Shelf life (days) | α-farnesene | MHO | α-farnesene | MHO | α-farnesene | MHO | α-farnesene | MHO | α-farnesene | MHO |
| 2 | 0 | 8.72 $^{a-h}$ | 5.75 $^{m-t}$ | 8.73 $^{a-g}$ | 5.59 $^{o-u}$ | 8.71 $^{a-h}$ | 5.65 $^{o-u}$ | 8.80 $^{a-d}$ | 5.74 $^{cx}$ | 8.82 $^{a-c}$ | 5.58 $^{p-u}$ |
| | 7 | 8.70 $^{a-i}$ | 5.33 $^{t-v}$ | 8.81 $^{a-c}$ | 5.30 $^{t-v}$ | 8.78 $^{a-e}$ | 5.37 $^{s-v}$ | 8.83 $^{a-c}$ | 5.47 $^{r-v}$ | 8.76 $^{a-f}$ | 5.37 $^{s-v}$ |
| | 14 | 8.77 $^{a-e}$ | 5.44 $^{r-v}$ | 8.74 $^{a-g}$ | 5.45 $^{r-v}$ | 8.72 $^{a-g}$ | 5.38 $^{s-v}$ | 8.37 $^{e-k}$ | 4.86 $^{w}$ | 5.74 $^{e-k}$ | 4.21 $^{x}$ |
| 3 | 0 | 8.64 $^{a-j}$ | 5.83 $^{l-s}$ | 8.66 $^{a-j}$ | 5.55 $^{q-u}$ | 8.53 $^{a-k}$ | 5.19 $^{u-w}$ | 8.61 $^{a-j}$ | 5.48 $^{r-v}$ | 8.75 $^{a-j}$ | 5.55 $^{r-v}$ |
| | 7 | 8.51 $^{b-k}$ | 5.31 $^{t-v}$ | 8.54 $^{a-k}$ | 5.05 $^{vw}$ | 8.53 $^{a-k}$ | 4.86 $^{u-w}$ | 8.51 $^{b-k}$ | 5.30 $^{t-v}$ | 8.54 $^{a-k}$ | 5.67 $^{n-u}$ |
| | 14 | 8.38 $^{e-k}$ | 5.54 $^{r-u}$ | 8.30 $^{h-l}$ | 5.19 $^{u-w}$ | 8.33 $^{g-l}$ | 5.26 $^{u-w}$ | 8.36 $^{e-k}$ | 5.25 $^{u-w}$ | 8.39 $^{d-k}$ | 5.48 $^{r-v}$ |
| 5 | 0 | 8.73 $^{a-g}$ | 7.02 $^{a-c}$ | 8.73 $^{a-g}$ | 6.82 $^{b-d}$ | 8.65 $^{a-j}$ | 6.03 $^{j-o}$ | 8.67 $^{a-j}$ | 7.09 $^{ab}$ | 8.75 $^{a-g}$ | 7.09 $^{ab}$ |
| | 7 | 8.29 $^{i-l}$ | 7.01 $^{a-c}$ | 8.81 $^{a-c}$ | 6.38 $^{e-j}$ | 8.63 $^{a-j}$ | 5.89 $^{l-r}$ | 8.60 $^{a-i}$ | 6.80 $^{b-d}$ | 8.54 $^{a-k}$ | 6.82 $^{b-d}$ |
| | 14 | 8.45 $^{c-k}$ | 6.79 $^{b-e}$ | 8.58 $^{a-k}$ | 6.28 $^{f-k}$ | 8.58 $^{a-k}$ | 5.63 $^{o-u}$ | 8.59 $^{a-j}$ | 6.18 $^{g-m}$ | 8.39 $^{d-k}$ | 6.60 $^{c-g}$ |
| 7 | 0 | 8.34 $^{f-k}$ | 6.69 $^{b-f}$ | 8.26 $^{j-l}$ | 6.23 $^{h-l}$ | 8.14 $^{l}$ | 5.24 $^{u-w}$ | 8.34 $^{e-k}$ | 6.29 $^{f-k}$ | 8.20 $^{kl}$ | 6.79 $^{b-e}$ |
| | 7 | 8.39 $^{e-k}$ | 6.46 $^{d-i}$ | 8.70 $^{a-i}$ | 6.15 $^{h-l}$ | 8.69 $^{a-i}$ | 5.76 $^{m-t}$ | 8.69 $^{a-i}$ | 6.09 $^{i-n}$ | 8.62 $^{a-i}$ | 6.01 $^{j-o}$ |
| | 14 | 8.62 $^{a-j}$ | 7.25 $^{a}$ | 8.86 $^{ab}$ | 6.52 $^{d-i}$ | 8.89 $^{ab}$ | 6.11 $^{i-m}$ | 8.95 $^{a}$ | 6.55 $^{d-h}$ | 8.71 $^{a-h}$ | 6.60 $^{c-f}$ |
| *Significance level Pr>F* | | | | | | | | | | | |
| | | *α-farnesene* | *MHO* | | | | | | | | |
| *Treatment* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *Period* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *Shelf life (days)* | | *0.3092* | *<0.0001* | | | | | | | | |
| *T X D* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *P X D* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *T X P* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *TXPXD* | | *<0.0001* | *<0.0001* | | | | | | | | |
| *LSD (5%)* | | *2.98* | *2.98* | | | | | | | | |

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by ILOS, ILOS+CA = controlled atmosphere preceded by ILOS, DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere.

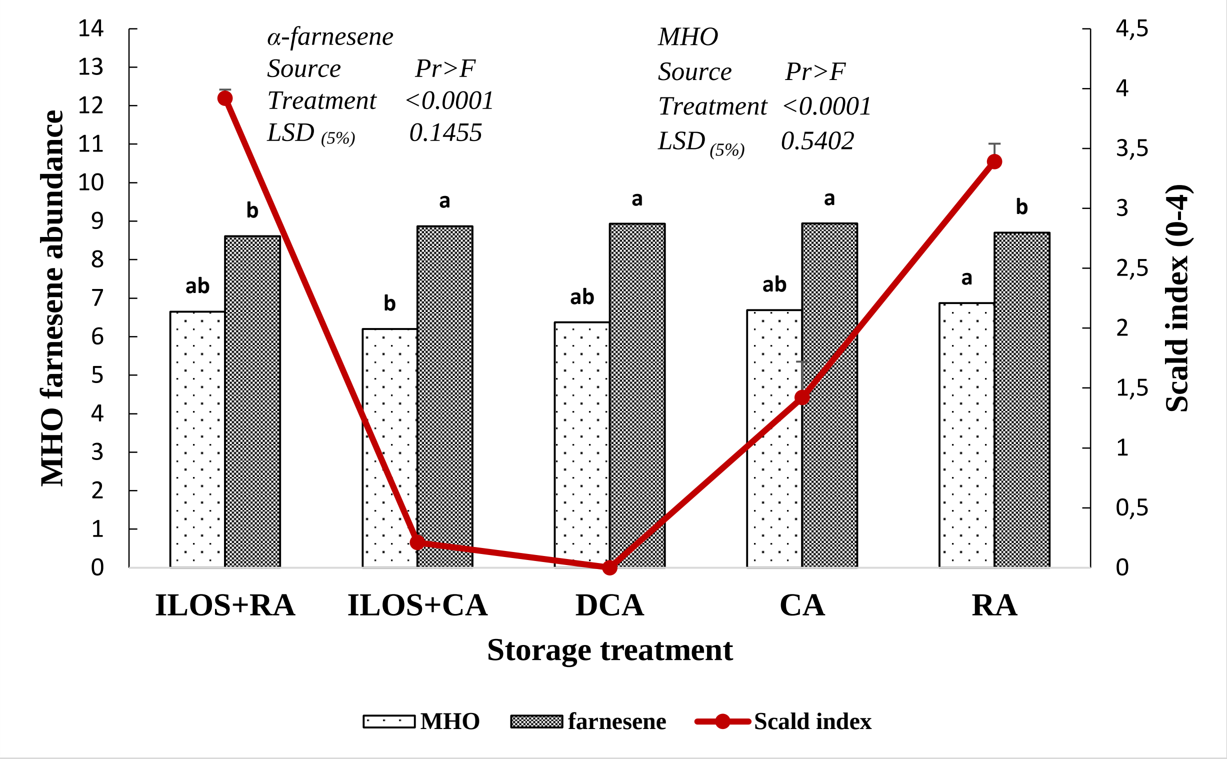


Figure 1. Effect of storage treatments on relationship of α-farnesene, MHO and scald severity index of pre-optimally harvested 'Granny Smith' apples at the end of shelf life (14 days) after 7 months cold storage period during the 2012 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = regular atmosphere preceded by ILOS, ILOS+CA = controlled atmosphere preceded by ILOS, DCA = Dynamic controlled atmosphere, CA = controlled atmosphere, RA = regular atmosphere.

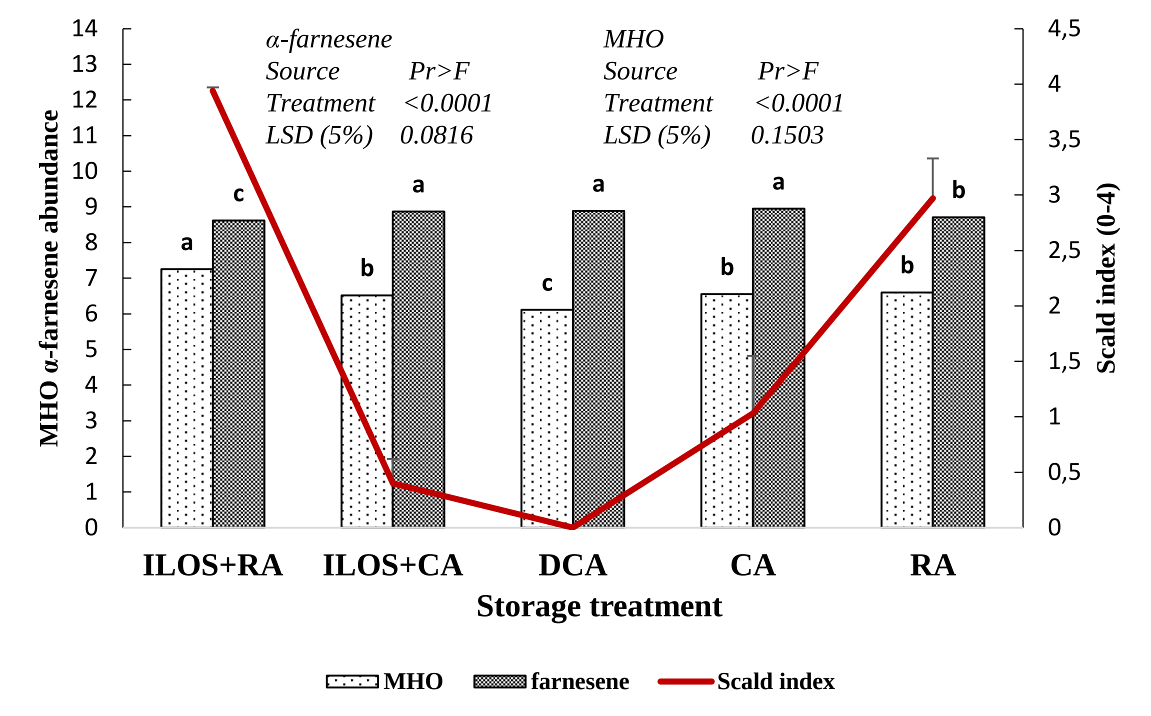


Figure 2. Effect of storage treatments on relationship of α-farnesene, MHO and scald severity index of optimally harvested ‘Granny Smith’ apples at the end of shelf life (14 days) after 5 months cold storage period during the 2013 harvest season.

Note: ILOS is a preconditioning once off 10d treatment, ILOS+RA = Regular atmosphere preceded by ILOS, ILOS+CA = Controlled atmosphere preceded by initial low oxygen stress, DCA = Dynamic controlled atmosphere, CA = Controlled atmosphere, RA = Regular atmosphere.

## GENERAL SUMMARY AND CONCLUSION

The research work in this thesis has been presented in five research papers covering the effects of the following five storage treatments:

- Dynamic controlled atmosphere (DCA)
- Controlled atmosphere preceded by initial low oxygen stress (ILOS+CA)
- Controlled atmosphere (CA)
- Regular atmosphere (RA) preceded by ILOS (ILOS+RA)
- RA as a control as alternative strategies for the control of superficial scald on ‘Packham’s Triumph’ pears and ‘Granny Smith’ apples.

The overall findings are summarised below:

**Research paper 1**

DCA treatment offers a promising alternative to the use of DPA as a long term storage scald inhibitor on ‘Packham’s Triumph’ pears. DCA treatment effectively suppressed superficial scald symptoms on ‘Packham’s Triumph’ pears for up to 7 months. Lafer (2011) reported similar findings on organically grown ‘Uta’ pears. CA preceded by ILOS effectively controlled superficial scald on ‘Packham’s Triumph’ pears up to 5 months in storage, thus offers an alternative as a short term treatment to inhibit superficial scald on ‘Packham’s Triumph’ pears without the use of DPA. These findings corroborate findings by Calvo et al., (2002) on ‘Beurre’d Anjou’ pears. CA effectively controlled superficial scald for 3 months only on ‘Packham’s Triumph’ pears. The onset and intensity of superficial scald in pome fruit may vary from season to season. The superficial scald symptoms may appear after transfer to warmer temperatures. When storage period is extended (7 months) symptoms of superficial scald may appear during storage on ‘Packham’s Triumph’ pears.

‘Packham’s Triumph’ pears appeared to be prone to shrivelling disorder. Fruit from all treatments (DCA, CA preceded by ILOS, CA, RA preceded by ILOS and RA) had neck shrivelling after 7 months in cold storage. Neck shrivelling due to moisture loss may be reduced by use of internal packaging (Watkins, 2008; Berry, 2013), edible coatings such as kafirin (Buchner, 2006), sealing of the fruit stem (Burger, 2005) and/or humidifying the storage room (Mitcham and Mitchel, 2002) to prevent loss of moisture from the fruit.

DCA treatment suppressed decay in ‘Packham’s Triumph’ pears better than short term storage of fruit under ILOS followed by long term CA storage. Development of superficial scald started from the stem end of the fruit and later spread toward the blossom end with increase in severity. The same pattern was observed for shrivelling. Defillipi et al. (2006) reported stem to blossom end pattern of scald development on ‘Wonderful’ pomegranates.

DCA, CA preceded by ILOS and CA treatments maintained TSS and TTA better than RA stored ‘Packham’s Triumph’ pears after 7 months cold storage followed by 7 days shelf life. The maintenance of TTA level by DCA has been reported by Lafer (2011) on ‘Uta’ pears and on ‘Beurre’d Anjou’ pears by Calvo et al. (2002).

**Research paper 2**

DCA and RA treated ‘Packham’s Triumph’ pears had significantly better firmness retention than CA preceded by ILOS, RA preceded by ILOS and CA after storage after 7 months as it was above the minimum acceptable limit of 50 N immediately after cold storage. The firmness level fell below acceptable human consumption (20 N) after 7 days shelf life following 2, 3, 5 and 7 months storage periods irrespective of treatment applied before or during storage. Apart from suppressing superficial scald on Packham’s Triumph’ pears DCA technology retains firmness better after extended storage of 7 months. Zanella et al. (2008) showed that DCA retains firmness better than ultra-low oxygen treatment followed by CA.

DCA and CA treated ‘Packham’s Triumph’ pears had better retention of skin colour (skin colour score and hue angle) after 7 months in storage compared to CA preceded by ILOS treated fruit. CA and CA preceded by ILOS treated ‘Packham’s Triumph’ pears were not significantly different in skin colour retention after 7 months in cold storage. CA preceded by ILOS treated fruit had the highest yellowing index (YI) but were not significantly different from RA preceded by ILOS and DCA ‘Packham’s Triumph’ pears.

DCA and RA preceded by ILOS treated ‘Packham’s Triumph’ pears had better taste scores than CA, CA preceded by ILOS and RA treated fruit after 7 months in storage during the 2012 season. RA fruit had the lowest taste score below acceptable levels for human consumption due to the presence of off flavours in the fruit. DCA and CA treated ‘Packham’s Triumph’ pears had better appearance scores than fruit treated with CA preceded by ILOS, RA preceded by ILOS and RA. Control fruit had the lowest appearance scores at the end of the 5 months storage period. Texture scores decreases with increased shelf life.

**Research paper 3**

The volatiles MHO and α-farnesene are implicated in the incidence of superficial scald on ‘Packham’s Triumph’ pears. CA preceded by ILOS and DCA treatments controlled superficial scald by perhaps suppressing autoxidation of α-farnesene to MHO. Superficial scald symptoms appeared on the ‘Packham’s Triumph’ pears when the α-farnesene concentrations were lower, and MHO concentrations were higher. DCA and CA preceded ILOS effectively control the incidence of superficial scald on ‘Packham’s Triumph’ pears by keeping the concentrations of MHO lower. The suppression of MHO by ILOS has been reported by Wang and Dilley, (2000) on ‘Law Rome’ and ‘Granny Smith’ apples and by Pesis et al. (2014) on ‘Spadona’ and ‘Bartlett’ pears. Although undetectable at harvest, MHO accumulates in the peel of ‘Packham’s Triumph’ pears after 2 months in cold storage.

There was a strong negative correlation between superficial scald incidence and α-farnesene, and the correlation between superficial scald and MHO was a strong positive on ‘Packham’s Triumph’ pears. ‘Packham’s Triumph’ pears treated with DCA, CA and CA preceded by ILOS may be marketed as organic products as they have no chemical residues or any postharvest chemicals provided only permissible organic pesticides were used during production as well.

**Research paper 4**

DCA treatment effectively controlled superficial scald on pre-optimal and optimally mature ‘Granny Smith’ apples for up to seven months. Pre-optimally harvested ‘Granny Smith’ apples were more prone to superficial scald than optimally mature fruit. Incidence and severity of superficial scald may vary from season to season. Erkan and Pekmezci (2004), Lotze et al. (2003) and Bauchot et al. (1999) reported that later harvested ‘Granny Smith’ apples were more resistant to superficial scald than earlier harvested fruit. DCA offers a promising alternative to the use of DPA for South African exporters as CA preceded by ILOS and CA treatments effectively controlled superficial scald on ‘Granny Smith’ apples for up to 3 months only. These results corroborate findings by Lau (1997) of the ineffectiveness of ILOS to control superficial scald on ‘Starkimson Delicious’ apples. Superficial scald increased from zero to seven days at shelf life conditions. When storage period is extended (seven months) symptoms of superficial scald appeared during cold storage in RA and RA preceded by ILOS treated fruit.

DCA and CA retained firmness better than ILOS treated fruit. ILOS followed by CA did not retain firmness better than CA on 'Granny Smith' apples. 'Granny Smith' apples stored in RA were firmer than RA preceded by ILOS fruit. 'Granny Smith' apples retain firmness during storage and none of the treatment had the firmness retention falling below 50 N, which is the minimum acceptable limit after storage (Little and Holmes, 2000). The end of storage life is mostly identified using presence of physiological disorders by marketers, retailers and consumers, who will not accept wastage in excess of 5 to 10% (Little and Holmes, 2000).

'Granny Smith' apples affected with superficial scald (RA and RA preceded by ILOS treated fruit) had the lowest appearance scores due to visible blemishes on the fruit peel. DCA treated fruit had no superficial scald and had the highest appearance score at the end of storage and at the end of shelf life after 7 months in cold storage. Taste scores increased with increased shelf life, the score increased significantly in all treatments from zero to seven days, due to intensification of flavours thereby increasing taste score. Texture scores decreased with time in shelf life but DCA, CA and CA preceded by ILOS maintained texture better than RA preceded by ILOS and RA treatments. DCA, CA and CA preceded by ILOS maintained sensory quality attributes (appearance, texture and taste) of 'Granny Smith' apples better than RA and RA preceded by ILOS after long term storage. Similar findings were reported by Escheverria et al. (2004).

**Research paper 5**

The volatiles MHO and α-farnesene were implicated in the incidence of superficial scald on 'Granny Smith' apples. DCA technology controlled superficial scald on Granny Smith apples by perhaps delaying autoxidation of α-farnesene to MHO as evidenced by the appearance of superficial scald on the fruit when the α-farnesene concentrations were high. MHO concentrations were suppressed by the scald controlling treatment of DCA on 'Granny Smith' apples. DCA without use of DPA effectively control the incidence of superficial scald on 'Granny Smith' apples.

## Conclusions

This research has provided South African growers and exporters and indeed the global fruit industry, with alternative technologies to DPA chemical treatment for controlling superficial scald on 'Packham's Triumph' pears and 'Granny Smith' apples. DCA technology offers an alternative for long term storage (up to seven months) of 'Packham's Triumph' pears and 'Granny Smith' apples. Furthermore, storing fruit under DCA retains firmness better than the control on 'Packham's Triumph' pears and 'Granny Smith' apples. Storing fruit at CA preceded by ILOS lends itself as an alternative for short term (5 months) inhibition of superficial scald on 'Packham's Triumph' pears. A major advantage of the ILOS storage regime found in this study is that it requires no installation of software as long as the room remains airtight; however, this treatment offers no control of superficial scald on 'Granny Smith' apples. It was found that DCA treatment inhibits superficial scald symptoms on 'Granny Smith' apples and 'Packham's Triumph' pears by suppressing the concentration of MHO in the fruit peel.

## References

Bauchot, A.D., Reid, S.J., Ross, G.S. & Burmeister, D.M. (1999). Induction of apple scald by anaerobiosis has similar characteristics to naturally occurring superficial scald in 'Granny Smith' apple fruit. *Postharvest Biology and Technology*, 16, 9-14.

Berry, T.M. (2013). Resistance to airflow and the effects on cooling efficiency of multi scale ventilated pome fruit packaging. MSc. Thesis, Stellenbosch University Stellenbosch University. http://scholar.sun.ac.za.

Buchner, S. (2006). Coating of pears (var. 'Packham's Triumph') with kafirin protein and its effect on postharvest physiology and shelf life. MSc. Thesis, University of Pretoria, Pretoria.

Burger, G.E. (2005). Factors affecting shrivelling and friction discolouration of pears (*Pyrus communis* L) MSc. Thesis, Stellenbosch University, Stellenbosch. http://scholar.sun.ac.za.

Calvo, G., Salvador, M.E. & Sanchez, E. (2002). Control of superficial scald in 'Beurre'd Anjou' pears with low oxygen levels. *Acta Horticulturae,* 596, 879-882.

Defillipi, B.G., Whitaker, B.D., Hess-Pierce, B.M. & Kader, A.A. (2006). Development and control of superficial scald on 'Wonderful' pomegranates during long-term storage. *Postharvest Biology and Technology*, 41, 234-243.

Echeverria, G., Lara, I., Fuentes, T., Lopez, M.L., Graell, J & Puy, J. (2004). Assessment of relationships between sensory and instrumental quality of controlled atmosphere stored 'Fuji' apples for multivariate analysis. *Journal of Food Science*, 69, 368-375.

Erkan, M. & Pekmezci, M. (2004). Harvest date influences superficial scald development in 'Granny Smith' apples during long term storage. *Turkish Journal for Agriculture*, 28, 397-403.

Lafer, G. (2011). Effect of different CA storage conditions on storability of 'Uta' pear. *Acta Horticulturae,* 909, 757-760.

Lau, O.L. (1997). Initial low oxygen stress offers no scald control benefits to 'Starkimson Delicious' apples in 0.7 kPa $O_2$ storage. *HortScience,* 32, 1239-1241.

Little, C.R. & Holmes, R.J. (2000). *Controlled atmosphere storage* in storage technology for apples and pears: A guide to production, postharvest treatment and storage of pome fruit in Australia (pp. 420-515). Knoxfield: Highway Press Pty Ltd.

Lotze, E., van der Merwe K. & Rogalska, S. (2003). Manual identification of post-harvest disorders of pome and stone fruit. ARC Infruitec-Nietvoorbij.

Pesis, E, Feygenberg, O., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J. & Ben-Arie, R. (2014). Low oxygen pre-storage treatment is effective in reducing chilling injuries of deciduous fruit. *International Journal of Postharvest technology and Innovation*, 4, 23-32.

Mitcham, E.J. & Mitchell, F.G. (2002). Postharvest handling systems: Pome fruits. In Kader A.A. (Ed.), *Postharvest technology of horticultural crops* (3rd ed., pp. 333-344). University of California Publication 3311.

Wang, Z. & Dilley, D.R. (2000). Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology*, 18, 201-213.

Watkins, C.B. (2008). Postharvest ripening and innovation in storage technology. *Acta Horticulturae*, 796, 51-58.

Zanella, A., Cazzanelli, P. & Rossi, O. (2008). Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apple. *Acta Horticulturae*, 796, 77-88.