THE INFLUENCE OF CELL WALL BOUND CALCIUM, CELL NUMBER AND SIZE ON THE DEVELOPMENT OF MEALINESS IN 'FORELLE' PEAR.

EVALUATION OF X-RAY CT AND NIR AS NON-DESTRUCTIVE TECHNIQUES FOR MEALINESS DETECTION.

By
Tavagwisa Muziri

Dissertation presented for the degree of Doctor of Philosophy Degree in the Faculty of

AgriSciences (Department of Horticultural Sciences) at the

University of Stellenbosch
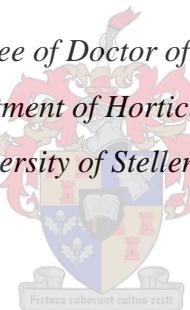
Supervisor: Dr E.M. Crouch
Dept. of Horticultural Science
University of Stellenbosch

Co-supervisor: Prof K.I. Theron
Dept. of Horticultural Science
University of Stellenbosch

March 2016

DECLARATION

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature…**T. Muziri**…………………..   Date…………2016…………………

SUMMARY

'Forelle' pear (*Pyrus communis* L.) is the second most planted pear and the second highest generator of foreign exchange for pears in South Africa. It is favoured for its red blush, melting texture, sweet taste and pear flavour. However, 'Forelle' develops mealiness, a floury, soft and dry texture with low extractable juice. Consumers dislike mealy fruit. 'Forelle' mealiness has been characterized by a loss of cell to cell binding during ripening in a previous study. This study aimed to further understand the role of cell wall bound and free Ca^{2+}, as well as the cell size and cell number in the development of mealiness in 'Forelle'. In addition, two non-destructive methods for the detection of mealiness in intact pears were examined.

It was found that free Ca^{2+} constituted about 49-73% of the total cell Ca^{2+}. Depending on farm origin, mealy fruit contained a lower free Ca^{2+} concentration compared to non-mealy pears. Plant growth regulators and selective blossom thinning that caused larger cells had a higher mealiness percentage. Scanning electron microscopy revealed larger intercellular spaces for treatments with a higher mealiness incidence. Macro X-ray computed tomography (X-ray CT) showed a higher percentage of defects in the neck of fruit that would become mealy after storage, and after softening. To our knowledge this is a first such finding. Micro (X-ray CT) found that cells of mealy fruit were larger and ellipsoidal in shape while non-mealy cells were smaller and more rounded. Mealiness was also associated with high fruit porosity.

A further study described physicochemical measurements which relate to mealiness. Mealy fruit were mostly larger with a higher total soluble solids (TSS), TSS:TA ratio and lower juice area and juice weight obtained by a confined compression method. Fourier transform near-infrared absorbance spectroscopy (FT-NIR) was employed to determine if spectra could be used to distinguish between mealy and non-mealy fruit using sensory and TSS based schemes. Classification was done using orthogonal partial least squares discriminant analysis (OPLS-DA). This study showed that FT-NIR spectra can indeed be used to discriminate between mealy and non-mealy 'Forelle' pears. Two–class (mealy and non-mealy) discriminant analysis produced models with accuracies ranging from 51% to 95%. Mealiness caused an increase in transmittance in specific regions of the spectra. FT-NIR was then evaluated for the quantification of TSS using partial least squares (PLS) regression. Validated models had root mean squared error of prediction (RMSEP) = 0.76-0.94 and relative prediction deviation (RPD) = 1.53-2.17, with the equator blush consistently giving better performance for three

farms making the model ideal for hand held FT-NIR applications. External validation results of farm location showed reduced model robustness. The decrease in prediction performance was attributed to the differing TSS ranges in locations and possibly seasons. It is recommended that future studies on FT-NIRs calibration models for 'Forelle' use fruit from wide origins with wide TSS ranges over various seasons.

OPSOMMING

'Forelle' pere (*Pyrus Communis* L.) is die tweede mees aangeplante peer en die tweede grootste inkomste genereerder van buitelandse valuta vir pere in Suid Afrika. Dit word verkies vir sy rooi blos, smeltende tekstuur, soet smaak en peer geur. 'Forelle' ontwikkel egter 'n melerige, sagte en droë tekstuur met lae ekstraheerbare sap. Verbruikers hou nie van melerige vrugte nie. 'Forelle' melerigheid is in 'n vorige studie verbind aan die verlies van sel tot sel verbindings gedurende rypwording. Die doel van hierdie studie was om die rol van selwand gebinde en vrye Ca^{2+}, asook selgrootte en selgetal in die ontwikkeling van melerigheid in 'Forelle' te verstaan. Laastens is twee nie-destruktiewe metodes vir die opsporing van melerigheid in intakte pere ondersoek.

Daar is gevind dat vrye Ca^{2+} omtrent 49-73% van die totale sel Ca^{2+} uitmaak. Afhangend van die boord van oorsprong, het melerige pere 'n laer konsentrasie van vrye Ca^{2+} bevat, in vergelyking met nie melerige pere. Plant groeireguleerders en selektiewe bloeiseluitdunning wat groter selle veroorsaak het, het 'n hoër persentasie van melerige vrugte gehad. Skanderings elektron mikroskopie openbaar dat groter intersellulêre spasies, in behandelings wat meer melerigheid gehad het, gevind is. Makro X-straal verwerkte tomografie (X-straal VT) het aangedui dat defekpersentasie hoër was in die nek van vrugte wat melerig sou word na opberging, asook na sagwording. Dit is volgens ons kennis die eerste diesulke bevinding. Mikro X-straal VT het gevind dat melerige selle groter en meer ellipsoïdaal in vorm was terwyl nie-melerige selle kleiner en meer rond was. Melerigheid was ook geassosieer met 'n hoër vrugporositeit.

'n Verdere studie het die fisikochemiese metings wat 'n verwantskap toon met melerigheid bepaal. Melerige vrugte was meestal groter vrugte met 'n hoër persentasie van totale oplosbare vastestowwe (TOVS), TOVS:titreerbare suur verhouding, en 'n laer sap area en sap gewig wat bepaal was met die begrensde kompressie metode. Fourier getransformeerde naby infrarooi absorpsiespektroskopie (FT-NIR) om te bepaal of spektra benut kan word om tussen melerige en nie-melerige vrugte te onderskei d.m.v. sensoriese en TOVS klassifikasie is gedoen met behulp van ortogonale gedeeltelike laagste kwadraat diskriminant analise (OPLS-DA). Die studie toon dat FT-NIR spektra wel gebruik kan word om tussen melerige en nie-melerige 'Forelle' pere te onderskei. Twee–klas (melerige en nie-melerige) diskriminant analises het modelle geproduseer met 'n akkuraatheid van 51% tot 95%. Melerigheid het 'n verhoging in

transmissie in sekere areas van spektra veroorsaak. FT-NIR is verder geëvalueer vir die kwantifisering van TOVS met die gebruik van gedeeltelike laagste kwadraat (PLS) regressie modelle. Die gevalideerde modelle het gemiddelde kwadraat fout van voorspelling (RMSEP) = 0.76-0.94 en relatiewe voorspellingsafwyking (RPD) = 1.53-2.17 gehad, met die ewenaar bloskant van die vrug wat konsekwent beter presteer het vir drie plase. Dit sou die model ideaal maak vir handgehoude tipe FT-NIR toepassings. Eksterne validasie resultate van plaas posisie toon verlaagte model robuustheid. Die verlaging in voorspellings prestasie was toegeskryf aan die TOVS verspreiding wat verskillend was vir plase en moontlik ook seisoene. Daar word aanbeveel dat toekomstige kalibrasie model studies op 'Forelle' vrugte van 'n wye oorsprong en TOVS verspreidings insluit oor verskeie seisoene.

ACKNOWLEDGEMENTS

Imbayago Nyika and wife, for giving good me company during the four years in Cape Town.

The Midlands State University, an institution that gave me the opportunity to pursue postgraduate studies.

My wife, Dr Dubekile Nyoni for your patience and support. You are a true pillar of strength.

Justice, Takudzwa, Tinotenda, Rudo and Anashe, my children, for giving me a purpose to live and work.

Rumbidzai Chikutiro-Muziri and Henry Hatirutyi Tungamirirai Muziri, my parents. I thank you for bringing me to this earth at a difficult moment in our country's history. You have been a source of encouragement to me throughout my life. Ndinotenda.

Finally, I would like to thank The Almighty God for bringing me this far.

DEDICATION

To my late brother, Takuranago Zhou.

TABLE OF CONTENTS

NOTE: The language and style used in the different chapters of this thesis are in accordance with different journal requirements. The review paper conformed to the South African Fruit Journal while the rest of the chapters were written to conform to the requirements of the Postharvest Biology and Technology Journal. Each chapter in this thesis is therefore an individual entity, itself a whole, but building into a compilation.

GENERAL INTRODUCTION AND OBJECTIVES

Pears are an important deciduous crop in South Africa, in terms of employment creation and foreign exchange earnings. In the 2012/13 season, the pear industry contributed about 16% (R2 billion) of the total gross income of deciduous fruit (Department of Agriculture, Forestry and Fisheries, 2014). Forelle is now the second highest exported pear cultivar from South Africa, accounting for 26% of the total pear area planted, compared to the highest of 33% of Packham's Triumph (HORTGRO, 2014). The planted area under 'Forelle' has grown from 2895 ha in 2005 to 3195 ha in 2014, the largest increase of all pear varieties during the period (Theron et al., 2008; HORTGRO, 2014). The success of 'Forelle' has mainly been a result of its exceptional blush which is favoured by consumers (Manning, 2009). 'Forelle's ability to develop the exceptional blush under South African conditions has put it well apart from its rivals, 'Rosemarie' and 'Flamingo' which seem to be sensitive to temperatures causing a decrease in pigmentation (Steyn et al., 2005). Some consumers like 'Forelle' for the crunchy texture, sweet taste and pear flavor when hard and ripe (Crouch and Bergman, 2013). Traditional consumers of 'Forelle' like it for its buttery texture when ripened under shelf life conditions (Cronje et al., 2015; Manning, 2009). In spite of the numerous advantages over other varieties, 'Forelle' pears are known for their resistance to ripen normally and develop mealiness when not stored under cold conditions of -0.5 °C for at least 12 weeks (Martin, 2002; Crouch et al., 2005). Mealiness causes fruit to develop low extractable juice content with a floury, soft and dry texture (Martin, 2002) which is disliked by the majority of pear consumers (Manning, 2009; Cronje et al., 2015).

The major challenge with mealiness is that it develops during shelf life, usually in the market or with the consumer, making it impossible to cull the mealy fruit before packing. Current practice to ensure 'Forelle' fruit of good quality with low mealiness is a mandatory cold storage period of 12 weeks at -0.5 °C prior to ripening at room temperature. Consistent with this cold storage requirement, the South Africa pear protocol for export fruit stipulates that fruit should be stored for a period of 8 weeks before release for shipment (Hurndall, 2011) with an additional 4 to 6 weeks at -0.5 °C in shipping and distribution to its main market, Europe. This cold storage requirement is a challenge from both the energy and logistics viewpoints. Firstly, the 8 week storage period increases the energy requirement for cold storage. Secondly, the length of the cold storage period means that South African fruit arrives late on to the European markets, prompting consumer migration to other competing varieties and suppliers (Crouch and Bergman, 2013). Apart from the above challenges, there is also the likelihood that mealiness

may eventually result in consumer dissatisfaction with 'Forelle'. For a cultivar like Forelle which is famous for its attractive bicolour and pear flavour, mealiness could result in consumer disappointment and lead to consumer aversion (Manning, 2009; Cronje et al., 2015).

In order to remain competitive, market 'Forelle' of superior quality and sustain consumer trust, a better understanding and management of mealiness is required. To reduce incidences of mealiness on markets, techniques for early detection of mealiness before marketing need to be developed. Research has concentrated mainly on understanding the biochemical and physiological basis of mealiness mostly in peaches and apples and a few in pears. Studies have been done on the role of enzymes, particularly polygalacturonase (PG) and pectin methyl esterase (PME) (Obenland and Carroll, 2000; Villalobos-Acuna and Mitcham, 2008), the role of ethylene (Martin et. al., 2003; Zhou et al., 2005) and pectin (Brummell et al., 2004; Crouch, 2011; Hobbs et al., 1991; Villalobos-Acuna and Mitcham, 2008). Compositional differences of cell wall material of mealy and non mealy fruit have also been studied in nectarines and pears. Recently, studies have been done by a number of researchers to try and reduce the mandatory cold storage period, however with varying successes (Carmichael, 2011; Crouch and Bergman, 2013; Martin, 2002). Work by Crouch and Bergman from 2010 to 2013 led to an alternative programme of supplying crunchy 'Forelle' pears to the European markets called the 'Forelle' early market access programme (FEMA) (Crouch and Bergman, 2013). Although the FEMA has improved farmers' incomes (Steenkamp, 2014), it has not completely solved the problem of mealiness. Fruit marketed through FEMA are hard and ripe yet a large segment of consumers, particularly of European origin prefer soft, sweet and juicy pears (Crouch and Bergman, 2013; Manning, 2009). There is need for more research on mealiness. The current study was therefore carried out to shed more light on mealiness, improve our understanding of mealiness in 'Forelle', and to examine non-destructive methods for mealiness detection in 'Forelle' pears.

An extensive literature study was done to examine research that has been done on mealiness in other fruits as well as on 'Forelle'. The review was followed by two experimental studies, which are reported in Papers 1 and 2. The first experiment (Paper 1) was motivated by findings from related studies where calcium has been linked to physiological disorders (Fallahi et al., 1997; De Freitas et al., 2010; De Freitas and Mitcham, 2012). The study specifically aimed to examine the role of bound and free Ca^{2+} in the development of mealiness. A sequential extraction method of determining cell wall bound and free Ca^{2+} was developed and three Ca^{2+} fractions were extracted and quantified from fresh fruit tissues. The second experiment (Paper 2) examined the role played by cell sizes and cell numbers in the development of mealiness in 'Forelle' pears.

The study was premised on the thesis that cell sizes and shapes influence cell packing, cell to cell bonding and debonding and therefore should have a bearing on mealiness, itself hypothesised to result from cell separation at the middle lamella. Electron microscopy examination of dried tissues was done followed by image analysis to count and measure cells of mealy and non-mealy 'Forelle' pears. The study also examined other histological (cell packing, intercellular spaces and cell walls) differences between mealy and non-mealy fruit.

Presently, mealiness determination is done by a sensory panel after the fruit has been ripened under shelf life conditions. There is no instrumental method to determine mealiness non-destructively, or maturity indices to predict the disorder. If a technique could be obtained that can determine mealiness or its marker at harvest or in shelf life it would be useful in the fruit industry to monitor and control mealiness. Optical techniques which utilise the visual and near-infrared wavelengths have gained prominence in fruit quality evaluations. These systems make it possible to grade fruit, determine internal quality parameters not detectable from outside, and predict future biochemical processes through marker biomolecules in the fruits (Alander et al., 2013; Cubero et al., 2011; Teyer et al., 2013). Their advantages include that the methods are objective, fast, can provide real-time information, provide substantial information in addition to the required characters and can be used in online monitoring of samples and avoid human error (Cubero et al., 2011; Alander et al., 2013; Teyer et al., 2013). The price of purchase and installing these techniques in online grading has gone down in recent years (Alander et al., 2013). Some of the widely used methods in the food industry include magnetic resonance imaging, X-ray computed tomography (X-ray CT), hyperspectral imaging and visible / near infrared spectroscopy (NIR).

No study has reported on non-destructive determination of 'Forelle' quality attributes. The third and fourth section of this study examined two techniques; NIR and X-ray-CT as non-destructive techniques for mealiness detection. NIR and X-ray-CT are widely regarded techniques for non-destructive determination of quality in agricultural produce and have received considerable attention during the last 2 decades (Bobelyn et al., 2010; Cantre et al., 2014a and b; Herremans et al., 2013; Louw and Theron, 2010; Nicolai et al 2007; Magwaza et al., 2012; Van Dalen et al., 2007). Paper 3 covers work on X-ray CT for detection of mealiness. X-ray macro CT was used to determine whether differences exist between mealy and non-mealy pears after storage and shelf-life while X-ray micro CT (destructive) was used to further describe the microstructural differences between mealy and non-mealy tissues after shelf-life.

Of the many optical techniques, the techniques based on the near infrared spectrum are more useful in food evaluation as the spectra are related to overtones and combination bands of chemical bonds such as C-H, O-H, and N-H, which have influence in most foods (Alander et al., 2013; Louw and Theron, 2013). It is also convenient for large batches where continuous inspections need to be done. Paper 4 therefore aimed to determine the physicochemical characteristics relating to mealiness and explored using NIR spectra to discriminate mealy and non-mealy fruit. Paper 5 discusses models where NIR spectra from various positions on the fruit and different orchards are used to predict TSS.

Literature Cited

Alander, J.T., Bochko, V., Martinkauppi, B., Saranwong, S., Mantere, T., 2013. A review of optical nondestructive visual and near infrared methods for food quality and safety. Int. J. Spectrosc. Hindawi Publishing Corporation. http://dx.doi.org/10.155 /2013/341 402. (accessed 22-10-2014).

Bobelyn, E., Serban, A.S., Nicu, M., Lammertyn, J., Nicolai, B.N., Saeys, W. 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133-143.

Brummell, D.A., DalCin V., Lurie, S., Crisosto, C.H., Labavitch, J.M., 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55, 2041-2052.

Cantre, D., East, A., Verboven, P., Araya, X, T., Herremans, E., Nicolai, B., Pranamornkith, T., Loh, M., Mowat, A., Heyes, J., 2014a. Microstructural characterization of commercial kiwifruit cultivars using X-ray micro computed tomography. Postharvest Biol. Technol. 92, 79-86.

Cantre, D., Herremans, E., Verboven, P., Ampofo-Asiama, J., Nicolai, B.M., 2014b. Characterisation of the 3-D microstructure of mango (*Mangifera indica* L. cv. Carabao) during ripening using x-ray computed microtomography. Innov. Food Sci. Emerg. Technol. 24, 28-39.

Carmichael, P.C. 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Cronje, A., Crouch, E.M., Muller, M., Theron, K.I., van der Rijst, M., Steyn, W. J., 2015. Canopy position and cold storage duration affects mealiness incidence and consumer

preference for the appearance and eating quality of 'Forelle' pears. Sci. Hortic. 194, 327-336.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L). PhD Thesis. Stellenbosch University.

Crouch, E.M., Holcroft, D.M., Huysamer M., 2005. Mealiness of 'Forelle' pears-quo vadis? Acta Hort. 671, 369-376.

Crouch, I., Bergman H., 2013.Consumer acceptance study of early marketed 'Forelle' pears in the United Kingdom and Germany. SA Fruit Journal, 11(6), 64-71.

Cubero, S., Alexos, N., Molto, E.,Gomex-Sanchis, J., Blasco, J., 2011. Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. Food Bioprocesses Technol. 4, 487-504.

De Freitas, S.T., Do Amarante, C.V.T., Labavitch, J. M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biol. Technol. 57, 6-13.

De Freitas, S.T., Mitcham, E. J., 2012. Factors involved in fruit calcium deficiency. Hort. Rev. 40, 107-145.

Department of Agriculture Forestry and Fisheries. 2014. A profile of the South African Pear Market Value Chain. Directorate Marketing, Arcadia. South Africa. www.webapps.daff.gov.za/mis. (accessed 02-12-2014).

Fallahi, E., Conway, W.S., Hickey, K. D., Sam, C.E., 1997. The role of calcium and nitrogen in post-harvest quality and disease resistance of apples. Hort. Sci. 32, 831-835.

Herremans, E., Verboven, P., Bongaers, E., Estrade, P., Verlinden, B.E., Wevers, M., Hertog, M.L.A.T.M., Nicolai, B.M., 2013. Characterisation on 'Braeburn' browning disorder by means of X-ray micro-CT. Postharvest Biol and Technol. 75, 114-124.

Hobbs, M.C., Easterbrook, K.M., Melton, L.D., 1991. Cell wall material composition of mealy fruit among ripening nectarines. J. Sci. Food Agr. 57, 141-145.

HORTGRO, 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

Hurndall, R.F., 2011. 'Forelle' dispensation procedures for 2011 season. http://www.decidous.co.za (accessed 10-09-2014).

Louw, D.E., Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina*), using NIR spectroscopy. Postharvest Biol. Technol. 58, 176-184.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolai, B., 2012. NIR spectroscopy Applications for internal and external quality analysis of citrus fruit-A review. Food Bioprocess Technol. 5 (2), 425-444.

Manning, N., 2009. Physical, sensory and consumer analysis of pear genotypes among South African consumers and preference of appearance among European consumers. MSc Food Science thesis. Department of Food Science, Stellenbosch University, Stellenbosch, South Africa.

Martin, EM. 2002. Ripening responses of 'Forelle' pears. MSc (Agric) Thesis Department of Horticultural Sciences, Stellenbosch University, South Africa.

Martin, E.M., Crouch, I.J., and Holcroft, D.M. 2003. Ripening and mealiness of 'Forelle' pears. Acta Hort. 600, 449-452.

Nicolai, B.M., Beullens, K., Bobelyn, E., Piers, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99-118.

Obenland, D.M., Caroll, T. R., 2000. Mealiness and pectolytic activity in peaches and nectarines in response to heat treatment and cold storage. J. Amer. Soc. Hort. Sci. 125 (6), 723-728.

Steenkamp, E.M., 2014. FEMA_ A blushing success. SA Fruit Journal, Aug/Sep 2014: 8-9.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., Jacobs, G. 2005. Red colour development and loss in pears. Acta Hort., 671, 79-85.

Teyer, E., Huang, S., Afoakwa, N., 2013. Review on the potential of near infrared spectroscopy (NIRs) for the measurement of chemical residues in food. Am. J. Food Sci. Technol. 1, 1-8.

Theron, K.I., van der Merwe, P.W., Ferrandi, C.H., 2008. Pear production in South Africa. Acta Hort. 800, 89-94.

Van Dalen, G., Nootenboom, P., van Vliet, L.J., Voortman, L., and Esveld, E., 2007. 3-D imaging, analysis and modelling of porous cereal products using X-ray microtomography. Image Anal. Stereol. 26, 169-177.

Villalobos-Acuna, M., Mitchan E.J. 2008. Ripening of European pears: The chilling dilemma. Postharvest Biol.Technol. 49, 187-200.

Zhou, H., Dong, L., Ben-Arie, R., Lurie, S., 2001. The role of ethylene in the prevention of chilling injury in nectarines. J. Plant Physiol. 158, 55-61.

LITERATURE REVIEW

Mealiness of 'Forelle' pear (*Pyrus communis* L.): a review

1 Introduction

1.1 The 'Forelle' pear history, origin and production

1.2 The history of mealiness in South African 'Forelle'

1.3 Implications of mealiness on liking: the consumer's perspective

1.4 Mealiness in the face of global competition

2.0 Biochemistry of ripening and mealiness

2.1 Role of enzymes in ripening and mealiness

2.2 The cell wall and mealiness development

2.3 Pectin involvement in fruit ripening and mealiness

2.4 The role of calcium in the development of physiological disorders and mealiness

2.4 Cell wall compositional differences between mealy and non mealy fruit

2.6 Role of cell size and cell number on mealiness development

2.7 Role of water and mealiness development

3.0 Environmental factors associated with mealiness

4.0 Conclusion

NOTE

This paper is intended for the South African Journal of Plant and Soil. The reference style is therefore in accordance with the journal requirements.

1. Introduction

Pears are an important deciduous crop in South Africa, in terms of employment creation and foreign exchange earnings. Forelle (*Pyrus communis* L.) is one of the most commonly grown pear cultivars from South Africa and is the most important blushed cultivar. It is now the second highest planted and exported pear cultivar from South Africa, accounting for 26 % of the total pear area planted (HORTGRO 2014). Over the past two decades, 'Forelle' pears have grown in both volumes of exports and area under cultivation, relative to other cultivars (HORTGRO 2014). In 2012, a total of 3 million cartons were exported in a trend that is expected to increase annually (Department of Agriculture Forestry and Fisheries 2012; Steenkamp 2014). Although 'Forelle's future looks promising, there are challenges in the post-harvest handling of the fruit. Like most European pear cultivars, 'Forelle' requires cold storage after harvesting in order to ripen uniformly (Crouch and Bergman 2013a; Villalobos-Acuna and Mitcham 2008). In addition to the costly cold storage requirement, 'Forelle' pears are prone to development of mealiness or astringency when not subjected to sufficient period of low temperature exposure after ripening (Carmichael 2011; Martin 2002).

Mealiness is common in apples, peaches, pears, nectarines and tomatoes (Barreiro et al. 2000; Lammertyn et al. 2002; Martin et al. 2003; Obenland and Carroll 2000). In 'Forelle' pears, mealiness occurs mostly in fruit harvested at the post optimum stage of maturity or in fruit that have not received sufficient time in cold storage at -0.5 °C (Carmichael 2011; Crouch et al. 2005). The standard commercial practice for 'Forelle' is that fruit is harvested at optimum maturity between 62.8 and 58.8 N firmness (6.4 – 6 kg) (Carmichael 2011), which is normally before fruit has reached the respiratory climacteric. The fruit is then stored for a minimum period of about 12 weeks at -0.5 °C for the fruit to ripen to a good eating quality during storage at ambient temperature (Carmichael 2011; Crouch 2011). The lengthy cold storage requirement results in arrival delays of South African fruit on to the European market, which prompts migration to other bicolour supplies by the importing supermarkets (Crouch and Bergman 2013a). Apart from the ripening related challenges, mealiness has the potential to tarnish the otherwise good image of 'Forelle' on the market and threaten its future marketability.

Mealiness has always been an important quality attribute for European consumers, so much so that in 1996 a consortium of seven partners with two universities and a commercial company from five countries was tasked to address the problem of mealiness in European apples (Lammertyn et al. 2002). Although these studies were based on apples, the commitment and investment shows the importance that European consumers put on mealiness. Coincidentally,

the majority of South African 'Forelle' are exported to Europe (HORTGRO 2014). Management of mealiness thus remains crucial for the success of 'Forelle' on the European markets. The objective of the current review therefore is to examine past research on aspects of 'Forelle' mealiness and examine differences, if any, with other fruit mealiness. The review further intends to point out research gaps that need to be addressed to fully understand and solve problems of mealiness in 'Forelle' pears.

1.1 The 'Forelle' pear history, origin and production

'Forelle' has a long history of cultivation dating back to the 17th century, with an origin claimed to be in Germany (Crouch and Bergman 2013a), where it was first commercially planted in 1846. The cultivar was introduced to South Africa in the 1800s (Fruits Unlimited 2009). 'Forelle' was first cultivated in Stellenbosch around 1893 and later at Two-A-Day in 1909 (Henk Griessel, Tru-Cape marketing, 2015 personal communication). Today, 'Forelle' features as one of the most important cultivars in South Africa, together with Packham's Triumph and William's Bon Chretien (Theron et al 2008). The four major cultivars grown in South Africa include Packham's Triumph (32 %), Forelle (26%), William's Bon Chretien (22%), and Abate Fetel (6%) (HORTGRO 2013). 'Forelle' is one of the three blushed cultivars grown in South Africa, characterised by soft, buttery flesh. The other two blushed cultivars are 'Rosemarie' and 'Flamingo' which are both early season cultivars. Locally bred, the new cultivar Cheeky, plus Rosemarie, contribute only 7% to total plantings (HORTGRO 2013). South African pear orchard age distribution shows that 40% of Packham's Triumph and 45% of William's Bon Chretien orchards are currently above 25 years while 'Forelle' orchards older than 25 years are only 12% (HORTGRO 2014) (Table 1). The majority of 'Forelle' orchards are younger than 15 years and the total area of 'Forelle' orchards younger than 25 years is greater than the total for 'Packham's Triumph' and 'William's Bon Chretien' in the same category. Statistics above suggest 'Forelle' is increasing in importance in terms of volume of exports and contribution to pear production.

Table 2. South Africa pear orchard age distribution in 2014 (hectares).

Cultivar	0-3 years	4-10 years	11-15 years	16-25 years	25 + years
Packham's Triumph	603	725	335	707	1611
Forelle	169	700	520	1434	370
William's Bon Chretien	53	184	384	845	1209
Early Bon Chretien	46	359	106	228	9
Abate Fetel	48	26	16	333	9
Rosemarie	0	5	2	62	188
Beurré Bosc	70	168	0	3	0
Doyenné du Comice	1	4	1	136	21
Flamingo	5	8	2	123	4
Golden Russet Bosc	0	1	4	114	2
Others	23	25	13	97	102
Total	1018	2204	1383	4082	3524
% OF TOTAL AREA	8%	18%	11%	33%	29%

Source: HORTGRO, 2014. Key Deciduous Fruit Statistics. Paarl, South Africa.

1.2 The history of mealiness in South African 'Forelle'

In South Africa, mealiness in 'Forelle' was first reported in the 1980s (Hurndall 2011). It was first recognized that if not sufficiently stored under cold storage prior to ripening, 'Forelle' tends to ripen mealy or develop astringency (Martin et al. 2003, Crouch et al. 2005). The first observations of this cold storage requirement of 'Forelle' were made by a company called Two-A-Day (Hurndall 2011). The observations were confirmed by Unifruco Research Services (URS) from 1989-1993 as well as by Infruitec in the mid-1990s and later by Stellenbosch University in 2001 (Hurndall 2011). Following this revelation, a measure was adopted which involves storing fruit under cold storage conditions before ripening. This has since been enforced as a standard for release of harvesting and export of 'Forelle'. The 'Forelle' Producer's Association and the Department of Agriculture, Forestry and Fisheries (DAFF) stipulate that the 8-week storage period must take place in South Africa before shipment. The shipping and distribution time at low temperatures can take from 4-6 weeks to Europe. The total period stored at low temperature is thus in accordance with the mandatory 12 week cold storage period at -0.5 °C. This standard practice has financial implications to producers and marketers. Hence, mealiness has created a challenge to both the marketing and competitiveness of 'Forelle'.

1.3 Implications of mealiness on liking: the consumer's perspective

The decision to purchase a fruit is usually guided by the appearance (colour, size, absence of blemishes), in addition to aroma, firmness and price (Hamadziripi 2012). However, the decision to buy again after experiencing a fruit is guided by the satisfaction one gets from eating the first one (Henderson 2000). Mealiness, internal browning or flesh bleeding or reddening have been identified as factors that deter consumers from purchasing fruit (Newman 2006). Mealiness does not appear in the early stages of the supply chain during cold storage but rather manifests itself in the hand of the consumer during ripening. In consumer behaviour, both the contrast theory and the generalized negativity theory posit that consumers' perception of a product will be much lower if they do not get the expected results from a highly perceived product (Anderson 1973; Donoghue and de Klerk 2006). Consumers are attracted to 'Forelle' due to its attractive red blush (Manning 2009), but when they experience the undesirable texture resulting from mealiness, they get easily disappointed. Such negative experiences on a highly regarded product are often accompanied by three major consumer responses. Some consumers may decide to covertly ignore the product but often tell others about their experiences, others may decide to publicly complain while others may demand redress and compensation (Donoghue and de Klerk 2006).

A study of South African consumer eating behaviour for pears was done by Steyn et al. (2011). The study showed that consumers prefer bright coloured pears with a strong pear flavour, sweet in taste, have a soft melting texture in the mouth and high amounts of juice. Their findings were congruent with preferences of traditional consumers of European pears and were also found to be in line with breeding objectives of the Agricultural Research Council (ARC) Infruitec Nietvoorbij (Von Mollendorf 2008). Both South African and European consumers were shown to dislike mealy 'Forelle' pears (Manning 2009). Mealy pears lack the buttery, melting character associated with pears of good eating quality, are devoid of free juice (Martin 2002) and the flavour is subdued. The South African and European consumers represent a large percentage of consumers of 'Forelle'. In fact, available statistics show that in the year 2012, 60% of 'Forelle' were exported to the EU and Russia while an additional 12% were exported to the United Kingdom (HORTGRO 2013). The prospect theory maintains that where a customer has high expectations of a product, as is the case with 'Forelle' which is liked in its traditional markets because of its exceptional blush and pear flavour (Steyn et al. 2011), any negative aspect of the product such as mealiness will be accompanied by a profound negative perception in future (Trepel et al. 2005). The resultant impact is magnified resentment of the product. It is

11

therefore crucial that 'Forelle' protocols to curb mealiness are followed to manage its occurrence and ensure a good consumer experience.

1.4 Mealiness in the face of global competition

In Chile, 'Forelle', together with 'Abate Fetel', 'Packham's Triumph' and 'Bosc' have been identified as holding the future of the Chilean pear industry and South Africa has been identified as a strategic competitor (FreshFruitPortal 2012). Sales of 'Forelle' in Chile grew by 11% in 2011 compared to 2010 (FreshFruitPortal 2012). In 2015, 12200 tonnes are expected to be exported, representing an increase of 9% compared to 2014 exports (SimFruit 2015). In Europe, attempts at finding alternative blushed varieties have been ongoing for some time. Presenting on the status of pear production in Europe, Deckers and Schoofs (2005) argued the need to have a bicoloured variety in Europe. Thus, although 'Forelle' from South Africa remains very competitive owing to its superior bicolour and pear flavour, there is growing competition from alternative varieties and supplies. The emergence of competing producers in the marketing of 'Forelle' means that the market will demand a product of high quality.

South Africa has recently looked at the possibility of supplying crisp, sweet and juicy fruit (FreshFruitPortal 2012). This has been tried in a successful programme called the 'Forelle' early market access (FEMA) programme (Crouch and Bergman 2013b, Steenkamp 2014). Although FEMA has improved farmer incomes, it has not tackled the problem of mealiness in its entirety as a large segment of consumers; especially of European origin prefer soft and juicy pears (Manning 2009). South Africa therefore has to consolidate the competitiveness of its fruit on the global market, particularly the European market where the majority of its 'Forelle' goes, if it has to remain competitive. 'FEMA' fruit carry a label 'crisp and sweet' to avoid consumer confusion between crisp and soft eating 'Forelle' pears. However, in order to satisfy the soft and sweet eating consumers and the complete 'Forelle' market, mealiness needs to be addressed in the pears that do soften.

2.0 Biochemistry of ripening and mealiness

Ripening of fruit involves biochemical processes of an irreversible nature. The processes involved in ripening are mediated by hormones and catalysed by various endogenous disassembly enzymes, often in a well-coordinated manner (Cantu et al. 2008). If ripening is a well-coordinated and genetically determined process, what then causes some fruit to ripen mealy when the others ripen juicy, with a buttery texture? In order to understand mealiness as an unusual form of a soft and dry texture during ripening, it is important to understand the

biochemistry and molecular biology of fruit softening. In the following section, major biochemical processes that precede ripening and softening of fruit are reviewed with particular reference to mealiness.

2.1 Role of enzymes in ripening and mealiness

The softening of fruit is a result of the breakdown of the cell wall and the dissolution of the middle lamella (Carpita and McCann 2000). It involves solubilisation and depolymerisation of pectins with accompanying loss of neutral sugars from pectin side-chains, and dissolution of the middle lamella (Rose et al. 2003). A range of enzymes is involved in fruit ripening and softening. The majority of these enzymes are hydrolytic in nature, including polygalacturonases, β-galactosidases, pectin methyl-esterases (PME), rhamnogalacturonases and pectinesterases (Dal Cin et al. 1999, Lurie and Crisosto, 2005). The most widely studied enzyme involved with fruit ripening is polygalacturonase (PG). PG is a hydrolase enzyme which is largely responsible for pectin depolymerisation (Dal Cin et al. 1999), a process that requires pectin to be de-methyl-esterified first by PME (Brummell and Harpster 2001). Another hydrolase, rhamnogalacturonase, is responsible for breaking down rhamnogalacturonic acid I (RG-I) type pectin backbones (Payasi et al. 2009).

Several studies have attempted to link enzyme activity to mealiness development (Brummell et al. 2004; De Freitas et al. 2010; Obenland et al. 2008). Obenland et al. (2008) linked mealiness in peaches to the accumulation of ACC oxidase, an enzyme responsible for ethylene biosynthesis. Brummell et al. (2004) on the other hand showed that endo-1, 4-β-glucanase, endo-1, 4-β-mannase, β-galactose, α-arabinosidase and endo-polygalactosidase were lower in mealy than in non-mealy fruit. Mealiness in peaches has been linked to a reduction in exo-polygalacturonase (exo-PG) (Lurie and Crisosto 2005). In peach, fruit treated with propylene to induce mealiness were shown to have low levels of endo-PG activity and a higher level of exo-PG (Yoshioka et al. 2010). This was accompanied by lower solubilisation and depolymerisation of polyuronides. PME is said to be an abundant enzyme associated with fruit ripening. The enzyme desterifies methoyxlated pectin in the cell wall, making it susceptible to further degradation, especially of polygaracturonan (Vicente et al. 2005). The inhibition or imbalance in the activity of this enzyme could result in formation of low methoxy pectin which binds water forming insoluble gels (Obenland and Caroll 2000). The activity of PME was evidenced by the high degree of highly methylated pectins in green peach, which suddenly decrease due to increased PME activity as fruit ripens (Ortiz et al. 2011). A reduction in PME was observed in

13

mealy fruit (Buescher and Furmanski 1978), though in other studies PME was shown to be unchanged by mealiness (Obenland and Caroll 2000).

The hydrolase enzyme, α-L-arabinofuranosidase (α-AFase) is one of the important enzymes that has been associated with mealiness. α-AFase, together with xylanases are responsible for degradation of xylan to component sugars (Saha 2000). In particular, α-L-arabinofuranosidase is involved in hyrolysis of arabinosyl residues of cell wall arabinofuranose containing polysaccharides (Yoshioka et al. 2010). The enzyme is found abundantly in hemicelluloses such as arabin, arabinoxylan, gum arabic and arabinogalactan (Saha, 2000). Arabinosyl and galactocyl-containing side chains determine cell wall porosity and may restrict access by pectinolytic enzymes (Ortiz et al. 2011). What then is the link between this enzyme and with mealiness? A comprehensive review and study by Nobile et al. (2011), on the role of the α-AFase gene associated with mealiness, reported its association with apple and peach mealiness.. A significant increase was seen in α-AFase before the onset of ripening of apple (Pena and Capita 2004). In 'Mondila Gala' the levels of α-AFase were shown to decrease during fruit development and to increase sharply in overripe fruit (Goulao et al. 2007). In peach, α-AFase activity was shown to increase 10 times in fruit that had been treated with propylene to induce mealiness (Yoshioka et al. 2010). An accompanying increase in loss of arabinosyl residues was observed in the same fruit. The same enzyme was evidently absent in the fruit at harvest but would appear later in shelf life in fruit that would become mealy.

In addition to the role played by ripening related enzymes in fruit ripening, Payasi et al. (2009) identified the protein expansin to have both direct and regulatory effects on ripening related enzymes. Its role in ripening is to increase accessibility of cell-wall modifying enzymes to cell wall polymers (Payasi et al. 2009). A decrease in expansin protein and mRNA has also been noticed in mealy peaches (Obenland et al. 2003).

2.2 The cell wall and mealiness development

Mealiness in apple fruit is commonly accepted to result from cell separation through cell to cell debonding (Harker and Hallet 1992; McAtee at al. 2009; Ng et al. 2013). Recent evidence from advanced electron microscopy has confirmed that indeed in fruit such as apples and 'Forelle' pears, mealiness is associated with cell separation (Crouch 2011; Segonne et al. 2014; McAtee et al. 2009;). Separation occurs at the middle lamella as cells fail to break at the cell wall due to the relative strength of the cell wall compared to that of the middle lamella (Harker and Hallett 1992). In order to understand events that precede this failure of cells to break during mastication

or the biochemistry that predisposes some fruits to this condition, an understanding of the cell wall and its various components is required. In addition, familiarity with the chemical composition of the cell wall is of vital importance.

Extensive studies and reviews have been done on the structure and composition of the cell wall, thanks to the advent of advanced microscopy and chemical elucidation techniques. The cell wall is the centre of all biochemical activities affecting fruit texture. It is responsible for controlling the rate and direction of cell growth (Cantu et al. 2008*;* Carpita and McCann 2000). It is composed of proteins and complex interacting polysaccharides, each performing unique functions, among them structural and regulatory functions (Cantu et al. 2008). Cellulose, pectins and hemicelluloses are the three major polysaccharides found in fruit cells (Voragen et al. 2009). Cellulose provides the basic structural strength of the cell. Pectins and hemicelluloses on the other hand reinforce the cellulose fibres into a heterogeneous polysaccharide matrix. Of the three polysaccharides, pectins are the most biologically active and the most abundant in cell walls of fruit (Caffall and Mohnen 2009; Carpita and McCann 2000; Harholt et al. 2010).

2.3 Pectin involvement in fruit ripening and mealiness

A review of the structural composition and function of pectin was done by several authors (Carpita and McCann 2000; Harholt et al. 2010; Orfila et al. 2001; Raffo et al. 2012; Voragen et al. 2009). Pectins are a mixture of different polysaccharides which are branched, hydrated and rich in D-galacturonic acid (Carpita and McCann 2000; Voragen et al. 2009; Orfila et al. 2001). They are found predominantly in the primary cell wall, conferring integrity and rigidity to the cell wall (Caffall and Mohnen 2009). They also determine wall porosity, modulate wall pH and regulate cell to cell adhesion at the middle lamella (Carpita and McCann 2000). Pectins serve in cell to cell communication as recognition sites for substances in the environment such as pathogens and insects and regulate cross membrane transport and permeability of cell walls to enzymes (Voragen et al. 2009). The major polysaccharides making pectins are homogalacturonan, xylogalacturonan, rhamnogalacturonan I (RG-I), arabinogalacturonan I, arabinogalacturonan II, rhamnogalacturonan II, and arabin. Reviews of these different pectin subunits have been done extensively in other studies (Caffall and Mohmen 2009, Campell and Braam 1999, Crouch 2011, Hobbs et al. 1991), and will not be expanded further in this review. The current review is limited to the role of pectins in fruit ripening, with particular reference to mealiness.

Pectin is the principal component of the middle lamella and the primary cell wall. Pectin content is higher in fruit cell walls and is found predominantly in the spaces between cellulose microfibrils and the cross-linking hemicelluloses (Payasi et al. 2009). Because of their presence in the cell wall, pectins invariably contribute to fruit texture and quality and determine how readily fruit softens (Payasi et al. 2009). Secondly, pectins are the most extractable compound from the cell wall such that activities which cause cell disassembly or swelling are more likely to target the pectins (Caffall and Mohnen 2009). Pectins are capable of regulating cell growth by limiting wall porosity, thereby influencing access to the cell wall by cell-modifying enzymes (Carpita and McCann 2000). Differences in cell to cell debonding resulting in the mealiness phenomenon could be a result of differences in predisposition to the activity of cell-modifying enzymes, hence could be linked to pectins (Payasi et al. 2009). Experimental evidence has also shown differences in pectin composition between mealy and non-mealy apples (Nobile et al. 2011). In mealy peaches, higher levels of insoluble pectins have been observed (Brummell et. al 2004; Lurie and Crisosto, 2005). In mealy 'Forelle' the water-soluble pectin was depolymerised at an earlier stage of ripening and no indication of less broken down, higher molecular weight polyuronides were found in the CDTA fraction, which were found in dry, soft peach fruit (Crouch, 2011). The pectins from mealy tissues were therefore more broken down and therefore in this pear cultivar mealiness was not associated with insoluble pectins (Crouch 2011).

2.4 The role of calcium in the development of physiological disorders and mealiness.

Calcium is a crucial regulator of growth and development in plants (Hepler 2005; White et al. 2003). It plays a vital role in plant cells and in maintaining structural integrity in plant tissues and organs (White at al. 2003). At the cellular level, calcium plays a very active role in regulating cell wall structure and function (White at al. 2003). Calcium confers structural rigidity to the cell wall through cross linking with negatively charged regions of pectin (Hepler and Winship 2010). More specifically, it cross links with homogalacturonan in what is called the egg-box model (Caffall and Mohnen 2009), conferring structural integrity to the cell wall. In addition to participating in cell wall integrity and strength, calcium is involved in cell to cell communication as well as communication between cytoplasm and the extracellular environment (Hong-Bo 2008; Xiong et al 2006). Because of its intimate involvement with the cell wall, calcium has been linked to a number of physiological disorders in plants (De Freitas and Mitcham 2012; Simon 1978; White et al. 2003) In fact, the majority of physiological disorders are associated with calcium deficiency (Gastol and Domagala-Swiatkiewicz 2006).

In studies done on apple, low levels of calcium in the apoplast are assumed to cause membrane weakening leading to cell death and bitter pit development (De Freitas et al. 2010). In this study, the authors demonstrated that depending on the apoplasm pH, pectins can bind Ca^{2+}, making it unavailable to the apoplastic solution. This in turn causes weakening of membranes and consequently development of deficiency symptoms. In addition, de-esterification of pectins may also lead to a general reduction in the apoplastic pool of calcium, leading to weakening of membrane and localised cell death. Bitter pitted fruit had a higher content of their calcium bound in the insoluble pectin fraction (De Freitas et al. 2010). Contrary to previous assertions that cell wall calcium is static, De Freitas et al. (2010) argue that there is a likelihood that the cell wall Ca^{2+} is in a state of constant flux, with Ca^{2+} moving in and out of the cell wall.

Recently, it has been shown that it is not the level of Ca^{2+} that is solely responsible for Ca^{2+} related physiological irregularities or deficiency symptoms. Rather, differences in accumulation of Ca^{2+} in the plasma membrane and cellular organelles may be the cause of some deficiency symptoms observed in certain fruits. The review by Saure (2005) showed that in some cases fruit with Ca^{2+} content equal to normal fruit may develop deficiency symptoms due to localisation of Ca^{2+} in organs such as vacuoles. A recent review by De Freitas et al (2012) has also shown that relative composition of calcium in the plasma membrane and organelles such as vacuoles and endoplasmic reticulum can be linked to some deficiency symptoms such as bitter pit and blossom end rot. In addition, it has been shown that the level of Ca^{2+} in cellular organelles such as vacuoles and endoplasmic reticulum depend on cell wall Ca^{2+} binding capacity and Ca^{2+} movement across membranes (De Freitas et al. 2010). The result of such movement and the relative ratios of Ca^{2+} between organelles, membranes and cell walls have not been studied in most crops.

Very few studies have been reported on the role of calcium in the development of mealiness. The role of Ca^{2+} in mealiness development has been explored though very little was found on the relation between mealiness and Ca^{2+} (Mignani et al. 1995; Saftner et al. 1998). However, the nature and occurrence of mealiness suggest a link with calcium. If the hypothesis that mealiness results from failure of cells to separate at the middle lamella as is claimed for apples (Ng et al. 2013) and pears (Crouch 2011) is true, then calcium is highly likely to be involved as it is found abundantly in this zone (Huxham et al. 1999). Also Ca^{2+} containing solutions increase the rigidity of the cell wall and firmness in fresh cut produce which has been demonstrated in apple and lettuce by Rupasinghe et al. (2005) and Martín-Diana et al (2005), respectively. The high porosity found in apples (Verboven et al. 2008) which also develop mealiness (De Smedt et al.

1998) suggests a link between mealiness and the area of cell to cell contact. Studies have linked calcium to retardation of leaf senescence, closure of stomata, elongation and rigidity of pollen tubes (Hepler 2005, Hepler and Winship 2010). The role of Ca^{2+} in cell to cell binding was demonstrated experimentally (Caffall and Mohnen 2009; Ortiz et al. 2011). Ortiz et al. (2011) showed that application of calcium improved cell to cell adhesion. It plays a role in the egg-box conformation and the homogalacturonan-Ca^{2+} complexes to strengthen cell walls (Caffall and Mohnen 2009; Carpita and McCann 2000).

Whilst it can be accepted that there is link between mealiness disorders and calcium, there are differences in both distribution and expression of symptoms between disorders of calcium and mealiness. In blossom end rot in tomato and bitter pit of apples, symptoms are localised (Simon, 1978; De Freitas et al. 2012). In mealiness however, the development of the disorder is not as localised. Secondly, in bitter pit and blossom end rot symptoms appear as water-soaked tissues, followed by tissue disintegration and dehydration while mealiness does not cause water soaked symptoms. At the cellular level there is also membrane leakage and plasmolysis while at the fruit surface, symptoms appear as depressed dark brown portions (De Freitas et al. 2012). Mealiness on the other hand does not occur as water soaked but as a dry textural disorder. In addition, whereas in bitter pit and blossom end rot symptoms are not reversible, the incidence of mealiness in 'Forelle' has been shown to decrease with extended shelf life (Martin 2002). This makes 'Forelle' mealiness unique from other calcium disorders, assuming calcium is involved in this textural disorder.

2.5 Cell wall compositional differences between mealy and non mealy fruit

Compositional differences between cell walls of mealy and non-mealy fruit have been studied (Crouch 2011, Hobbs et al. 1991). Crouch (2011) as previously reported in this review found significantly lower galacturonic acids associated with the middle lamella of mealy pears. The study also did not find high molecular weight polyuronides, as is observed in peach studies in the CDTA fraction (Brummell et al. 2004). Hobbs et al. (1991) found differences in the composition of xylose and galactose and no differences in the amount of pectin depolymerisation and solubilisation between mealy nectarines compared to non mealy fruit. Brummell et al. (2004) however found that high molecular weight pectins do play a role in peach. The study by Crouch (2011) concluded that cell to cell disintegration in 'Forelle' pear was a result of a broken down middle lamella plus plasmodesmata and not due to high molecular weight pectate gels found in some peach and nectarine studies. The mechanism of

mealiness development in 'Forelle' seems therefore more related to the disintegration of cell to cell adhesion during ripening.

2.6 Role of cell size and cell number on mealiness development

Cell shape and size have been identified as important determinants of fruit size and fruit texture (McAtee et al. 2009). These have been studied in apple disorders, including in apple mealiness (Harker and Hallett 1992; Harpartap et al. 2005; Lammertyn et al. 2002). Studies on apple have shown a link between cell sizes and the readiness of the cell to separate (McAtee et al. 2009). Predisposition to respiratory related disorders in apples was shown to be related to cell size and packing (Verboven et al. 2008). Intercellular airspace size was also found to play a role in apple mealiness (De Smedt et al. 1998). Although mealiness occurs in apples as it occurs in pears, generalisations cannot be made because of the profound differences that exist between apples and pears in fruit growth and ripening behaviour. Apples differ from pears in that there is growth of intercellular spaces during fruit development (Mendoza et al. 2007), an attribute which is absent in pear fruit (McAtee et al. 2009). Secondly, when apples are ripe, they retain a crispy juicy texture for good quality while pears of good quality are juicy and buttery in the mouth (Harpatarp et al. 2005). Independent studies of the roles played by cell sizes, number and intercellular airspaces on pear texture during ripening are therefore critical for the understanding of mealiness in 'Forelle' pears. Micro X-ray computed tomography opens up avenues for 3-D visualisation and quantification of these parameters as well as the cell to cell topography (Mendoza et al. 2007) which should also be employed in further describing the mechanism of mealiness development in pears.

2.7 Role of water and mealiness development

Water plays an important role in plants. In fruit and other succulent organs, water provides structural rigidity through turgor (Jarvis et al. 2003). Its loss from cells through osmosis leads to loss of turgor resulting in flaccid cells. Both conditions seem to be related to mealiness, though the exact role played by water in mealiness has not been established. On the one hand, it is hypothesied that turgor pressure can lead to cell separation at the middle lamella (Jarvis 1998). This is beacause as cells take up water and become turgid, they tend to form a sphere which pulls cells away from cell corners where they are joined (Jarvis 1998). Basing on the previous theories that opinion that mealiness result from cell separation without rupture at cell surface, if turgor leads to separation then it could result in mealiness. However, turgor may also also predispose cells to rupture at the surface when a force is applied (Harker and Hallet 1992; Ozga

et al. 2002), a condition that is often associated with non-mealy fruit. In other studies, a reduction in turgor was found to be associated with severe mealiness in apples (Iwanani et al. 2008). It is argued that a reduction in turgor may lead to flaccid cells which do not break easily upon a force such as mastication hence contents are not released, leading to a dry mealiness sensation. A study of the influence of osmotic potential using mannitol in apples and potatoes showed that more cell-to-cell debending occurred at low turgor while cell rupture occurred at high turgor (Lin and Pitt 1986). More studies need to be done to establish if mealiness could be linked with turgor induced cell separation.

3. Environmental factors associated with mealiness

A number of studies have been done on the role of environmental factors on mealiness. Factors that have been linked with mealiness include exposure to cool temperature in the field (Murayama et al. 1999), storage duration after harvest (Carmichael 2011; Crouch 2011; Martin 2002) and chilling injury (Obenland and Caroll 2000). High temperatures prior to harvest or insufficient cold storage duration have also been linked to mealiness in 'Forelle' (Martin et al. 2003). Environmental factors such as maximum temperatures 6 weeks prior to harvest (Mellenthin and Wang, 1976), harvest maturity (Murayama et al. 1998), storage duration and condition (Chen et al. 1983; Murayama et al. 2002) played a role in mealiness development in 'La France', 'd'Anjou' and 'Marguerite Marillat'. A review by Obenland and Caroll (2000) showed that mealiness in peaches could be a result of chilling abnormalities which cause breakdown of pectin with subsequent accumulation of insoluble, low methoxy pectic substances that can form gels. These subsequently cause cell moisture to gel in the apoplast, resulting in dry, mealy fruit.

Mealiness in peach, nectarine and plum cultivars has been linked to chilling injury and ethylene inhibition (Crisosto et al. 1999; Manganaris et al. 2008; Zhou et al. 2001). Low temperature exposure after harvesting is responsible for stimulating ethylene biosynthesis in 'Forelle' pear during subsequent ripening at room temperatures around 20 $^{\circ}$C (Martin 2002; Crouch 2011). Longer storage duration after harvest was proven to reduce the incidences of mealiness in 'Forelle' pear (Cronje et al. 2015; Martin 2002; Carmichael 2011). However, when 'Forelle' pear fruit were treated with ethylene after a short storage period fruit ripened all mealy (Martin, 2002) and maximum mealiness occurs when ethylene production is at a maximum when ripened after 6-8 weeks of cold storage at -0.5 °C. Longer storage in this case inhibits further ethylene production during ripening, hence perhaps reducing the ability of the fruit to ripen and have lower levels of mealiness. 'Forelle' seems therefore unique in this respect, even when compared

to other pears. Canopy position was also shown to influence development of mealiness (Cronje et al. 2015). Outside canopy fruit were found to be more susceptible to mealiness development than inside canopy fruit. Late harvest, 14-28 days after the optimum time of harvest caused mealiness in 'Marguerite Marillat' and 'La France' pears (Murayama et al. 1998). In 'Forelle' pear Carmichael (2011) also demonstrated that post-optimum maturity fruit had a much higher mealiness incidence. High temperatures 6 weeks prior to harvest caused mealiness in 'd'Anjou' pear (Mellenthin and Wang et al. 1976). Overhead cooling trials indicated that high temperatures 6 weeks prior to harvest did not play a role in 'Forelle' mealiness development (Crouch et al. 2005). In addition to harvest conditions, fruit size has been identified as one of the factors involved in mealiness development (De Smedt et al. 2002). Late harvest and larger size in particular have been shown to promote mealiness in apples.

Reviews done by Crouch (2011) and Carmichael (2011) have expounded on the similarities and differences between mealiness in 'Forelle' and other disorders, with regards particularly to the role played by environmental factors. The two reviews show that while extensive studies have been done on mealiness or wooliness in other fruit, little is known regarding the mechanism that causes mealiness in 'Forelle' pears. The reviews also highlight major disparities that exist between mealiness in 'Forelle' pears and other fruit. Firstly, it is noted that in apples and nectarines, mealiness or wooliness increase with storage duration under cold temperatures, whereas in 'Forelle' mealiness decreases with storage longer than 12 weeks at -0.5 °C (Crouch 2011). Secondly, mealiness in other pear cultivars is influenced by total heat units accumulated six weeks prior to harvest (Mellenthin and Wang 1976), post optimum harvest maturity (Murayama et al. 1998) and prolonged cold storage (Murayama et al. 2002). Except for harvest maturity (Carmichael 2011, Martin 2002), these factors do not cause mealiness in 'Forelle' pears (Crouch et al. 2005; Crouch 2011).

Under laboratory conditions, it was shown that apple mealiness could be induced by applying room temperature (20 °C) and high relative humidity (95%) which in turn is a method to induce senescence (Barreiro et al. 1998). Although ripening due to higher temperatures expresses the disorder it does not cause the disorder as after extended storage all fruit ripen with no or very little mealiness during these conditions (Crouch 2011). No method or technique is currently known that can induce mealiness in 'Forelle' pear. However, harvesting fruit over mature (6.0-5.0 kg) and storing fruit for 6 weeks at -0.5 °C increased the risk for mealiness to occur (Carmichael 2011, Crouch 2011). The inability to induce mealiness or to know whether fruit is going to be mealy is one of the biggest challenges to studies of mealiness.

4. Conclusion

In conclusion mealiness in 'Forelle' pears is a textural disorder which occurs in fruit that have not received sufficient cold storage after harvest or are harvested at post-optimum maturity. The disorder has potential to damage the image of 'Forelle' which has over the years enjoyed the sought after status on the market due to non mealy blushed varieties. Strides have been made to try and manage mealiness by providing sweet, crispy and juicy pears to markets where these fruit are preferred by consumers. While a significant amount of research has been done with apples, peaches and nectarines, pear mealiness research has received relatively little attention. Research on 'Forelle' mealiness has focussed on temperatures 6 weeks prior to harvest, harvest maturity, fruit position in the canopy, storage temperature, ripening, ethylene treatments and cell wall compositional differences between mealy and non mealy fruit. The mechanism behind 'Forelle' mealiness development is not fully understood and more research is needed. Challenges in studying mealiness in 'Forelle' emanate from the fact that the condition cannot be detected from outside the fruit and that ripening needs to occur before mealiness develops. Monitoring of fruit that will become mealy is difficult, as no prior knowledge of its eventual occurrence is available. In addition, there is no instrumental method today that can be used to measure mealiness. Although quantification of extractable juice with a Chylofell has been tried in the past, it is not reliable for very soft fruit. Sensory panels are therefore currently the most widely used method although the method has limitations of being subjective, time consuming and not reliable for batch monitoring. 'Forelle' to our knowledge is the only fruit where mealiness decreases with an extended storage period. It is therefore a unique textural disorder which merits a lot more research in order to further elucidate the mechanism of its development.

Literature Cited

Anderson RE. 1973. Consumer dissatisfaction. The effect of disconfirmed expectancy on perceived product performance. *Journal of Marketing Research,* 10 (1): 38-44.

Barreiro P, Ortiz C, Ruiz-Altisent M, Ruiz-Cabello J, Fernandez-Valle ME, Recasens I, Asensio M. 2000. Mealiness assessment in apples and peaches using MRI techniques. *Magnetic Resonance Imaging,* 18, 1175-1181.

Barreiro P, Ortiz, C, Ruiz-Altisent M, De Smedt V, Schotte S, Andani Z, Wakeling I., Beyts PK. 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples: a collaborative experiment. *Journal of Texture Studies*. 29: 509-525.

Ben-Arie R, Sonego L. 1980. Pectolytic enzyme activity involved in wooly breakdown of stored peaches. *Phytochemistry*, 19: 2553-2555.

Brummell DA, Cin VD, Lurie S, Crisosto CH, Labavitch JM. 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. *Journal of Experimental Botany,* 55: 2041-2052.

Brummell DA, Harpster MH. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology,* 47 (1-2): 311-40.

Brummell DA. 2006. Cell wall disassembly in ripening fruit. *Functional Plant Biology*, 33: 103-119.

Buescher RW, Furmanski RJ. 1978. Role of pectinesterase and polygalacturonase in the formation of wooliness in peaches. *Journal of Food Science*, 43: 264-266.

Caffall KH, Mohnen D. 2009. The structure, function, and biosynthesis of plant cell polysaccharides. *Carbohydrate Research*, 344: 1879-1900.

Campell P, Braam J. 1999. Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall modifying functions. *Trends in Plant Science*, 4: 361-382.

Cantu D, Vicente AR, Labavitch JM, Bennett AB, Powell ALT. 2008. Strangers in the matrix: plant cell walls and pathogen susceptibility. Cell Press. *Trends in Plant Science*, 13(11): 610-617.

Carmichael PC. 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Carpita N, McCann M. 2000. The cell wall. In: Biochemistry and Molecular Biology of Plants. Chapter 2, 52-108. Eds. Buchanan B.B., Gruissem, W. and Jones, R.L.. Amer. Soc. Plant Physiologists, Rockville, Maryland.

Chen PM. Mellenthin WM, Borgic DM. 1983. Changes in ripening behavior of 'd'Anjou' pears (*Pyrus communis* L.) after cold storage. *Scientia Horticulturae*, 21: 137-146.

Chin LH, Ali MZ, Lazan H. 1999. Cell wall modifications, degrading enzymes and softening of carambola fruit during ripening. *Journal of Experimental Botan*y, 50 (335):767-775.

Crisosto CH, Mitchell FG, Ju Z. 1999. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. *HortScience,* 34(6): 1116-1118.

Cronje A, Crouch EM, Muller M, Theron KI, van der Rijst M, Steyn WJ. 2015. Canopy position and cold storage duration affects mealiness incidence and consumer preference for the appearance and eating quality of 'Forelle' pears. *Scientia Horticulturae*, 194:

327-336.

Crouch EM, Holcroft DM, Huysamer M. 2005. Mealiness of 'Forelle' pears_qouvadis? *Acta Horticulturae,* 671: 369-376.

Crouch I, Bergman H, Van Zyl S, Moelich D. 2013. Evaluation of modified atmosphere packaging and other technologies to assist in decreasing the 12 week mandatory cold storage period of Forelle pears. *South Africa Fruit Journal,* 12 (1): 66-69.

Crouch I, Bergman H. 2013a. Feedback on the 2013 Forelle early market access programme. *South Africa Fruit Journal*. Oct/ Nov, 72-80.

Crouch I, Bergman H. 2013b.Consumer acceptance study of early marketed 'Forelle' pears in the United Kingdom and Germany. *South Africa Fruit Journal*, 11 (6): 64-71.

Crouch IJ, Bergman H. 2012. Forelle pears. Post-harvest manipulations to enable versatile marketing of good quality fruit. *South African Fruit Journal*, 9 (4): 35-37.

Crouch, EM, 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L). PhD Thesis. Stellenbosch University.

De Freitas ST, Do Amarante CVT, Labavitch JM, Mitcham EJ. 2010. Cellular approach to understand bitter pit development in apple fruit. *Postharvest Biology and Technology,* 57: 6-13.

De Freitas ST, Mitcham EJ. 2012. Factors involved in fruit calcium deficiency. (1st edn). Jules, J. *Horticultural Reviews*, 40: 107-145. John Willey and Sons, Inc.

De Smedt V, Barreiro P, Verlinden BE, Veraverbeke EA, De Baerdemaeker J, Nicolai BM. 2002. A mathematical model for the development of mealiness in apples. *Postharvest Biology and. Technology,* 25: 64-71.

De Smedt V, Pauwels E, De Baerdemaeker, J, Nikolaï B. 1998. Microscopic observation of mealiness in apples: a quantitative approach. *Postharvest Biology and. Technology,* 14: 151-158.

Deckers T, Schoofs H. 2005. Status of pear production in Europe. Proceedings of the IXth International pear symposium. *Acta Horticulturae,* 671: 47-55.

Department of Agriculture Forestry and Fisheries. 2012. A profile of the South African Pear Market Value Chain. *Directorate Marketing*, Arcadia. South Africa. www.webapps.daff.gov.za/mis.

Donoghue S, de Klerk HM. 2006. Dissatisfied consumers' complaint behavior concerning product failure of major electrical household appliances- a conceptual framework. *Journal of Family Ecology and Consumer Sciences.* Vol 34. ISSN 0378-5254.

FreshFruitPortal. 2012. Apples and pears from Chile. Season Projections for 2012. simFRUIT.www.freshfruitportal.com/wp-content/uploads/.../pears_apples_chile.pdf. (Accessed on 13/10/2014).

Gastol M, Domagala-Swiatkiewicz I. 2006. Effect of foliar sprays on potassium, magnesium and calcium distribution in fruits of the pear. *Journal of Fruit and Ornamental Plant Research,* 14(2): 169-176.

Goulao LF, Santos J, de Sousa I, Olivera CM. 2007. Patterns of enzymatic activity of cell modifying enzymes during growth and ripening of apples. Postharvest Biology and Technology, 43: 307-318.

Hamadziripi ET. 2012. The effect of canopy position on the fruit quality and consumer preference of apples. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Harholt J, Suttangkakul A, Scheller HV. 2010. Biosynthesis of pectin. *Plant Physiology*, 153: 384-395.

Harker FR, Hallett IC. 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. *HortScience,* 27 (12): 1291-1294.

Harpartap M, Bedford D, Luby J, Vickers Z, Tong C. 2005. Relationship of instrumental and sensory texture measurements of fresh and stored apples to cell numbers and size. *HortScience,* 40 (6): 1815-1820.

Henderson JD. 2000. Innovative methods to measure consumer acceptability of fresh and formulated foods. Msc Thesis. The University of Georgia.

Hepler KP, Winship, LJ. 2010. Calcium at the cell wall cytoplast interface. *Journal of Integrative Plant Biology*, 52: 147-160.

Hepler KP. 2005. Calcium: A central regulator of plant growth and development. *The Plant Cell*, 17: 2142-2155.

Hobbs MC, Easterbrook KM, Melton LD. 1991. Cell wall material composition of mealy fruit among ripening nectarines. *Journal of the Science of Food and Agriculture,* 57: 141-145.

Hong-Bo S, Wei-Yi S, Li-Ye, C. 2008. Advances of calcium signals involved in plant anti-drought. *C.R. Biologies*, 331: 587-596.

HORTGRO. 2013. Key Deciduous Fruit Statistics, Paarl, South Africa, www.hortgro.co.za (accessed 23/03/2015).

HORTGRO. 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

25

Hurndall RF. 2011. 'Forelle' dispensation procedures for 2011 season. http://www.decidous.co.za (accessed 10/09/2014).

Huxham IM, Jarvis MC, Shakespeare L, Dover CJ, Johnson D, Knox JP and Seymour GB. 1999. Electron-energy-loss spectroscopic imaging of calcium and nitrogen in the cell walls of apple fruits. *Planta,* 208: 438-443.

Isabel S, Luis FG, Joáo S, Monic OC. 2007. Patterns of enzymatic activity of cell-modifying enzymes during growth and ripening of apples. *Postharvest Biology Technology,* 43 (3): 307-308.

Iwanani H, Moriya S, Kotoda N, Abe K. 2008. Turgor closely relates to postharvest fruit softening and can be useful index to select a parent for producing cultivars with good storage potential. *HortScience,* 43(5): 1377-1381.

Jarvis CM, Briggs SPH, Knox JP. 2003. Intercellular adhesion and cell separation in plants. *Plant Cell and Environment*, 26: 977-989.

Jarvis MC. 1998. Intercellular separation forces generated by intercellular pressure. *Plant, Cell and Environment,* 21: 1307-1310.

Lammertyn J, Verlinden BE, Nicolaï BM. 2002. Applying advanced instrumental methods: mealiness in fruit. In: Jongen W. (eds). *Fruit and vegetable processing. Improving quality*. Chapter 9: 170-187. CRC Press, Boca Raton, Florida, USA and Woodhead Publishing Limited, Abington Cambridge, England.

Lurie S, Crisosto CH. 2005. Chilling injury in peach and nectarine. *Postharvest Biology and Technology*, 37: 195-208.

Manganaris GA, Vicente AR, Crisosto CH, Labavitch JM. 2008. Cell wall modifications in chilling injured plum fruit (*Prunus salicina*). *Postharvest Biology and Technology,* 48: 77-83.

Manning N. 2009. Physical, sensory and consumer analysis of pear genotypes among South African consumers and preference of appearance among European consumers. Thesis: MSc. Food Science. *University of Stellenbosch*, *Department of Food Science*. Stellenbosch. South Africa.

Martin EM. 2002. Ripening responses of 'Forelle' pears. MSc (Agric) Thesis, *Department of Horticultural Sciences*, Stellenbosch University, South Africa.

Martin-Diana AB, Rico D, Barry-Ryan C, Frias JM, Mulcahy J, Henehan GTM, 2005. Calcium pectate washing treatments for salad-cut Iceberg lettuce: effect of temperature and

concentration on quality retention parameters. *Food Research Intenational,* 38: 729-740.

McAtee PA, Hallett IC, Johnston J, Schaffer RJ. 2009. A rapid method of fruit cell isolation for cell size and shape measurements. *Plant Methods*, 5: 5.

Mellenthin WM, Wang CY. 1976. Preharvest temperatures in relation to postharvest quality of 'd'Anjou' pears. *Journal of the American Society for Horticultural Sciences,* 101: 302-305.

Mendoza F, Verboven P, Mebatsion HK, Kerckhofs G, Wevers M, Nicolai B. 2007. Three-dimensional pore space quantification of apple tissue using X-ray computed microtomography. *Planta*, 226: 559-570.

Mignani I, Greve LC, Ben-Arie, R, Stotz HU, Li C, Shackel KA, Labavitch JM. 1995. The effect of GA_3 and divalent cations on aspects of pectin metabolism and tissue softening in ripening tomato pericarp. *Physiologia Plantarum*, 93: 108-115.

Murayama H, Takahashi T, Honda R, Fukushima T. 1998. Cell wall changes in pear fruit softening on and off the tree. *Postharvest Biology and Technology*, 14: 143-149.

Murayama H, Takahashi T, Honda T, Fukushima T. 1999. Cell wall changes in pear softening and cell wall polysaccharides in pears after different storage periods. *Postharvest Biology and Technology,* 26: 15-21.

Murayama H, Takahashi T, Horiuchi O, Fukushima T. 2002. Relationship between fruit softening and cell wall polysaccharides in pears after different storage periods. *Postharvest Biology and Technology,* 26: 15-21.

Newman S. 2006. Stonefruit mealiness- the quiet destroyer. Netpro Update. NSW Department of Primary Industries. Australia.

Ng JKT, Schroder R, Sutherland PW, Hallett IC, Hall MI, Prakash R, Smith BG, Melton LD, Johnston JW. 2013. Cell wall structures leading to cultivar softening rates develop early during apple (*Malus x domestica*) fruit growth. *BMC Plant Biology,* 13: 183. (http://.biomedcentral.com/147-2229/13/183).

Nguyen TA, Drasselaers, T, Verboven P, D'hallewin, G, Culeddu N, Van Hecke P, Nicolai BM. 2006. Finite element modelling and MRI validation of 3D transient water profiles in pears during postharvest storage. Journal of Science of Food and Agriculture. 86: 745-756.

Nobile MP, Wattebled F, Quecini V, Girardi CL, Lormeau M, Laurens F. 2011. Identification of a novel α-L-arabinofurasidase gene associated with mealiness in apple. *Journal of Experimental Botany,* 62 (12): 4309-4321.

Obenland DM, Caroll TR. 2000. Mealiness and pectolytic activity in peaches and nectarines in response to heat treatment and cold storage. *Journal of the American Society for Horticultural Science,* 125 (6): 723-728.

Obenland DM, Crisosto, CH, Rose, JKC. 2003. Expansin protein levels decline with the development of mealiness in peaches. *Postharvest Biology and Technology*, 29: 11-18.

Obenland DM, Vensel WH, Hurkman WJ. 2008. Alterations in protein expression associated with the development of mealiness in peaches. *Journal of Horticultural Science and Biotechnology*, 83 (1): 85-93.

Obenland DM, Vensel WH, Hurkman WJ. 2008. Alterations in protein expression associated with the development of mealiness in peaches. *Journal of Horticultural Science and Biotechnology*, 83 (1): 85-93.

Orfila C, Seymour GB, Willats WGT, Huxham JM, Jarvis MC, Dover CJ, Thompson AJ. 2001. Altered middle lamella homogalacturonan in the pericarp of Cnr, a ripening mutant of potato. *Plant Physiology*, 126 (1): 220-221.

Ortiz A, Graell J, Lara I. 2011. Preharvest calcium applications inhibit some cell wall-modifying enzyme activities and delay cell wall disassembly at commercial harvest of 'Fiji Kuku-8' apples. *Postharvest Biology and Technology*, 62: 161-167.

Payasi A, Mishra NN, Chaves AL, Singh R. 2009. Biochemistry of fruit softening: an overview. *Physiology and Molecular Biology of Plants*, 15(2): 103-113.

Pena MJ, Capita NC. 2004. Loss of highly branched arabins and debranching of rhamnogalacturonan I accompany loss of texture and cell separation during prolonged storage of apple. Plant Physiology, 135: 1305-1313.

Raffo MD, Ponce NMA, Sozzi GO, Stortz CA, Vicente, AR. 2012. Changes on the cell wall composition of tree-ripened "Bartlett" pears (*Pyrus communis* L.). *Postharvest Biology and Technology,* 73: 72-79.

Rose JKCR, Catala C, Gonzalez ZH, Roberts JA. 2003. Cell wall disassembly. In: Rose JKCR. 2003. (eds). *The plant cell wall*. Blackwell Publishing. CRS Press. New York, USA. pp 264-305.

Rupasinghe HPV, Murr DP, De'Ell JR, Odumeru J. 2005. Influence of 1-methylcyclopropene and Natureseal on the quality of fresh-cut 'Empire' and 'Crispin' apples. *Journal of Food Quality*, 28: 289–307.

Saftner AA, Conway, WS, Sams, CE. 1998. Effect of postharvest calcium chloride treatments on tissue water relations, cell wall calcium levels and postharvest life of 'Golden

Delicious' Apples. *Journal of the American Society for Horticultural Science*, 123 (5): 893-897.

Saha BC. 2000. α-L-arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. *Biotechnology Advances*, 18: 403-423.

Saure MC. 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control, *Scientia Horticulture*, 105: 65-89.

Segonne SM, Bruneau M, Jean-Marc GC, Le S, Francin-Allami M, Juchaux M, Laurens F, Orsel M, Jean-Pierre R. 2014. Multiscale investigation of mealiness in apple: an atypical role for a pectin methylesterase during fruit maturation. *BMC Plant Biology*, 14: 375. DOI 10.1186/s12870-014-0375-3.

SimFruit. 2015. Chile. Pear exports to increase in 2015. http://www.freshplaza.com/article/133968 (accessed on 15/04/2015).

Simon EW. 1978. The symptoms of calcium deficiency in plants. *New Phytology*, 80: 1-15.

Steenkamp E. 2014. FEMA_ A blushing success. *South African Fruit Journal*, Aug/Sep: 8-9.

Steyn WJ, Manning N, Muller N, Human JP. 2011. Physical, sensory and consumer analysis of eating quality and appearance of pear genotypes among South African consumers. *Acta Horticulturae,* 909: 579-586.

Theron KI, van der Merwe PW, Ferrandi CH. 2008. Pear Production in South Africa. *Acta Horticulturae,* 800: 89-93.

Trepel C, Fox CR, Poldrack RA. 2005. Prospect theory on the brain: Towards cognitive neuroscience of decision under risk. *Cognitive Brain Research,* 23: 34-50.

Verboven P, Kerckhofs G, Mebatsion HK, Ho QT, Temst K, Wevers M, Cloetens P, Nicolai BM. 2009. Three- dimensional gas exchange pathways in pome fruit characterised by synchroton x-ray computed tomography. *Plant Physiology,* 147: 518-527.

Vicente RA, Costa ML, Martínez GA, Chaves AR, Civello PM. 2005. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology Technology*. 38: 213-222.

Villalobos-Acuna M, Mitcham EJ. 2008. Ripening of European pears: the chilling dilemma. *Postharvest Biology Technology,* 49:187-200.

Von Mollendorf L. 2008. *New redflush pear in South Africa*. http://www.agriworldsa.com/article-archive/deciduous-fruit/new-redflush-pear-in-south-africa/ (accessed on 10/12/2015).

Voragen GJ, Coenen JG, Verhoef RP, Schols HS. 2009. Pectin, a versatile polysaccharide present in plant cells. *Structural Chemistry,* 20: 263-275.

White PJ. 2003. Calcium in Plants. *Annals of Botany*, 92: 487-511.

Xiong T, Bourque S, Lecourieux D, Amelot N, Grat S, Briere C, Mazars C, Pugin A, Ranjeva R. 2006. Calcium signalling in plant cell organelles delimited by a double membrane. *Biochimica et Biophysica Acta*, 1763: 1209-1215.

Yoshioka H, Hayama H, Tatsuki M, Nakamura Y. 2010. Cell wall modification during development of mealy texture in the stony-hard peach 'Odoroki' treated with propylene. *Postharvest Biology and Technology*, 55: 1-7.

Zhou H, Dong L, Ben-Arie R, Lurie S. 2001. The role of ethylene in the prevention of chilling injury in nectarines. *Journal of Plant Physiology*, 158: 55-61.

Zhou H, Luri S, Lers, A, Khatchitski A, Sonego L, Ben- Arie R. 2000. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent wooliness. *Postharvest Biology Technology*. 18: 133-144.

PAPER 1

The role of 'free' and cell wall bound Ca^{2+} on the development of mealiness in 'Forelle' pear (*Pyrus communis*. L.)

Abstract

'Forelle' pears are susceptible to developing mealiness, a textural disorder characterised by dry and soft tissue. Mealiness in 'Forelle' pears occurs mostly in fruit stored for between 6 and 12 weeks at -0.5 °C and ripened. The aim of this study was to investigate whether a link between calcium (Ca^{2+}) and the development of mealiness in 'Forelle' pear exists. Fruit were harvested at optimum harvest maturity (ca. 6.4 kg). Fruit samples were flash frozen in liquid nitrogen and milled. Cell walls were extracted using 96% v/v ethanol, washed with 100% v/v acetone to dry and used for a 24 h water extraction. Three fractions of cell wall Ca^{2+} were determined; alcohol/acetone soluble Ca^{2+}, water soluble Ca^{2+} and cell wall bound Ca^{2+}. Alcohol soluble and water soluble fractions were together considered as available Ca^{2+} (symplastic and apoplastic). Alcohol soluble $Ca^{2+,}$ water soluble Ca^{2+} and total Ca^{2+} differed significantly due to the interaction of location and mealiness. Results suggest that alcohol soluble Ca^{2+} and to a lesser degree total Ca^{2+} in 'Forelle' pears differed with mealiness, however this was depended on the location.

Key words

 Pyrus communis L., cell wall extraction, mealiness, sequential extraction, cell wall bound calcium, free calcium, texture

1. Introduction

Mealiness or low extractable juice content is a dry textural disorder of apples, nectarines, pears and other freshly harvested horticultural commodities (Barreiro et al., 2000; Lammertyn et al., 2002; Obenland and Carroll, 2000). The disorder has been linked to separation of cells at the cell wall due to a weak middle lamella (Harker and Hallet, 1992, McAtee et al., 2009), and to differences in cell wall breakdown (Crouch, 2011). 'Forelle' pears develop mealiness during shelf life when not cold stored sufficiently (-0.5 °C) prior to ripening under shelf life conditions. Research has been reported on 'Forelle' mealiness during the past two decades, covering the environmental conditions causing mealiness, the influence of rootstocks, effect of intermittent post-harvest warming, controlled and regular atmosphere storage conditions, post-optimum harvest maturity, storage duration after harvest as well as cell compositional differences between mealy and non-mealy fruit (Carmichael, 2011; Crouch, 2011; de Vries and Hurndall, 1993; Martin et al., 2003). Very few studies have looked at mealiness at the cellular or biochemical level.

Mealiness, like many other fruit textural disorders has been linked to the cell wall structure and the strength of adhesion between neighbouring cells (Harker and Hallet, 1992). In 'Forelle' pears, mealiness is associated with cell separation between neighbouring cells (Crouch, 2011) as opposed to breaking of cell walls in non-mealy fruit during mastication (Harker and Hallet, 1992). In other pears, cells stay intact even in soft, juicy and overripe fruit. Ca^{2+} has been suggested that the Ca2+ bridges may be involved in binding strength at the middle lamella (Crouch, 2011). Why then is the middle lamella of mealy 'Forelle' weaker than that of other pears? Research with the calcium chelator EDTA enabled researchers to establish that by removing Ca^{2+} from the pectates holding cells together, cell-to-cell adhesion is weakened, as is observed in mealy tissues (Hepler, 2005; McAtee et al., 2009). Calcium plays a vital role in plant cells and in maintaining structural integrity in plant tissues and organs and in regulating cell wall structure and function. Calcium confers structural rigidity to the cell wall through cross linking with negatively charged regions of pectin (Hepler and Winship, 2010). Fruit with higher Ca^{2+} levels have been shown to exhibit lower respiration rates and longer shelf life than fruit with low calcium (Gastol and Domagala-Swiatkiewicz, 2006). In addition, fruit softening is also retarded by higher concentration of Ca^{2+} (Johnston et al., 2002). Thus, the influence of Ca^{2+} on the disorder is based on its role in maintaining cell wall integrity and structure.

Calcium is an integral component of the cell wall where it plays a significant role in determining the structure and function of the wall (Hepler and Winship, 2010). The cell wall is comprised of carbohydrate polymers known as polysaccharides (Carpita and McCann, 2000; Caffall and Mohnen, 2009). The major polysaccharides are cellulose microfibrils which are cross-linked with glycans, xyloglucans and glucuronoarabino-xylans. Embedded within this matrix are pectins whose negatively charged regions are cross-linked with Ca^{2+} (Hepler and Winship, 2012). At least 60% of the total Ca^{2+} in some plant cells is associated with the cell wall fraction while the remainder is found predominantly in the vacuole (Fallahi et al., 1997). Because of its abundance and central role in maintaining cell wall integrity, Ca^{2+} has been linked to ripening disorders. Even where other minerals have been found to associate with postharvest fruit disorders like bitter pit in apples, Ca^{2+} has been found to play a synergistic or antagonistic role (Fallahi et al, 1997).

Managing fruit disorders linked to Ca continues to be a challenge in spite of decades of research. This has mainly been due to the complexity of processes and factors governing Ca translocation within fruit (Saure, 2005). Where attempts have been made to understand the role of Ca in bitter pit, results showed that it is not total Ca^{2+} per se that causes Ca deficiency in some fruit, but differences in deposition in the vacuole or binding to the cell wall (De Freitas et al., 2010). Compounding the problem is that Ca^{2+} continues to be translocated within the fruit after harvest and during storage (Holdaway-Clarke et al., 2000). Mealiness in 'Forelle' is one disorder where the role of Ca^{2+} has not been adequately explored. Strategies for control of mealiness therefore require an understanding of the roles of Ca^{2+} at the cellular level. We therefore aimed to establish the role played by cell wall yield, free Ca^{2+} in the cell, as well as cell wall bound Ca^{2+} on the development of mealiness in 'Forelle' pears.

2. Materials and Methods

2.1 Fruit material

Fruit for the study were harvested in March 2012 from four farms located in the Warm Bokkeveld area, South Africa, namely Koelfontein (lat. 33.00°S, long. 19.33°E), Buchuland (lat. 33.47°S, long 19.31°E), La Plaisante (lat. 33.41°S, long 19.19°E) and Achtertuin (lat. 33.39°S, long 19.34°E). Fruit was harvested at commercial harvest maturity (ca. 6.4 kg firmness) from 10 randomly selected plots consisting of 2 trees in each farm/ orchard. Twenty fruit were harvested from each tree, around shoulder height on either side of the canopy. Fruit were packed in high

density polyethylene (37.5 µm) lined pear boxes and stored at -0.5 °C until required for evaluations.

2.2 Maturity evaluations

The first maturity evaluation was done a day after harvest (0w+0d), using a subsample of 100 fruit (10 reps of 10 fruit each). Another subsample of 100 fruit from each farm was evaluated after cold storage of 8 weeks at -0.5 °C and finally a last evaluation at the end of seven days of ripening at 20 °C. Average fruit diameter (mm), fruit weight (g), titratable acidity (TA), total soluble solids concentration (TSS) and fruit firmness (kg) were measured. Fruit firmness was measured equatorially on opposite, pared sides of the fruit using a universal fruit texture analyzer (FTA 2007, Guss, Strand, South Africa), fitted with a 7.9 mm diameter probe. Fruit diameter and fruit weight were measured using a Cranston gauge and electronic balance, respectively, both fitted to the fruit texture analyser. Total soluble solids (TSS) were measured in a drop of expressed juice from 10 fruit using a hand held digital refractometer (PR-32, Atago, Tokyo, Japan). Titratable acidity was measured by titration of individual fruit juice using an automated titrator (Metrohm AG 760; Harison, Switzerland). Ten gram (10 g) of expressed juice from each individual fruit was titrated with 0.1 M NaOH to an end-point of pH 8.2.

2.3 Mealiness evaluation and milling

Mealiness evaluations were done at the end of shelf life using two methods. The first involved an organoleptic assay by a panel of three trained evaluators. Secondly, a confined compression test was done to determine the expressible juice, which in turn was used to score for mealiness (Barreiro et al., 1998). Fruit were categorised as mealy and non-mealy based on both the organoleptic tests and expressible juice released. After mealiness determination, all mealy fruit and an equal number of non-mealy fruit were taken for calcium determination. The fruit were peeled using a potato peeler and diced into small pieces, flash frozen in liquid nitrogen and stored at -80 °C. Frozen tissue was then milled using a laboratory blender (Waring Commercial, United Scientific Ltd., Cape Town) and packed in 50 mL centrifuge bottles and stored at -80 °C until required for cell wall extractions.

2.4 Sequential cell wall extraction

Milled samples were weighed (ca. 36 g) and placed in 500 mL conical flasks. Ethanol (160 mL of 96% (v/v)) was added and the flask was covered with aluminium foil. The sample was boiled for 20 min, in order to inactivate wall-modifying enzymes (Carrington et al., 1993). The sample

was allowed to cool down for 15 min and homogenised using an Ultra-Turrax blender (Ultra-Turrax, IKA T18 basic, Germany), for 30 sec. Cell wall debris remaining on the Ultra-Turrax was washed using 2 x 10 mL alcohol. The homogenised sample was then vacuum filtered through glass microfiber filter (Whatman^{TM}, GE Healthcare, UK). The alcohol filtrate was collected. The residue remaining after filtration was washed with 2 x 100 mL ethanol (96% v/v) through the filter paper and again with 2 x 50 mL acetone (100% v/v). The alcohol/acetone filtrate plus acetone and alcohol used for washing the cell wall pellet were collected in conical flasks and mixed. The residue remaining on the filter paper was gently scraped off and air dried to a constant weight at room temperature (ca. 48 h). The dry pellet was weighed and the cell wall yield determined and called the alcohol insoluble residue (%AIR). This was expressed as a proportion of total dry cell wall to the fresh tissue weight, as a percentage.

$$\%AIR = \frac{\text{dry cell wall after extraction with alcohol and acetone}}{\text{weight of milled fresh tissue used}} \text{ X } 100\%$$

2.5 Determination of alcohol soluble Ca^{2+}

The collected filtrate (30 mL) was concentrated in a savant (Model SC 210A, Speed Vac Concentrator, Thermo Scientific, USA) until the liquid volume dropped to 1 mL, in order to get rid of the alcohol and acetone. Distilled water (14 mL) was added to make up the volume to 15 mL. Ca^{2+} was determined from the mixture by calorimetric method using the Thermo ICAP 6300 ICP-AES instrument (Thermo Scientific, USA). The Ca^{2+} determined was called the alcohol soluble Ca^{2+}.

2.6 Determination of water soluble Ca^{2+}

The AIR was weighed (ca. 0.2 g) and placed in a 50 mL centrifuge tube. Distilled and de-ionized water (Merck-Millipore, Germany) was added (30 mL) and the mix was shaken overnight on a rotary shaker (Model KS 500, Janke & Kunkel, Germany) for 16 h. The mixture was then centrifuged at 3220 g (4000 rpm) for 10 min using the Eppendorf AG 22331 centrifuge (Centrifuge 5810R, Hamburg, Germany). After centrifuging, a supernatant was gently collected in a beaker. A second volume of 30 mL distilled water was added to the pellet and centrifuged for the second time. The two supernatants were mixed and a 15 mL aliquot was collected for Ca^{2+} determination using the Thermo ICAP 6300 ICP-AES instrument. The Ca^{2+} determined from this fraction was called water soluble Ca^{2+} fraction. Free cel wall Ca^{2+} was defined as that

component of Ca^{2+} that is not bound in the cell wall and could be extracted by ethanol and water. The following formula was used to determine free cell wall Ca^{2+}.

$$Free\ Ca^{2+} = alcohol\ soluble\ Ca^{2+} + water\ soluble\ Ca^{2+}$$

2.7 Determination of cell wall bound Ca^{2+}

The cell wall pellet which remained after centrifuging the alcohol insoluble residue with distilled water was dried at room temperature until it reached a constant weight. The dried residual cell wall was weighed (ca. 0.1 g) and was digested with nitric acid (HNO_3) for Ca^{2+} determination. Nitric acid (7 mL) was added to 0.1 g of the cell wall residue, and allowed to stand for 20 min, after which the sample was put in a MARS digester (CEM Corporation, US). Samples were digested at 1600 W, at a pressure of 800 psi and temperature of 180 °C. The time taken to reach the target temperature, also referred to as ramp time was set at 20 min. After digestion, the sample was allowed to cool down for 25 min. Deionised water was added to the digested sample to make up the volume to 50 mL. Calcium was then determined using the Thermo ICAP 6300 ICP-AES instrument. A schematic illustration of the sequential extraction process is shown in Fig. 1.

2.8 Data analysis

Factorial analysis of variance (ANOVA) was performed on Ca^{2+} data using Statistica 11 (StatSoft, Tulsa, OK, USA). Mean separation was done using Fisher's least significant difference ($LSD_{0.05}$).

3. Results

3.1 Mealiness and cell wall yield (%AIR)

Analysis of variance of cell wall yield showed no significant interaction between location and mealiness on cell wall yield (Table 1). Farm and mealiness had no significant effect on cell wall yield (P>0.05).

3.2 Alcohol soluble Ca^{2+}

The alcohol soluble Ca^{2+} concentration was affected by the interaction of location and mealiness (P=0.0466) (Table 2). At Achtertuin and La Plaisante, non-mealy fruit had higher alcohol

soluble Ca^{2+} compared to mealy fruit. At Buchuland and Koelfontein, differences were, however, not significant.

3.3 Water soluble Ca^{2+}

There was a significant interaction between location and mealiness on the water soluble Ca^{2+} (P=0.00033) (Table 2). Significantly higher water soluble Ca^{2+} was observed in mealy fruit compared to non-mealy fruit at Koelfontein. At the other three locations however, differences were not significant.

3.4 Total soluble or free Ca^{2+}

Total soluble Ca^{2+} was considered as the sum of the Ca^{2+} concentration in the alcohol soluble and water soluble fractions. There was a significant interaction between location and mealiness on soluble / free Ca^{2+} (P=0.0466) (Table 2). Non-mealy fruit had significantly higher available Ca^{2+} compared to mealy fruit at Achtertuin and La Plaisante (Table 2). However, differences were not significant at Buchuland and Koelfontein.

3.5 Cell wall bound Ca^{2+}

The Ca^{2+} concentration of the cell wall pellet remaining after the ethanol and water extractions represented the Ca^{2+} bound in the cell wall. The interaction between location and mealiness had a significant effect on the level of Ca^{2+} in the remaining cell wall residue (P=0.0135) (Table 2). The concentration of the cell wall Ca^{2+} in mealy and non-mealy fruit was variable and not significantly different between mealy and non-mealy fruit at the majority of sites, except at La Plaisante where non-mealy fruit had higher cell wall bound Ca^{2+} than mealy fruit.

3.6 Total Ca^{2+}

Farm and mealiness interaction had a significant effect on total Ca^{2+} (P=0.023) (Table 2). Non-mealy tissues had a higher Ca^{2+} concentration compared to mealy fruit at Achtertuin, Buchuland and La Plaisante (Table 2). At Koelfontein, the difference between total Ca^{2+} composition of mealy and non-mealy was not significant.

3.7 Relationship between different Ca^{2+} fractions

The contribution of alcohol soluble Ca^{2+} to the total Ca^{2+} was variable across locations, ranging between 38.7 % at Koelfontein and 70 % at La Plaisante (Fig. 2). The water soluble Ca^{2+} fraction was relatively small, ranging between 3.4 % at La Plaisante to 10.5 % at Koelfontein.

37

The relationship between soluble or free Ca^{2+} and the total Ca^{2+} concentration was further expressed as a ratio (Fig. 3). The ratio of soluble Ca^{2+} to total Ca^{2+} was higher in non-mealy compared to mealy fruit, though results were not significant (P = 0.5725). Ratios of soluble Ca^{2+} to the total Ca^{2+} ranged from 0.49 for mealy fruit at Koelfontein to 0.73 for non-mealy fruit at La Plaisante (Fig. 3). Non-mealy fruit had relatively larger percentages of 'soluble Ca^{2+}' to total Ca^{2+} compared to mealy fruit. The contribution of soluble Ca^{2+} to total Ca^{2+} in mealy and non-mealy fruit was not different at Buchuland.

4. Discussion

4.1 Cell wall yield

Cell wall yield (%AIR) was affected neither by mealiness nor by location. The average cell wall yield was around 2.46% of the fresh cell tissue, indicating that about 97% of the fruit is water. Cell walls in fruit such as pear are composed of cellulose, pectins and xylans (Renard, 1995). Results from this study are in agreement with findings by Crouch (2011) who found a comparable cell wall yield between mealy and non-mealy fruit. Renard (2005) also found cell walls representing 23g DW. kg^{-1} FW, equivalent to 2.3% of tissue mass. Previous findings also indicated that there is no difference in cell wall yield between mealy and non-mealy tissues, but that cell wall yield rather decreases with storage time during ripening especially after longer term storage (Cronje, 2014; Crouch, 2011; Ng et al., 2013). Harker and Hallet (1992) observed cell wall yields of apple fruit decreasing in cold storage, which they attributed to degradation of cell wall material during ripening.

4.2 Role of mealiness on soluble, bound and total Ca^{2+}

Four fractions of 'Forelle' pear cell wall Ca^{2+} were determined. Ca^{2+} determined from the alcohol and acetone filtrate was termed alcohol soluble Ca^{2+}. The Ca^{2+} extracted by water was called water soluble Ca^{2+}. Alcohol soluble and water soluble Ca^{2+} together represented 'soluble or free' Ca^{2+}' while cell wall residue Ca^{2+} represented the 'cell wall bound' Ca^{2+}. Together, the three fractions made up the total Ca^{2+}. Non-mealy fruit had significantly more total Ca^{2+} compared to mealy fruit at Achtertuin, Buchuland and La Plaisante farms. The highest Ca^{2+} was observed at La Plaisante (7.31 mg.g^{-1} FW) in non-mealy fruit followed by Achtertuin in non-mealy fruit (7.19 µg.g^{-1} FW). The lowest Ca^{2+} concentration was observed at Buchuland in mealy fruit (3.89 µg. g^{-1} FW). At Koelfontein where results were not significantly different, non-mealy fruit had 6.2 % more total Ca^{2+} when compared to mealy fruit. The Ca^{2+} content observed in this study was low comparable to that observed in apples (Saure, 2005). The

38

maximum concentration of fruit Ca^{2+} is normally reached shortly after flowering and decreases as the fruit enlarges mainly because fruit growth outpaces Ca^{2+} translocation (Saure, 2005).

Although at all locations it seems fruit with higher total Ca^{2+} concentration were non-mealy, it could not be said that total Ca^{2+} alone was responsible for mealiness. Different farms had different levels of Ca^{2+}, irrespective of their mealiness incidence. At Achtertuin for example, fruit with a concentration of 5.92 µg. g^{-1} FW were found to be mealy while at Buchuland fruit with a low concentration of 4.34 µg. g^{-1} FW, were non-mealy. These results suggest that apart from total Ca^{2+} content, other locational factors also contribute to mealiness. Therefore it can be argued that it is not solely the absolute Ca^{2+} composition that causes mealiness, but probably its association with other factors.

Cell wall soluble / free Ca^{2+} was statistically higher in non-mealy than in mealy fruit at Achtertuin and La Plaisante. The same trend was observed at Buchuland though results were not significantly different. The difference in soluble/ free Ca^{2+} between mealy and non-mealy fruit was attributed mainly to the alcohol soluble Ca^{2+} fraction, which was significantly higher in non-mealy than mealy fruit at the three locations. Soluble or free Ca^{2+} is presumed to be located predominantly in the middle lamella and in organelles such as vacuoles. This fraction can easily be washed out from the fresh tissues (Fallahi, et al., 1997) and the cell wall part of this fraction contains oligosaccharides with a degree of polymerisation of less than five (Martin-Cabrejas et al., 1994). Free Ca^{2+} is said to continue to be translocated within the fruit even after harvest and throughout storage and shelf life (Saure, 2005). This component is associated with water soluble compounds such as organic acids, chlorides and nitrates, exchangeable Ca^{2+}, adsorbed on pectins, such as calcium phosphates, carbonates and oxalates.

De Freitas and Mitcham (2012) explained the role played by free / soluble Ca^{2+} in the development of bitter pit. The authors attributed deficiency symptoms to low water soluble Ca^{2+} concentration in the apoplast. Others argue that elevated alcohol soluble Ca^{2+} or free Ca^{2+} composition in the intercellular spaces causes a negative feedback effect on enzymes responsible for dissolution of middle lamella, particularly pectin methylesterases, pectate lyases, β-galactosidases, α-t-arabinofuranosidase and β-xylosidases (Hepler and Winship, 2010; Ortiz et al. 2011). Once enzymes are inhibited, it is assumed that strong binding occurs between cells as the middle lamella is not weakened resulting in cells breaking at the cell wall upon mastication, as evidenced by non-mealy fruit. Conversely, where low levels of free calcium were found in

mealy tissues, enzymes responsible for middle lamella depolymerisation and dissolution would continue uninhibited by Ca^{2+}, producing a very weak middle lamella which could cause cells to slide upon mastication.

In summary, our data showed that mealiness is associated with the alcohol soluble Ca^{2+} or cell wall free Ca^{2+} fraction, though this depended on location. The cell wall bound Ca^{2+}, represented by the Ca^{2+} which remains in the cell wall fraction after extraction with alcohol and water had no influence on mealiness. Total Ca^{2+} however, seems to have an influence on mealiness development, though the critical levels required for mealiness to occur may differ across locations. Results from the current study and other previous research increasingly reinforce the theory that free Ca^{2+} is more active biologically and mediates physiological disorders such as mealiness.

5. Conclusion

The role of different Ca^{2+} fractions within the cell wall of mealy fruit was investigated. Results showed a link between mealiness and different Ca^{2+} fractions of the fruit. Depending on location, low levels of Ca^{2+} were associated with mealy fruit. Cell wall free Ca^{2+} constituted between 49% and 73% of the total Ca^{2+}. The flux of Ca^{2+} across organelles, cell-to-cell adhesion, strength of the cell wall, permeability of the plasma membrane as well as the activity of disassembly enzymes may all be influenced by the free Ca^{2+} and Ca^{2+} may therefore play an indirect role in the development of the mealiness disorder. Although free Ca^{2+} seems to differ for mealy and non-mealy fruit, a clear mechanism of the involvement of Ca^{2+} in the development of mealiness and free Ca^{2+} associated reactions as well as the minimum level of free Ca^{2+} associated with mealiness requires further research. .

Literature Cited

Barreiro, P., Ortiz, C., Ruiz_Altisent, M., Ruiz_Cabello, J., Fernandez_Valle, M.E., Recasens, I., Asensio, M., 2000. Mealiness assessment in apples and peaches using MRI techniques. Magn. Reson. Imag. 18, 1175-1181.

Barreiro, P., Ortiz, C., Ruiz-Altisent., M., De Smedt, V., Schotte, S., Andani, Z., Wakeling, I., Beyts, P.K., 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples. A collaborative test. J. Texture Stud. 29, 509-525.

Caffall, K.H., Mohnen, D., 2009. The structure, function, and biosynthesis of plant cell polysaccharides. Carbohydr. Res. 344, 1879-1900.

Carmichael PC. 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Carpita, N. and McCann, M, 2000. The cell wall. In: Biochemistry and Molecular Biology of Plants. Chapter 2, 52-108. Eds. Buchanan B.B., Gruissem, W. and Jones, R.L.. Amer. Soc. Plant Physiologists, Rockville, Maryland.

Carrington, C.M.S., Greve, C.L., Labavitch, J.M., 1993. Cell wall metabolism in ripening fruit. VI. Effect of antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. Plant Physiol. 103, 429-434.

Cronje, A., 2014. Effect of canopy position on fruit quality and consumer preference for the appearance and taste of pears. MSc (Agric) Dissertation. Department of Food Science, Stellenbosch University, Stellenbosch, South Africa.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L.). PhD Dissertation. Stellenbosch University.

De Freitas, S.T., Do Amarante, C.V.T., Labavitch, J.M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biol. Technol. 57, 6-13.

De Freitas, S.T., Mitcham, E.J., 2012. Factors involved in fruit calcium deficiency. Hort. Rev. 40, 107-145.

De Vries, P.J., Hundall, R.F., 1993. Maturity parameters and storage regimes to obtain 'Forelle' pears of an acceptable quality. Unifruco Research Services Report. 1993, 95-99.

Fallahi, E., Conway, W.S., Hickey, K.D., Sam, C.E., 1997. The role of calcium and nitrogen in post-harvest quality and disease resistance of apples. Hort. Sci. 32, 831-835.

Gastol, M., Domagala-Swiatkiewicz, I., 2006. Effect of foliar sprays on potassium, magnesium and calcium distribution in fruits of the pear. J. Fruit Ornam. Plant Res. 14, 169-176.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. Hort. Sci. 27, 1291-1294.

Hepler, K.P., 2005. Calcium: A central regulator of plant growth and development. Plant Cell. 17, 2142-2155.

Hepler, K.P., Winship, L.J., 2010. Calcium at the cell wall cytoplast interface. J. Integr. Plant Biol. 52, 147-160.

Holdaway-Clarke, T., Walker, N.A., Hepler, P.K., Overall, R.L., 2000. Physiological elevations in cytoplasmic free calcium by cold or ion injection result in transient closure of higher plant plasmodesmata. Planta. 210, 329-335.

Johnston, J.W., Hewett, E.W., Hertog, M.L.A.T.M., 2002. Postharvest softening of apple (*Malus domestica*) fruit: A review. N. Z. J. Crop Hortic. Sci. 30 (3), 145-160.

Lammertyn, J., Verlinden, B.E., Nicolai, B.M., 2002. Applying advanced instrumental methods: mealiness in fruit. In: Jogen, W. 2002. Fruit and Vegetable processing: Improving quality. Woodhead Publishing in Food Science. Cornwall, England. Pp 170-187.

Martin, E.M., Crouch, I.J., and Holcroft, D.M., 2003. Ripening and mealiness of 'Forelle' pears. Acta Hort. 600, 449-452.

Martin-Cabrejas, M.A., Waldron, K.W., Selvendran, R.R, Parker, M.L., Moates, G.K., 1994. Ripening related changes in cell walls of Spanish pear (*Pyrus communis*). Physiol. Plants. 91, 671-679.

McAtee, P.A., Hallet, I.C., Johnston, J.W., Schaffer, R.J., 2009. A rapid method of fruit cell isolation for cell size and shape measurements. Plant Methods. 5 (5). 1-7.

Ng, J.K.T., Schroder, R., Sutherland, P.W., Hallett, I.C., Hall, M.I., Prakash, R., Smith, B. G., Melton, L.D., Johnston, J.W., 2013. Cell wall structures leading to cultivar softening rates develop early during apple (*Malus x domestica*) fruit growth. BMC Plant Biol. 13 (183), 1-16.

Obenland, D.M., Caroll T.R., 2000., Mealiness and pectinolystic activity in peaches and nectarines in response to heat treatment and cold storage. J. Am. Soc. Hortic Sci. 125 (6), 723-728.

Ortiz, A., Grael, J., Lara, I. 2011., Pre-harvest calcium applications inhibit some cell wall modifying enzyme activities and delay cell wall disassembly at commercial harvest of 'Fugi' Kiku-8- apples. Postharvest Biol.Technol. 62 (2), 161-167.

Renard, C.M.G.C., 2005. Effects of conventional boiling on the polyphenols and cell walls of pears. J. Sci. Food Agric. 85, 310-318.

Saure, M.C., 2004. Calcium translocation to fleshy fruit: its mechanism and endogenous control. Sci. Hortic. 105, 65-89.

Table 1. Effects of farm and mealiness on cell wall yield (%AIR). Fruit were harvested from Achtertuin, Buchuland, Koelfontein and La Plaisante farms in March 2013.

Location	Mealiness	Alcohol insoluble residue (%AIR)
Achtertuin	Non-mealy	2.26
	Mealy	2.51
Buchuland	Non-mealy	2.31
	Mealy	2.46
Koelfontein	Non-mealy	2.66
	Mealy	2.48
La Plaisante	Non-mealy	2.61
	Mealy	2.35
Significance:		Pr>F
Location		0.46934
Mealiness		0.90023
Location x mealiness		0.05962

Table 2. Effects of location and mealiness on Ca^{2+} concentration ($\mu g \cdot g^{-1}$ FW) of different cell wall fractions of 'Forelle' pear harvested from Achtertuin, Buchuland, Koelfontein and La Plaisante. Means were separated using Fishers protected least significant difference ($LSD_{0.05}$).

Location	Mealiness	Alcohol soluble Ca^{2+}	Water soluble Ca^{2+}	Total soluble Ca^{2+}	Cell wall bound Ca^{2+}	Total Ca^{2+}
		($\mu g \cdot g^{-1}$ FW)				
Achtertuin	Non-mealy	4.75a	0.33bc	5.06a	2.13a	7.19a
	Mealy	3.45b	0.4ab	3.84b	2.08a	5.92bc
Buchuland	Non-mealy	2.39c	0.34bc	2.73c	1.61b	4.34c
	Mealy	2.13c	0.29cd	2.43c	1.46b	3.89d
Koelfontein	Non-mealy	2.53c	0.21f	2.74c	1.91a	4.47c
	Mealy	1.63cd	0.44a	2.08cd	2.14a	4.21cd
La Plaisante	Non-mealy	5.11a	0.25ef	5.36a	1.90a	7.31a
	Mealy	2.81bc	0.24ef	3.04bc	1.49b	4.54c
Location		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Mealiness		0.0005	0.0044	0.001	0.125	0.0015
Location x mealiness		0.0466	0.0003	0.0466	0.0135	0.0235

Means in the same column followed by different letters are significantly different at p≤ 0.05.
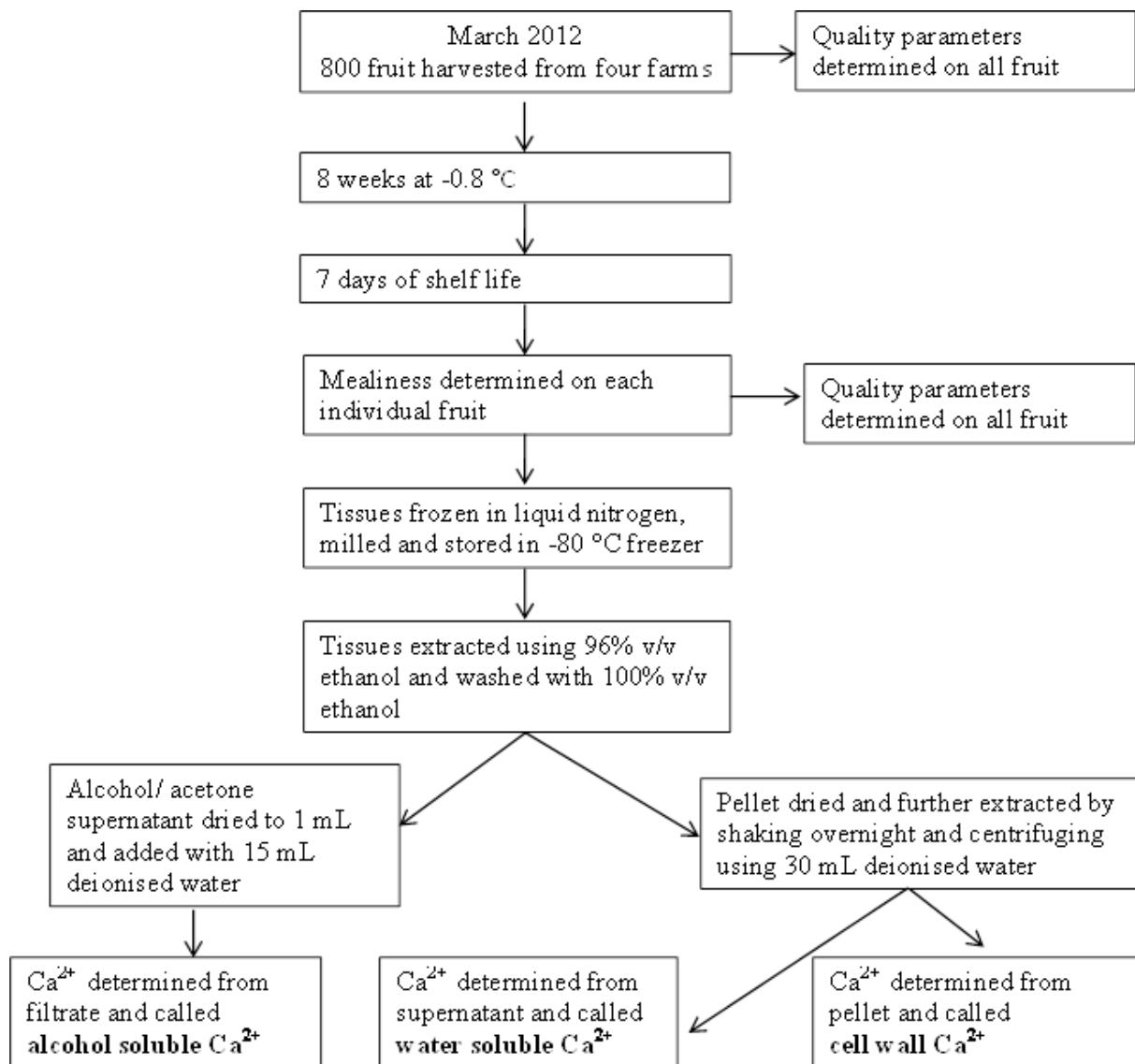
Fig. 1. Flow diagram showing the sequential process of extracting Ca^{2+} fractions from the cell walls.
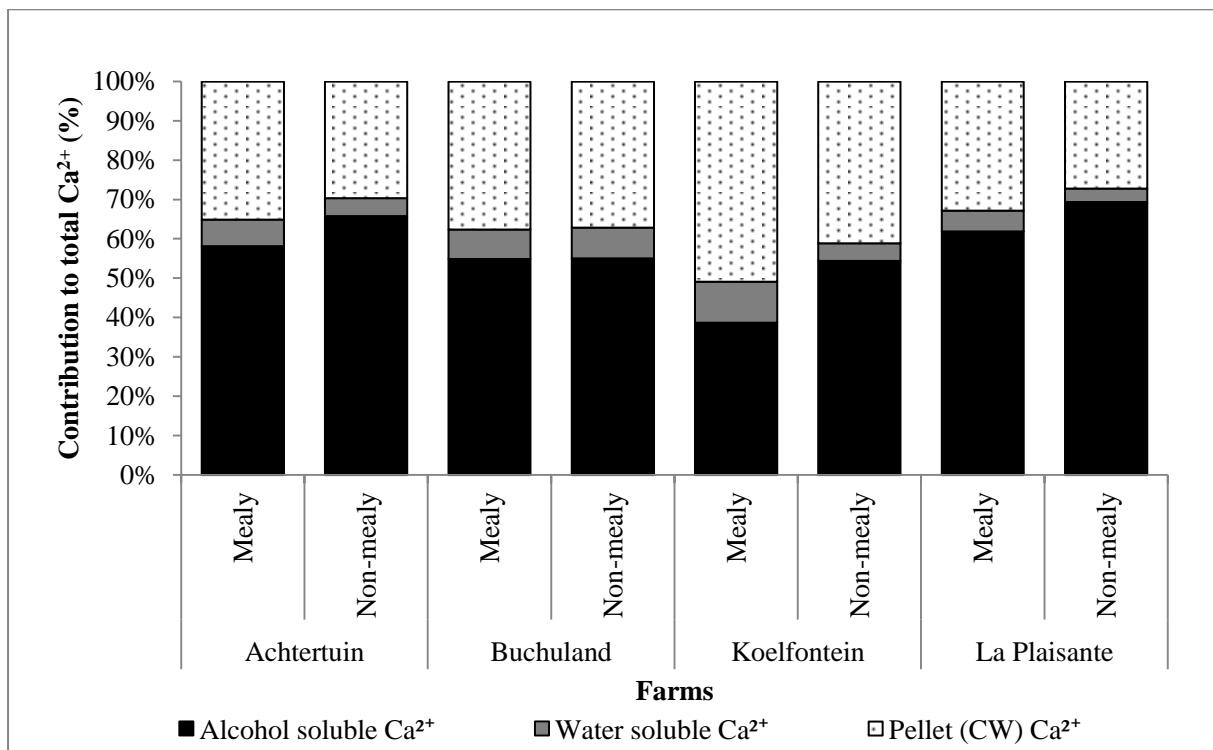
Fig. 2. Relative contribution of the different Ca^{2+} fractions to the total Ca^{2+} of mealy and non-mealy 'Forelle' pear tissues. Fruits were harvested from Achtertuin, Buchuland, Koelfontein and La Plaisante farms.
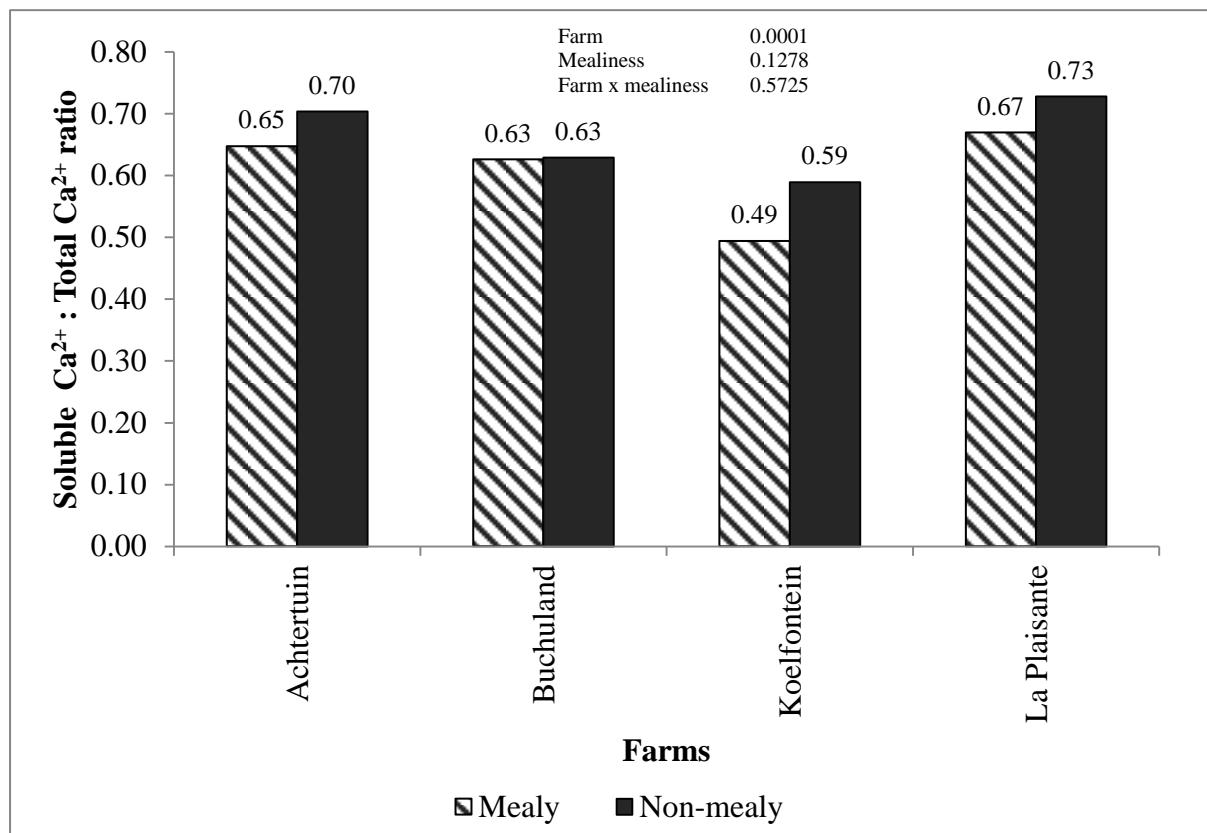
Fig. 3. Ratios of soluble Ca^{2+} to the total Ca^{2+} of mealy and non-mealy 'Forelle' pears harvested from Achtertuin, Buchuland, Koelfontein and La Plaisante farms.

PAPER 2

The influence of cell sizes and cell numbers on the development of mealiness in 'Forelle' pears (*Pyrus communis* L.).

Abstract

'Forelle' pears are prone to development of mealiness during shelf life. Mealiness is a textural disorder characterised by dry and soft texture deterioration, occurring mostly in fruit stored for less than the mandatory 12 weeks at -0.5 °C. This study investigated the role of cell numbers and cell size in the development of mealiness in 'Forelle' pear. Variations in cell numbers and sizes among fruit were incited by applying plant growth regulators: 2,4-dichlorophenoxyacetic acid, prohexadione-Ca, gibberellin$_{4+7}$ and forchlorfenuron at different stages following bloom during the 2011 season. In 2012, hand thinning of fruit was done on blossom clusters, leaving fruit from the "king" blossom and one fruit from a smaller blossom. Cell numbers, cell size and other cell characteristics were examined on dried sections of the fruit hypanthium using scanning electron microscopy. Treatments that caused fruit to develop larger cells had higher mealiness percentage. Forchlorfenuron and its combination with gibberellin$_{4+7}$ produced fruit with significantly ($P=0.038$) higher mealiness percentage. 2,4-D amine had the lowest mealiness percentage compared to other treatments. Cell volume and cell diameter exhibited a positive linear relationship with mealiness percentage. Plant growth regulator application did not significantly affect cell numbers. Examination of histological differences between mealy and non-mealy fruit revealed differences in size of intercellular spaces with mealy fruit having larger spaces.

Key words

Pyrus communis L., scanning electron microscopy, mealiness percentage, cell diameter, cell volume, texture

1. Introduction

Mealiness is a post-harvest storage disorder of pome fruit characterised by texture deterioration of fruit during inappropriate storage, resulting in soft, dry and mealy fruit (Lammertyn et al, 2002; Nobile et al., 2011). The disorder is common in apples (De Smedt et al., 1998; Harker and Hallet, 1992), peaches (Brummell et al., 2004; Luza et al, 1992), pears (Crouch et al., 2005; Manning, 2009), nectarines (Dawson et al., 1992) and tomatoes (Jackman et al. 1992). In 'Forelle' pears, mealiness occurs in fruit harvested at the post-optimum maturity stage (<5.5 kg firmness) (Carmichael, 2011), or in fruit that have not received sufficient time in cold storage prior to ripening (Crouch et al., 2005). The development of mealiness poses a number of practical challenges to the pear industry, particularly in South Africa where the majority of 'Forelle' are grown, in preparing their fruit for the market (Crouch and Bergman, 2013). Current practices to reduce mealiness in 'Forelle' include storing the fruit at -0.5 °C for a minimum period of 12 weeks (Crouch and Bergman, 2013).

Various attempts to understand and manage mealiness have been explored, in pear, peaches and apples. Studies have been done on the role of enzymes, particularly polygalacturonase (PG) and pectin methyl esterase (PME) in peach and nectarine (Obenland and Carroll, 2000), the role of ethylene in 'Forelle' pear (Crouch, et al., 2005), and cell walls in peach (Brummell et al., 2006), nectarines (Hobbs et al., 1991) and 'Forelle' pear (Crouch, 2011). Although cell shape and size are important determinants of fruit size and fruit texture (McAtee et al., 2009), there are no studies that examined the role of these attributes in mealiness of pears. The objective of this work was therefore to determine the role of cell sizes on mealiness in 'Forelle' pears and to examine cell packing and geometry in mealy and non-mealy fruit tissues. Scanning electron microscopy studies were employed to measure and discriminate cell sizes and other histological differences between mealy and non-mealy fruit tissues.

2. Materials and methods

2.1 Fruit material

'Forelle' pear trees were treated with different plant growth regulators (PGRs) on four farms in the Ceres and Wolsley areas, South Africa, namely Koelfontein (lat. 33.00°S, long. 19.33°E), Buchuland (lat. 33.47°S, long 19.31°E), La Plaisante (lat. 33.41°S, long. 19.19°E) and Achtertuin (lat. 33.39°S, long. 19.34°E). The PGR treatments as summarised in Table 1, were applied in order to incite variations in cell division and cell growth. The particular combinations of plant growth regulators were chosen due to commercial protocols to improve fruit set and

size which were studied and explained by Dreyer (2013). In the second year (2012 season), selective thinning of blossom clusters was done on selected trees, leaving the 'king' blossom fruit and one fruit from a smaller blossom at seven days after full bloom, again to incite variations in cell growth. For both experiments, harvesting was done at commercial harvest maturity (ca. 6.4 kg). Harvested fruit were packed in polyethylene (37.5 µm) lined pear boxes and stored for a period of 21 weeks at -0.5 °C, followed by 7 days of ripening at 20 °C. Evaluations were done at harvest and at the end of shelf life.

2.2 Maturity indexing

Average fruit diameter, fruit weight, titratable acidity (TA), total soluble solids (TSS) and fruit firmness (kg) were measured at harvest, then again at the end of shelf life following 21 weeks storage at -0.5 °C. Ten replications of 10 fruit each were analysed in each treatment. Fruit firmness was measured on opposite sides of the fruit using a universal fruit texture analyzer (FTA 2007, Guss, Strand, South Africa), fitted with a 7.9 mm diameter probe. Fruit diameter and fruit weight were measured using a Cranston gauge and electronic balance, respectively, both fitted to the fruit texture analyser. TA was measured by automatic titration of pooled juice sample (10 g) from 10 fruit for each replicate (Metrohm AG 760. Harison, Switzerland). TSS was measured using a digital refractometer (PR-32, Atago, Tokyo, Japan) on a pooled sample of 10 equatorial discs from 10 fruit for each replicate.

2.3 Tissue preparation for scanning electron microscopy

Two fruit from each replication in each treatment were used to make sectional cuts measuring 30 mm x 5 mm x 2 mm from the endocarp region of the fruit at harvest (Fig. 1). The tissue wedges were fixed in formalin- acetic acid- alcohol (FAA) (2 mL of 37% formaldehyde; 10 mL of 95% ethanol; 1 mL of glacial acetic acid; 7 mL of deionised water). After two weeks in FAA, the samples were sequentially dehydrated with ethanol (35%, 50%, 75%, 95%, and finally 100%v/v for 10 min each). The tissues were dried using an E3000 Critical Point Dryer (CPD) (Quorum Technologies, Kent, UK) following the procedure for drying soft tissues (described below). The same procedure was used for fruit that was stored for 21 weeks at -0.5 °C and ripened for 7 days at 20 °C. However, poor sectional cuts were obtained from ripened fruit as they were soft and could not be dried in the critical dryer.

2.4 Procedures for drying of tissue samples using the critical drying point apparatus

The procedure preparing tissue for microscope is described in two major steps.

50

Step 1

Samples were taken out of ethanol (100% v/v) and put in the CPD sample holding boat with three compartments filled with acetone (100% v/v). Transfer of samples was done expeditiously to avoid tissue desiccation. The boat was then put in the CPD drying compartment and secured with a tightly screwed lid. Temperature was maintained at ≤ 20 °C during the initial phase. When temperatures exceeded 20 °C, cold water was run through the chamber to cool it. Once the sample was secured in the chamber, CO_2 was introduced in the drying compartment, displacing acetone from the sample chamber. The displaced acetone was allowed to escape. Continued circulation of CO_2 was maintained for 3-5 min during which time the tissue was impregnated with CO_2 gas. Pressure was then maintained at ca. 750 kPa for 1h, during which the sample was fixed and the cell walls remained in position.

Step 2

 Step 1 was repeated. Hot water was allowed to flow slowly through the chamber to raise pressure and temperature to 1200 kPa and ≥ 30 °C, respectively. When the temperature reached 30 °C and pressure reached ca. 1100 kPa, this was regarded as the critical dry point. Once the critical dry point was reached, trapped gas was gently released, at which time the internal pressure was 0 kPa. Care was taken to gently release the pressure as rapid release may damage the sample. Temperature was also gradually lowered by way of opening the cold water tap. The dry sample was taken out, ready for coating and viewing.

2.5 Scanning Electron Microscope Imaging

Dried samples were mounted on glass slides completely covered with a double sided adhesive carbon tape. The samples were placed in the Gold Coater (S150A Sputter Coater, Edwards, UK) for 3 min to allow impregnation / coating with gold. The samples were then placed in the scanning electron microscope (SEM) (Zeiss EVO®MA15 Scanning Electron microscope (Oxford Instruments) for viewing and imaging. Beam conditions during the quantitative analysis were set at -20 kV, with a working distance of 8.5 mm and beam current of around -20nA. Images were taken on two positions on the cut section, the outer cortex and the mid cortex (Fig. 1A and B). All images used for analysis were captured at 100X resolution (Fig. 1D).

2.6 Image Analysis

Cell dimensions were measured on SEM images using ImageJ 1.46r (Wayne Rasband, National Institute of Health, USA), and NIS Elements (Nikon Instruments Inc., Melville, USA) software. A total of ten distinct cells from the mid-cortex were randomly chosen from each image for cell

anatomical measurements. Using NIS Elements, two diameters were measured on each of the ten cells, the first diameter on the longer axis and the second diameter taken orthogonal to the first. A third diameter was an average of the two diameters. Cell volume was determined using the method by Drazéta (2002), described by Voltź et al. (2003). The following formula was used for cell volume (CV, μm^3) determination, without modifications.

$$CV = \frac{\pi(d1.d2.d3)}{6}$$

where d1, d2 and d3 are the measured cell diameters (μm)

2.7 Mealiness determination

Mealiness evaluations were done at the end of 21 weeks at -0.5 °C and 7 days of shelf life at 20 °C. A sensory panel comprising of three trained evaluators with a minimum of 8 years of mealiness determination experience tasted each individual fruit at the end of shelf life. Small wedges of tissue were cut in the equator of each fruit and evaluated organoleptically. The tissues were further hand squeezed to look for the presence or absence of free juice, after which fruit were scored mealy or non-mealy. Periodically during testing, the evaluators cleaned their pallet with dry water biscuits and water.

2.8 Data analysis

Data were analysed using one way analysis of variance (ANOVA), using STATISTICA (StatSoft, Inc. 200, Tulsa, USA). Mean separation of cell size, cell diameter, cell area and fruit diameter as well as mealiness data was performed using the Fischer's Least Significant Difference ($LSD_{0.05}$) method. Mealiness data were log transformed before analysis to ensure normality. The relationships between mealiness with cell area, cell diameter and cell volume were modelled using regression analysis.

3. Results

3.1 Effect of plant growth regulators on fruit diameter

Average fruit diameter at Achtertuin was significantly smaller when treated with prohexadione-Ca + Gibberellins$_{4+7}$, compared to the control, forchlorfenuron and the combination of gibberellins$_{4+7}$, prohexadione-Ca and forchlorfenuron treated fruit. This lower fruit diameter did not differ significantly from the 2,4-Dichlorophenoxyacetic acid (2,4-D amine) treated fruit and

the latter did not differ significantly from the rest of the treatments (Table 2). At Buchuland all treatments except for 2,4-D amine resulted in a slightly but significantly larger fruit diameter compared to the control (Table 3). At La Plaisante forchlorfenuron treated fruit had the smallest diameter and prohexadione-Ca treated fruit the largest diameter (Table 4). Although these treated fruit differed significantly from each other, neither differed from the untreated control nor from the 2,4-D amine and the combination of gibberellins$_{4+7}$, prohexadione-Ca and forchlorfenuron. At Koelfontein the fruit diameter was not affected by individual growth regulators or their combination treatments (Table 5).

3.2 Effect of plant growth regulators on cell diameter, cell area and volume

Cell diameter (P=0.00045), cell area (P<0.0001) and cell volume (P<0.00034) were significantly affected by treatment application at Achtertuin (Table 2). Forchlorfenuron and the combination of prohexadione-Ca + gibberellins$_{4+7}$ + forchlorfenuron produced fruit with the largest cell diameter (140.1 μm and 140.3 μm, respectively) as well as cell volume (Table 2). Cell diameter and cell volume of 2,4-D amine treated fruit was comparable to that of the control fruit as well as the prohexadione-Ca treated fruit. The cell area was the highest when fruit were treated with forchlorfenuron and the lowest for the untreated control. However, the control did not differ from the 2,4-D amine treated fruit nor did the prohexadione-Ca treated fruit. At Buchuland, La Plaisante and Koelfontein, plant growth regulators had no effect on cell diameter and cell volume (Tables 3, 4 and 5).

3.3 Effect of plant growth regulators on cell number

Variable responses to treatment application were observed with respect to cell number. At Achtertuin the cell numbers were the highest in 2,4-D amine treated fruit compared to all other treatments. The lowest number of cells was recorded for the untreated control fruit, but did not differ significantly from the gibberellins$_{4+7}$ + prohexadione-Ca treatment nor the combination of gibberellins$_{4+7}$ + prohexadione-Ca + forchlorfenuron treatment. Forchlorfenuron had higher cell numbers compared to the control but lower cell numbers compared to the 2,4-D amine treated fruit. At Koelfontein, the combination treatment of gibberellins$_{4+7}$ + prohexadione-Ca + forchlorfenuron had the highest cell number but only differed significantly with the 2,4-D amine treatment with the lowest cell number. However, at Buchuland and La Plaisante, cell number was not significantly affected by treatment application.

3.4 Effects of plant growth regulators on mealiness

Mealiness response to treatment application was variable across sites. At Achtertuin, the highest mealiness percentage was observed in fruit treated with forchlorfenuron (86%) while the lowest mealiness was recorded in 2,4-D amine treated fruit (10%) (Table 2). At Buchuland, the combination prohexadione-Ca + gibberellins$_{4+7}$ and the control produced fruit with the highest mealiness percentages (Table 3). The least mealiness was observed in fruit treated with 2,4-D amine and the combination treatment of prohexadione-Ca, gibberellins$_{4+7}$ and forchlorfenuron. When compared to the control, 2,4-D amine and the combination treatment prohexadione-Ca + gibberellins$_{4+7}$ + forchlorfenuron suppressed mealiness by 52.6% and 49.1%, respectively at Buchuland (Table 3). Mealiness percentage was not significantly affected by PGRs at La Plaisante and Koelfontein (Tables 4 and 5).

3.5 Effect of fruit position following preferential thinning on cell anatomical characters and mealiness

Fruit diameter, average mass, cell diameter and cell volume were significantly affected by thinning ($P<0.05$) (Table 6). Cell diameter of the 'king' blossom fruit was 13% bigger than the cell diameter of small blossom fruit while cell volume was 48.2% larger. Mealiness percentages for both the 'king' blossom and smaller fruit were generally low (8.3 % and 3.3 %, respectively) (Table 6). Mealiness was high in fruit with large cell numbers i.e. 'king' blossom fruit. No relationship existed between mealiness and cell numbers (data not shown).

3.6 Cell anatomical properties revealed by scanning electron microscopy

Tissues were visualized under scanning electron microscopy. Based on visual examinations, comparisons were made of fruit from treatments with higher mealiness percentages and those with lower mealiness. Forchlorfenuron treated fruit had large intercellular spaces compared to those from 2,4-D amine (Fig. 2A and 2B), which had low incidences of mealiness. Although we could not quantify differences in intercellular spaces, visual examination of SEM images shows clear differences in intercellular spaces between mealy and non mealy tissues. Apart from intercellular spaces, examination of SEM images revealed other anatomical differences that could be ascribed to mealiness. Cells from mealy fruit were shown to slide along the cutting blade during incision while cells from mealy fruit had fractures on their surfaces (Figs. 3A and B).

54

4. Discussion

PGRs are used in fruit production to increase fruit set (Davies, 2004; Greene, 1989; Lavee, 1989), increase fruit size (Bohner and Bangerth, 1988), in the regulation of ripening (Tingwa and Young, 1975) and to improve the fruit appearance (El-Otmani et al., 2000). In the current study, the application of PGRs resulted in variations in cell numbers and cell sizes of 'Forelle' pears. The largest cells were observed in fruit treated with forchlorfenuron and the combination of prohexadione-Ca, gibberellins$_{4+7}$ and forchlorfenuron (Fig 4A). The combination of prohexadione-Ca and gibberellins$_{4+7}$ produced comparable cell sizes and mealiness with forchlorfenuron. The smallest fruit with the smallest cell size were those treated with 2,4-D amine. The cell size of 2,4-D amine treated fruit was, however, comparable with control fruit. Mealiness too was least in the 2,4-D amine and forchlorfenuron (Fig. 4A). Our findings on forchlorfenuron treated fruit are consistent with its perceived role of improving cell division and expansion (Curry and Greene, 1993; Theron and Steyn, 2008). However, when forchlorfenuron was combined with prohexadione-Ca and gibberellin$_{4+7}$, the effects changed. Effects of the combined PGRs were variable, probably due to the combined or independent effects of the different PGRs (Coenen and Lomax, 1997). Dreyer (2013) also found variable results when she used the same growth regulators in 'Forelle' and 'Abate Fetel'.

The highest mealiness percentage was observed on forchlorfenuron, gibberellins$_{4+7}$ and control treated fruit. The combination of prohexadione-Ca + gibberellins$_{4+7}$ + forchlorfenuron resulted in a low mealiness percentage comparable with that obtained in fruit treated with 2,4D amine. Forchlorfenuron had the largest cells, while 2,4-D amine had both smaller fruit and smaller cells In another 'Forelle' pear study it was also found that mealy and partly mealy fruit were mostly larger compared to non-mealy fruit (Paper 4). Our results support an earlier hypothesis that smaller fruit are less prone to mealiness (Barreiro et al., 1998). Further, correlation studies showed a weak but positive linear relationship between cell volume and mealiness (Fig. 4B). This correlation improved dramatically for both cell volume and cell diameter when outlier farms were excluded (data not shown). Model performance can therefore be improved by more in depth statistical analysis and perhaps studying mealiness over a ripening time as in this study mealiness was evaluated after 7 days of ripening and mealiness is known to increase and then decrease during the shelf life period (Martin, 2002) which may differ for farms as ripening potential may differ. Various mechanisms have been put forward to explain the high incidences of mealiness in larger celled fruit. Some argue that because of the large cell sizes, cell contact between neighbouring cells is reduced making the cells prone to cell-to-cell debonding during

55

ripening (Harker and Hallet, 1992; Crouch, 2011), leading to tissue breakdown when masticated. Other authors argue that as cells enlarge, vacuoles tend to occupy a greater proportion of the cell volume, compromising the load bearing capacity of the cell (Ozga et al., 2002), leading to tissue breakdown. Both mechanisms may be involved and act synergistically in mealiness.

Results from this experiment were not consistent in terms of both cell diameter and cell numbers. A number of reasons could have caused these inconsistencies. One contributing factor could have been the position of the cutting plane, especially as it relates to the distribution of branchysclereids. Because cells of pears are not spherical in shape, the position of sampling/ plane of cutting determines how many cells could be counted (McAtee et al., 2009). Secondly, there were differences in terms of uniformity of cell size across treatments. The size of cells found in the control and 2,4-D amine were almost uniformly distributed while that of forchlorfenuron and prohexadione-Ca + gibberellins$_{4+7}$ + forchlorfenuron followed a bimodal distribution with large cells and small ones, attributed to position of incision or distribution of stone cells. These had an influence on the sizes of cells in the cut section. Secondly, the effect of different hormonal treatments on cell shape was different. Cells of fruit treated with 2,4-D amine were more circular while those from fruit treated with other hormones, particularly forchlorfenuron, the combination of prohexadione-Ca + gibberellins$_{4+7}$ + forchlorfenuron and the control had angular shaped cells, typical of pear cells.

No relationship could be established between mealiness percentage and cell numbers. This could have been caused by a number of reasons. Firstly, smaller cells (with diameter ≤ 30 µm) were not counted and measured. Smaller cells surrounding the stone cells were thus not counted. In treatments that caused skewed distribution of cell sizes due to the presence of stone cells, it was difficult to properly account for the numbers of cells, hence the poor relationship. Although attempts were made to avoid regions with stone cells, these would frequently encroach in the measurement plane. McAtee et al. (2009) noted the pitfalls of counting cells using the sectioning method and developed a digestion method using enzymes that dissolve the cell walls and release individual cells into solution. Still, the method has its limitations, among them how to measure the cut cells in solution. Recently, methods have been developed and used on large scale in measuring cells non-destructively using x-ray computed tomography (Herremans et al. 2013; Herremans et al., 2015).

SEM revealed that there were observable differences between the intercellular spaces of mealy fruit and those of non mealy fruit. Fruit with low incidences of mealiness were found to be associated with smaller intercellular spaces. For example, forchlorfenuron treated fruit had large intercellular spaces compared to those from 2,4-D amine, which in turn had low incidences of mealiness. Apart from intercellular spaces, examination of SEM images of mealy and non-mealy fruit sectioned after shelf life revealed other anatomical differences that could be associated with mealiness. Cells from mealy fruit slide along the cutting blade during incision while cells from mealy fruit had fractures on the surface. The behaviour of mealy cells could have been because cells had lost cell fluids. Such cells would readily move with the cutting blade as they are flaccid and not intact.

5. Conclusion

Cell size was positively correlated to mealiness development. Secondly, the treatment that promoted larger cell sizes had a higher mealiness incidence. Examination of scanning electron micrographs further revealed that in addition to cell sizes, larger intercellular spaces and differences in degree of cell-cell bonding seem to be associated with mealy fruit. Further work needs to be done to establish the effect of sizes of intercellular spaces on mealiness.

Literature Cited

Barreiro, P., Ortiz, C., Ruiz-Altisent., M., De Smedt, V., Schotte, S., Andani, Z., Wakeling, I., Beyts, P.K., 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples. A collaborative test. J. Texture Stud. 29, 509-525.

Bohner, J., Bangreth, F., 1988. Effect of fruit set sequence and defoliation on cell number, cell size and hormone levels of tomato fruits (*Lycopersicon esculentum* Mill.) within a truss. J. Plant Growth Regul. 7, 141-155.

Brummell, D.A., Dal Cin, V., Lurie, S., Crisosto, C.H. and Labavitch, J.M., 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55(405), 2041-2052.

Brummell, D.A., 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol., 33: 103-119.

Carmichael, P.C., 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, Department of Horticultural Sciences, Stellenbosch University, Stellenbosch. South Africa.

Coenen, C., Lomax, T.L., 1997. Auxin-cytokinin interactions in higher plants: old problems and new tools. Trends Plant Sci. 2, 351-356.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L.). PhD Thesis, Department of Horticultural Sciences. Stellenbosch University, Stellenbosch. South Africa.

Crouch, E.M., Holcroft, D.M., Huysamer M., 2005. Mealiness of 'Forelle' pears-quo vadis? Acta Hort. 671, 369-376.

Crouch, I.J., Bergman H., 2013. Consumer acceptace study of early marketed Forelle pears in the United Kingdom and Germany. SA Fruit Journal. 11 (6), 64-71.

Curry, A.C. and Greene, D.W., 1993. CPPU influences fruit quality, fruit set, return bloom, and postharvest drop of apples. HortSci. 28 (2), 115-119.

Davies, P.J., 2004. The plant hormones. Their nature, occurrence, and functions. In: Davies, P.J 2004 (eds). Plant Hormones: biosynthesis, signal transduction, action. Klumer Academic Press. Boston. London, UK. Pp 1-15.

Dawson, D.M., Melton, L.D., Watkins, C.B., 1992. Cell wall changes in nectarines (*Prunus persica*). Solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit. Plant Physiol. 100, 1203-1210.

De Smedt, V., Pauwels, E., De Baerdemaeker, J., Nikolaï, B., 1998. Microscopic observation of mealiness in apples: a quantitative approach. Postharvest Biol. Technol. 14, 151-158.

Dreyer, C., 2013. Fruit set and fruit size studies on 'Forelle' and 'Abate Fetel' pear (*Pyrus communis*. L). MSc (Agric) thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

El-Otmani, M., Coggins (Jr), C.W., Agusti, M., Lovatt, C.J., 2000. Plant growth regulators in citriculture: World current uses. Crit. Rev. Plant. Sci. 19(5), 395-447.

Greene, A.W., 1989. Regulation of fruit set in tree fruits with plant growth regulators. Acta Hort. 239, 323-334.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortSci. 27 (12), 1291-1294.

Herremans, E., Verboven, P., Bongers, E., Estrade, P., Verlinden, B., Wevers, M., 2013. Isolation of single cells and pores for the characterization of 3D fruit tissue microstructure based on X-ray micro-CT image analysis. Proc. InsideFood Symposium, 9-12 April, Leuven, Belgium. www.insidefood.eu/INSIDEFOOD_WEB/UK/.../ proceedings/037O.pdf. (accessed 2015/12/10).

Herremans, E., Verboven, P., Verlinden, B., Cantre, D., Abera, M., Wevers, M., Nicolai, B., 2015. Automatic analysis of the 3-D microstructure of fruit parenchyma tissue using x-ray CT explains differences in aeration. BMC Plant Biol. 15, 264. Doi:10.1186/s12870-015-0650-y.

Hobbs, M.C., Easterbrook, K.M., Melton, L.D., 1991. Cell wall material composition of mealy fruit among ripening nectarines. J. Sci. Food. Agric. 57, 141-145.

Jackman, R.L., Gibson, H.J., Stanley, D.W., 1992. Effects of chilling injury on tomato fruit texture. Plant Physiol. 86, 600-608.

Lammertyn, J., Verlinden, B.E., Nicolai, B.M., 2002. Applying advanced instrumental methods: mealiness in fruit. In: Jogen, W. 2002. Fruit and Vegetable processing: Improving quality. Woodhead Publishing in Food Science. Cornwall, England. Pp 170-187.

Lavee, S., 1989. Involvement of plant growth regulators and endogenous growth substances in the control of alternate bearing. Acta Hort. 239, 311-321.

Luza, J.G., van Gorsel, R., Polito, V.S. and Kader, A.A., 1992. Chilling injury in peaches: a cytochemical andultrastructural cell wall study. J. Amer. Soc. Hort Sci. 117, 114-118.

Martin, E.M., 2002. Ripening responses of 'Forelle' pears. MSc (Agric) Thesis. Department of Horticultural Sciences, Stellenbosch University, South Africa.

Manning, N., 2009. Physical, sensory and consumer analysis of pear genotypes among South African consumers and preference of appearance among European consumers. Thesis presented for the degree of Master of Science (Agric) in Food Science. Department of Food Science, Stellenbosch University.

McAtee, P.A., Hallet, I.C., Johnston, J.W., Schaffer, R.J., 2009. A rapid method of fruit cell isolation for cell size and shape measurements. Plant Methods, 5: 5. 1-7.

Nobile, P.M., Wattebled, F.., Quecini, V., Giradi, C. L.., M. Lormeau, Francois, L., 2011. Identification of a novel Alpha-L-arabinofuranosidase gene associated with mealiness in apple. J. Exp. Bot. 62 (12), 4309-4321.

Obenland, D.M., Caroll, T.R., 2000. Mealiness and pectolytic activity in peaches and nectarines in response to heat treatment and cold storage. J. Amer. Soc. Hort. Sci. 125 (6), 723-728.

Ozga, J.A., van Huizen, R., Reineke, D.M., 2002. Hormone and Seed-Specific Regulation of pear fruit growth. Plant Physiol. 128, 1379-1389.

Theron, K.I., Steyn, W.J., 2008.Girdling.Science behind the age-old technique. Acta. Hort. 800, 51-59.

Tingwa, P.O., Young, R.E., 1975. The effects of indole-3-acetic acid and other growth regulators on the ripening of avocado fruits. Plant Physiol. 55, 937-940.

Voltź, R.K., Harker, F.R., Lang, S., 2003. Firmness decline in 'Gala' during fruit development. J. Amer. Soc. Hort. Sci. 128 (6), 797-802.

Tables and figures

Table 1. Spraying dates for the plant growth regulators used on 'Forelle' pear. 2,4-dichlorophenoxy acetic acid (2,4-D) amine salt formulation (Amine) [a] was applied at 12 mg.kg^{-1} 14 days after full bloom (DAFB), forchlorfenuron[b] at 20 mg.kg^{-1} at 14 DAFB, gibberellins$_{4+7}$[c] at 70 mg.kg^{-1} at 65 and 80 DAFB; prohexadione-Ca[d] was applied at 20 mg.kg^{-1} at 65 and 80 DAFB. 2,4-D amine and forchlorfenuron were applied with breakthrough wetting agent at 2L 100 L^{-1}.

Farms	Full Bloom	Forchlorfenuron/ (2,4-D amine)	Prohexadione-Ca / Gibberellins$_{4+7}$	
Achtertuin	11/9/2011	26/9/2011	16/11/2011	30/11/2011
Buchuland	13/9/2011	26/9/2011	16/11/2011	30/11/2011
La Plaisante	21/9/2011	4/10/2011	23/11/2011	6/12/2011
Koelfontein	12/9/2011	27/9/2011	16/11/2011	30/11/2011

[a] 2,4-D amine 480 SL, DOW AgroSciences South Africa (Pty) Ltd.

[b] SITOFEX 10EC; Philagro South Africa (Pty) Ltd.

[c] Regulex® 10SG; Philagro South Africa (Pty) Ltd.

[d] Regalis™; BASF South Africa (Pty.) Ltd.

61

Table 2. Effect of different combinations of growth regulators on fruit weight, fruit diameter, cell diameter, cell area, cell volume and mealiness percentage on 'Forelle' pear at Achtertuin.

Treatment:	Average fruit diameter (mm)	Cell numbers/ 10^6 μm^2	Average cell diameter (μm)	Average Cell area (μm^2)	Average cell volume (μm^3)	Mealiness (%)[x] 21w+7d
Untreated control	69.2 a[z]	62c	114.48b	9534b	699541b	35 b
2,4-D amine	68.2 ab	81a	115.33b	14089ab	802724b	10 c
Prohexadione-Ca + Gibberellins$_{4+7}$	65.7 b	66bc	121.76b	13240ab	905361b	40 b
Forchlorfenuron	68.6 a	74b	140.31a	18708a	1503093a	86 a
Gibberellins$_{4+7}$ + Prohexadione-Ca + Forchlorfenuron	69.2 a	64bc	140.34a	17690ab	1444334a	33 b
			Pr>F			
	0.002	*0.002*	*0.00045*	*0.0001*	*0.00034*	0.0001

[t]Treatment means in the same column followed by the same letter are not significantly different at 5 % level (LSD).

[x]LSD determined on logit transformed data.

Table 3. Effect of different combinations of growth regulators on fruit weight, fruit diameter, cell diameter, cell area, and cell volume and mealiness percentage on 'Forelle' pear harvested at Buchuland.

Treatment:	Average fruit diameter (mm)	Cell numbers/ 10^6 μm^2	Average cell diameter (μm)	Average Cell area (μm^2)	Average cell volume (μm^3)	Mealiness (%)[x] 21w+7d
Untreated control	67.1 b[z]	75	121.5	14914.6	974962	57 a
2,4-D amine	68.3 ab	61	119.5	16935.6	902025	27 b
Prohexadione-Ca + Gibberellins$_{4+7}$	68.6 a	66	130.1	15549.9	1254937	54 a
Forchlorfenuron	70.7 a	61	129.0	16508.8	1208125	43 a
Gibberellins$_{4+7}$ + Prohexadione-Ca + Forchlorfenuron	69.9 a	70	115.8	15348.1	808298	29 b
Pr>F						
	0.0040	*0.1887*	*0.5048*	*0.192*	*0.4635*	*0.0071*

[z]Treatment means in the same column followed by the same letter are not significantly different at 5 % level (LSD).

[x]LSD determined on logit transformed data.

Table 4. Effect of different combinations of growth regulators on fruit weight, fruit diameter, cell diameter, cell area, and cell volume and mealiness percentage on 'Forelle' pear harvested at La Plaisante.

Treatment:	Average fruit diameter (mm)	Cell numbers/ 10^6 μm^2	Average cell diameter(μm)	Average cell area (μm^2)	Average cell volume (μm^3)	Mealiness (%)[x] 21+7
Untreated control	68.72 ab	64	135.33	17297a	1377436	46
2,4 D Amine	68.5ab	60	139.08	19123a	1536447	61
Prohexadione-Ca + Gibberellins$_{4+7}$	66.49b	61	140.02	19317a	1627236	67
Forchlorfenuron	69.15a	58	142.21	19079a	1376044	47
Gibberellins$_{4+7}$ + Prohexadione-Ca + Forchlorfenuron	67.53ab	73	126.58	13302b	1428743	53
Pr > F						
0.047	*0.0983*	*0.1424*	*<0.0001*	*0.7558*	*0.7870*	

[t]Treatment means in the same column followed by the same letter are not significantly different at 5 % level (LSD).

[x]LSD determined on logit transformed data.

Table 5. Effect of different combinations of growth regulators on fruit weight, fruit diameter, cell diameter, cell area, and cell volume and mealiness percentage on 'Forelle' pear harvested at Koelfontein.

Treatment:	Average fruit diameter (mm)	Cell numbers/ 10^6 μm^2	Average cell diameter (μm)	Average cell area (μm^2)	Average cell volume (μm^3)	Mealiness (%)[x] 21+7
Untreated control	63.77	59ab	136.51	17456	1308427	8
2,4 D Amine	65.95	52b	142.37	20731	1425004	1
Prohexadione-Ca + Gibberellins$_{4+7}$	65.85	59ab	145.29	20409	1436194	18
Forchlorfenuron	66.39	64ab	139.35	17201	1484370	7
Gibberellins$_{4+7}$ + Prohexadione-Ca + Forchlorfenuron	64.47	71a	138.96	19601	1011234	8
Pr > F						
	0.176	*0.0049*	*0.8038*	*0.1747*	0.077	*0.50157*

[t]Treatment means in the same column followed by the same letter are not significantly different at 5 % level (LSD).

[x]LSD determined on logit transformed data.

Table 6. Effect of preferential thinning of the four flower cluster leaving the king blossom and the smaller blossom on physicochemical and cell anatomical properties of 'Forelle' pear fruit. Means are values ± SE.

Parameter measured	Fruit type		
	Smaller fruit	King Blossom fruit	Significance
Firmness (kg)	6.37±0.12	6.38±0.13	ns
Fruit diameter (mm)	58.8±2.94	64.71±2.16	*
Average mass (g)	136.01±16.94	174.17±12.03	*
Cell numbers/ $10^6 \mu m^2$	64.87±2.63	58.93±2.67	ns
Cell diameters (µm)	137.33±3.64	155.4±3.7	***
Cell volume (μm^3)	1378988±127792.2	2043630±129976.9	***
Mealiness percentage	3.3%	8.3%	ns

Titratable acidity, total soluble solids, firmness, fruit diameter and mass were measured on seven replicates of 10 fruit each (n=70). Cell diameter, cell numbers and cell volume were measured on 30 fruit from each category.

ns, *, ***< Non significant or significant at $P<0.05$, 0.01 or 0.001, respectively.

66
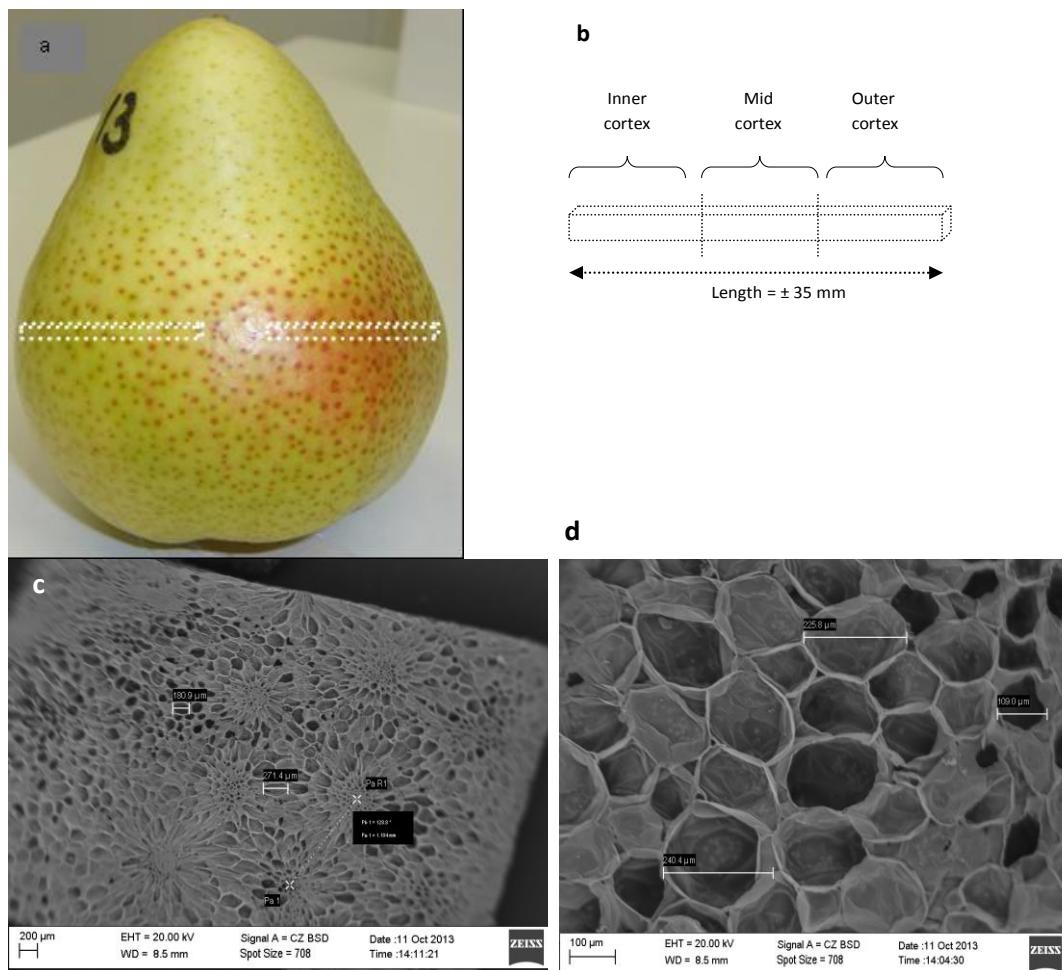
Fig. 1. Schematic view of the sample extractions and image processing. a) shows positions where the wedges of tissues were excised from the equator of the fruit, b) shows the tissue section extracted with the different sections from where spot images were taken i.e edge, mid cortex and inner cortex. Analysis was done of the mid cortex spot, c) mid cortex taken at x19 resolution showing the branchysclereids (vascular tissues) which were avoided during spot analysis and imaging, d) shows the final image of selected spot taken x100 resolution.
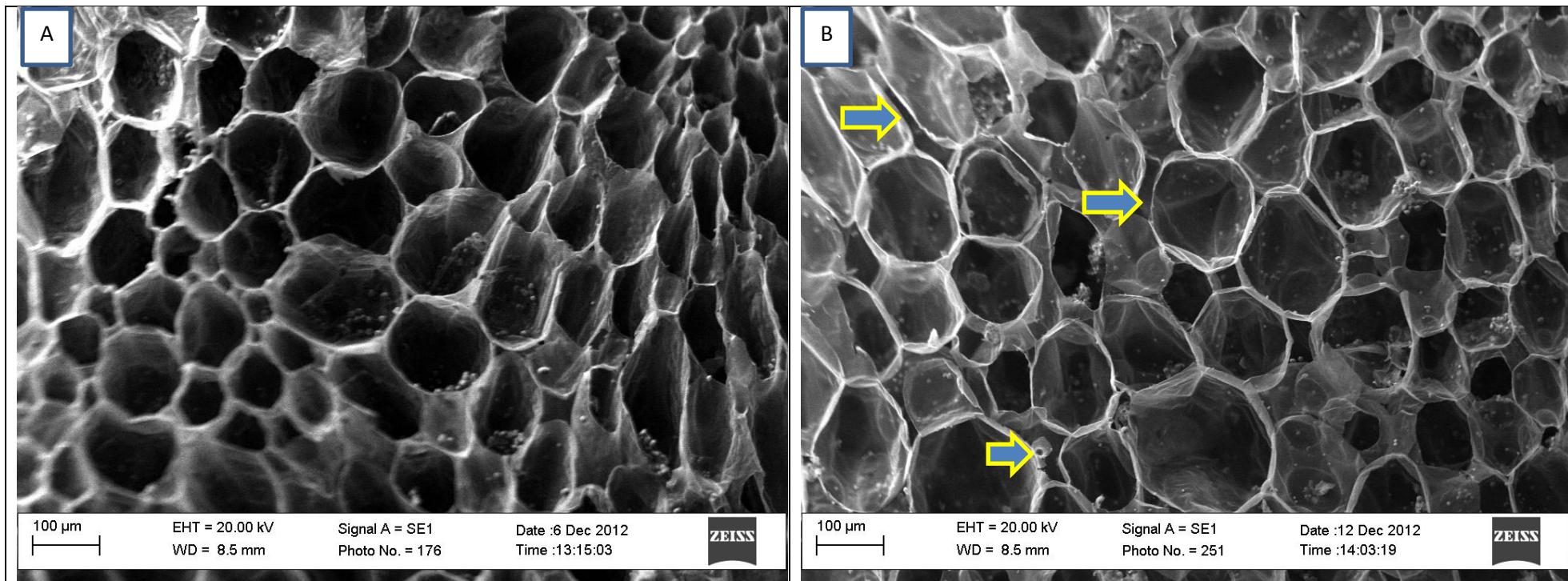
Fig. 2. Cell anatomical properties as revealed by scanning electron microscopy images taken at 100X resolution. **A** Shows the cells from Achtertuin fruit treated with 2,4-D amine, at harvest, showing the cells closely packed with small intercellular spaces; **B** shows cells taken from forchlorfenuron treated fruit harvested at Achtertuin with relatively larger intercellular spaces [arrows].
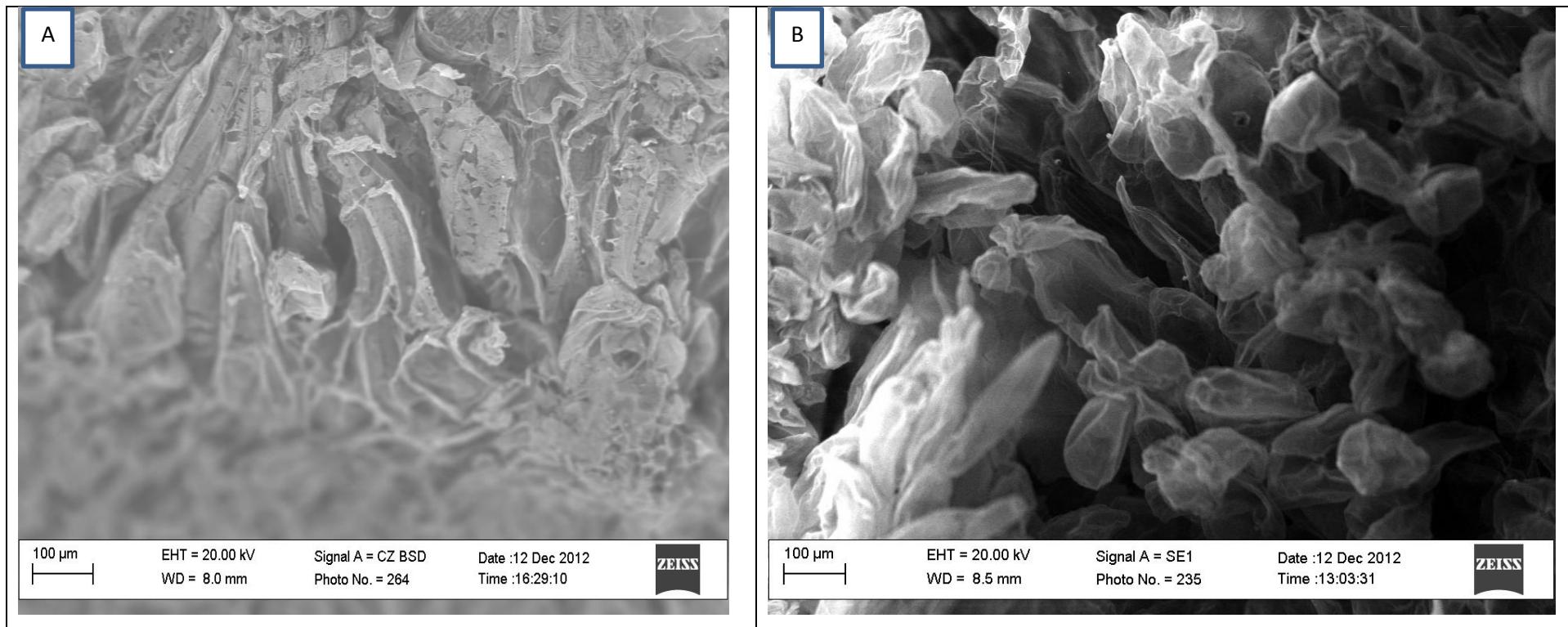
Fig. 3. Scanning electron micrographs of [A] non mealy fruit cells, and [B] mealy fruit cells. Non mealy fruit cells show breakage in the cell wall which is absent in mealy cells. Mealy cells slid whilst attempting to cut with the cutting blade.
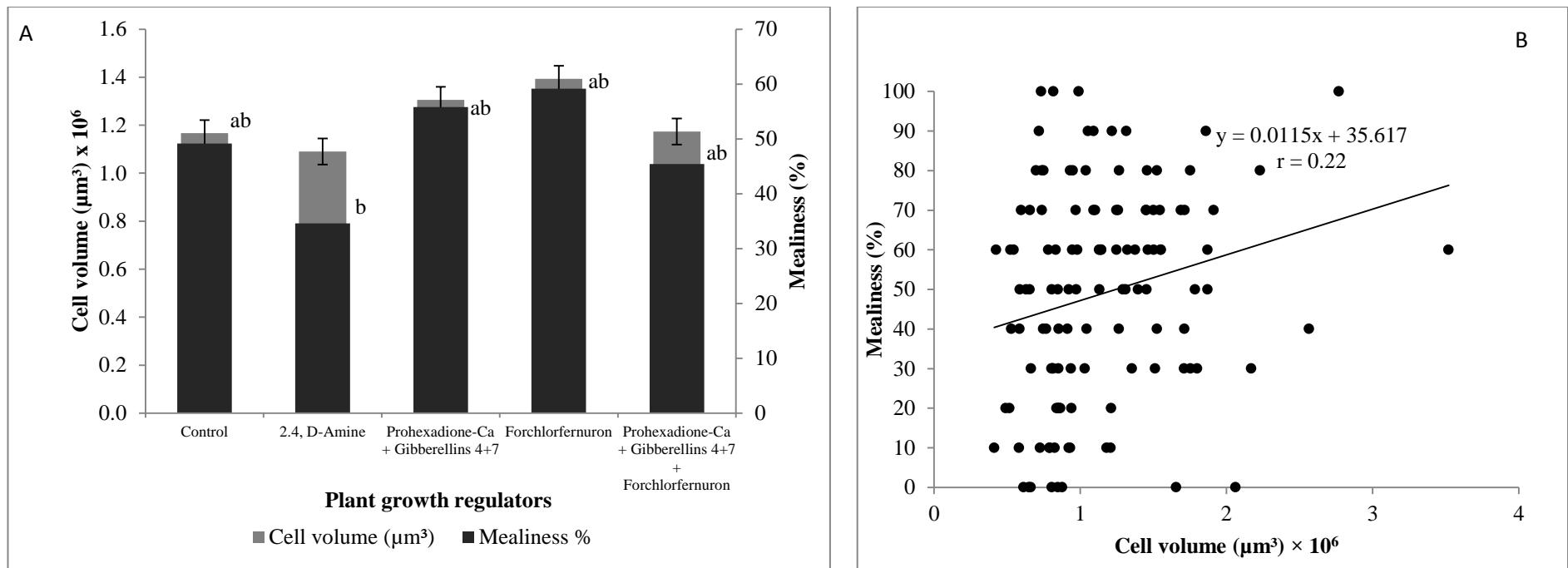
Fig. 4. Plant growth regulators on cell volume and mealiness percentage for all the farms (A). Shows significant differences in mealiness percentage between treatments (P<0.05). Letters show significant differences between mealiness at p ≤ 0.05. Correlation between cell volume and mealiness percentage (B).

PAPER 3

Microstructure analysis and detection of mealiness in 'Forelle' by means of X-ray computed tomography

---

Abstract

Mealiness causes fruit to develop a soft and dry texture during ripening. No technique currently exists to detect mealiness non-destructively. Two studies were conducted to i) determine the potential of X-ray macro computed tomography (macro-CT) to detect mealiness in 'Forelle' pears non-destructively, and to ii) establish histological differences between mealy and non-mealy fruit using X-ray micro computed tomography (micro-CT). Scans were made after 8 and 11 weeks of cold storage at -0.5 °C and at the end of shelf life of 7 days. Total porosity, pore size distribution and connectivity of cells were measured on regions of interest (ROI) cubes excised from the neck and equator of individual fruit. Macro-CT scans showed the presence of structural disorders in the cortex tissue, identified by micro-CT as large pores, in mealy fruit. These pores were already present at the end of cold storage before fruit would become mealy. Statistical differences with fruit which would ripen and become juicy were significant ($P<0.05$). The disorder appeared more clearly in the neck than in the equator region of the cortex. At the end of shelf life, porosity was significantly higher in mealy than non-mealy fruit. Micro-CT results confirmed that tissues of mealy fruit were more porous than those of non-mealy fruit, with a larger proportion of pores greater than 56.53 µm. Evidence was found of lysigenous pore formation in mealy fruit. Cells of mealy fruit were larger and oval-shaped while non-mealy fruit cells were more spherical. X-ray computed tomography was shown to be a promising technology for the non-destructive determination of mealiness at an early stage of fruit ripening.

Key words

Pyrus communis L., Forelle pears, X-ray CT, image analysis, lysigenous pores, non-destructive.

1. Introduction

Forelle is the second most planted and exported pear cultivar from South Africa (HORTGRO, 2014). The cultivar is mainly favoured for its exceptional blush, crunchy texture combined with sweet flavour when hard and ripe, but also by traditional consumers of pears who like ripened 'Forelle' for its soft, buttery or melting texture. However, like most European pears, 'Forelle' requires cold storage after harvesting in order for fruit to ripen uniformly (Villalobos-Acuna and Mitcham, 2008). When not stored sufficiently long under low temperatures, 'Forelle' pears may develop mealiness or astringency or both when fruit ripen at ambient temperatures during the shelf life period (Carmichael, 2011; Martin, 2002). Mealiness in 'Forelle' results in a soft, dry texture which is not desired by the majority of pear consumers (Manning, 2009). The standard practice to reduce mealiness therefore involves storing fruit for a minimum of 12 weeks at -0.5 °C before ripening in shelf life. This practice however, does not guarantee that mealiness does not develop.

Around the world, consumers are increasingly becoming conscious of the quality of foods they consume, particularly those from high income societies where the majority of fruit are consumed (Van Dalen et al., 2007). Mealiness is described in some literature as a 'quiet destroyer', as it does not show in the early stages of the supply chain but rather manifests itself in the hand of the consumer (Newman, 2006). No technique currently exists that can detect mealiness non-destructively and before fruit ripen. Techniques that rely on destructive sampling are not very effective in monitoring batch mealiness because the development of mealiness in one fruit in a batch does not mean occurrence in another (Barreiro et al., 2000). Therefore, a technique that can determine the condition of each individual fruit will be the most suitable for determining mealiness in a batch. Because mealiness has been described as an internal quality condition which is situated at the histological level (Lammertyn et al., 2002), techniques that examine the microstructure of the tissue may provide new perspectives to our understanding of mealiness.

Techniques that have received attention in the past few decades for determining conditions not detectable from outside such as mealiness include magnetic resonance imaging (MRI) (Barreiro et al., 1999; Barreiro et al., 2000, Lammertyn et al., 2003a), ultrasound (Bechar et al., 2005) and X-ray computed tomography (Herremans et al., 2014a, Van Dalen et al., 2007, Bechar et al., 2005). X-rays are electromagnetic radiation ranging from 0.01 to 10 nm (Kotwaliwale et al., 2014). Their use in visualising material properties is premised on the

72

material's ability to impede penetration by X-rays. As X-rays traverse an object of heterogeneous material composition, they are attenuated differently by the different components in the material. This difference in the materials ability to attenuate X-rays, also known as a material's radiodensity is reflected in the shadow images produced on the detector (Johansson, 2004; Fonseca et al., 2009). The shadow images produced can then be transformed into 3-dimensional (3-D) images through mathematical operations (reconstruction) with accompanying software (called computed tomography). Tomography reconstructs two-dimensional (2-D) cross- sectional images or slices through a 3-D object. Consecutive two-dimensional slices are then stacked into a volumetric dataset, forming a virtual 3-D image.

The number of applications of X-ray computed tomography (CT) in plant and food science has increased due to its ability to provide microstructural detail without destroying the structure of the material (Mendoza et al., 2007, Herremans et al., 2013a), with little or no sample preparation or chemical fixation required as in techniques such as scanning electron microscopy. Recently, studies have covered a variety of fruits including mango, kiwi, 'Conference' pear, apple and pomegranates (Cantre et al., 2014a; Cantre et al., 2014b; Lammertyn et al., 2003b; Herremans et al., 2013a; Magwaza and Opara, 2014). The applicability of X-ray CT in detecting internal browning disorder in 'Braeburn' apples was demonstrated by Herremans et al., (2013b). The researchers also compared the use of X-ray micro-CT and MRI in detecting watercore disorder and established that micro-CT produces superior classification of watercore in apples when compared to MRI. Earlier, Sonego et al., 1995 showed detection of wooliness in peaches using x-ray computed tomography.

Currently, the mechanism of mealiness development in 'Forelle' pear is not clearly understood, and can only be investigated destructively after ripening (Crouch, 2011). A first objective of this study was therefore, to determine whether X-ray CT can be used as a non-destructive imaging method to detect the mealy structure of intact 'Forelle' pears before ripening in comparison to the structure after ripening. We therefore performed X-ray CT measurements and analysed the fruit both at the start and end of 7 days shelf life after 8 weeks of cold storage. Mealiness classes were determined after ripening using a sensory panel of three trained judges and confirmed using a confined compression test. A second objective was to describe microstructural differences between mealy and non-mealy fruit. In this second experiment, the tissue microstructure of samples excised from 'Forelle' pears was

visualised and analysed with micro-CT.

2. Materials and methods

2.1 Fruit material

Fruit were harvested in March 2013 within the commercial harvest window for 'Forelle' in South Africa, from two farms, namely Koelfontein (lat. 33.00°S, long. 19.33°E) and La Plaisante (lat. 33.41°S, long 19.19°E), located in the Ceres and Wolseley regions in the Western Cape Province. The fruit were harvested at shoulder height from each tree, from either side of the canopy. Fruit were stored in polyethylene bag (37.5 µm) lined commercial cartons to reduce moisture loss and shrivelling and stored for a period of 8 weeks at -0.5 °C before evaluations. Twenty four fruit were scanned at Stellenbosch University using X-ray macro-CT after 8 weeks of cold storage at -0.5 °C before ripening and again after 7 days after ripening at room temperature. After 8 weeks of cold storage, 100 fruit were shipped to KU Leuven, Belgium where micro-CT scanning was performed after a further 3 weeks of storage at -0.5 °C and again after 7 days shelf life. Fig. 1 shows the sample collection, storage and CT scanning done in the study.

2.2 Fruit quality evaluation

As an indication of harvest maturity, a sample of 100 fruit from each farm were destructively evaluated for fruit weight, fruit firmness, total soluble solids (TSS) and titratable acidity (TA) (Table 2). An additional 100 fruit per farm were evaluated for maturity (section 2.3) after 8 weeks at -0.5 °C plus 7 days at 20 °C. After the last tomographic acquisitions fruit weight, firmness, total soluble solids content, titratable acidity, as well as juiciness and mealiness (section 2.3), were measured on each individual fruit.

Fruit diameter and fruit weight were measured using a Cranston gauge and electronic balance, respectively, both fitted to the fruit texture analyser (FTA 2007, Guss, Strand, South Africa). Fruit firmness was measured equatorially on opposite sides of the fruit on a peeled (1 mm) surface using a universal fruit texture analyser, fitted with a 7.9 mm diameter probe. TSS content was measured on a drop of expressed juice from each individual fruit using a hand held digital refractometer (PR-32, Atago, and Tokyo, Japan). TA was measured by titration of individual fruit juice using an automated titrator (Metrohm AG 760. Harison, Switzerland). Maturity indices (TSS, TA) at harvest were measured on 10 reps of 10 fruit each from each farm.

2.3 Mealiness and juiciness evaluation

Mealiness was determined using sensory analysis. Briefly, mealiness was evaluated on each individual fruit at the end of shelf life by a sensory panel comprising three trained evaluators with a minimum of 8 years of mealiness determination experience. Wedges of tissue were cut and evaluated organoleptically and hand squeezed looking for the presence or absence of free juice and were rated for mealiness. Periodically during testing, the evaluators cleaned their pallet with dry biscuits and water. For the micro-CT scanned fruit, fruit were evaluated in the same way by one trained panel member who accompanied fruit to Belgium.

A confined compression juiciness test (Barreiro et al., 1998) was also performed on the same fruit evaluated for mealiness in order to validate clear differences in fruit perceived mealy, partly mealy and non-mealy by the trained panel. Expressible juice from each individual fruit was measured using the confined compression test which measures juice released upon compression of a 1 cm high and 1 cm in diameter tissue wedge. This tissue wedge was excised from the equator region of each fruit and was compressed using a Texture Analyser (model TA. xTPlus, Stable Micro Systems, Inc, Surrey, UK). The instrument was set to move at 1 mm s^{-1}, and compress tissue to a distance of 2 mm from the surface, and return back at 10 mm s^{-1}. The machine was calibrated using a 10 kg steel block. A Bechkote protector filter paper (Whatman No. 2300 916, GE Healthcare, Burkingharmshire, UK) was used as sample holding paper, in order to collect juice released upon compression. After reaching the maximum deformation, the probe returned to the original position. The filter paper was weighed and then air-dried for 48 h after which it was oven dried at 40 °C for 24 h to develop the colour on the area covered by juice. The area covered by the released juice was measured using the ImageJ programme (Wayne Rasband, National Institute of Health, USA), after scanning the filter paper. For each fruit, there was a mealiness score as well as area and weight of juice.

2.4 Macro-CT

Twenty four 'Forelle' pears comprising 12 fruit from Koelfontein and 12 from La Plaisante were randomly selected at the end of 8 weeks at -0.5 °C. Prior to scanning, fruit were allowed to stand in the X-ray room overnight to equilibrate to room temperature. Tomographic scans were done using the General Electric Phoenix V │Tome│ X L240 (240 kV) (General Electric, Wunstorf, Germany) with an additional nanofocus tube up to 180 kV, housed at the

Department of Forestry, Stellenbosch University. Scans were taken again after a shelf life of 7 days. Fruit were mounted in a cup of low density polystyrene during scanning. A total of 3000 images were captured using a rotation of 360°, in a total time of 27 min. The resulting X-ray images were 16-bit per element images digitised as 2014 x 2014 pixel images with a pixel size of 54 μm. After scanning, tomographic reconstruction of the macro-CT images was done using the system supplied by Datos reconstruction software. The modified Feldkamp cone-beam procedure which uses the filtered back-projection algorithm was implemented. Preliminary image analysis was done using VG Studio Max Release 2.1 (Volume Graphics, GmbH, Germany). For further processing, the images were converted to an amenable 8-bit format using ImageJ software (Wayne Rasband, National Institute of Health, USA). The resulting 3D images were matrices of 2014 x 2014 x 2024 voxels with a voxel size of 54 μm.

Gray scale CT images were processed to detect structural disorders (defects) in selected cortex regions in the neck and on the equator of the fruit. These disorders were apparent as small areas of low grey scale values thus indicative of a locally high porosity. The high porosity voxels were segmented using auto-thresholding procedures described by Herremans et al. (2013b). Regions of interest (ROI) were selected from the virtual fruit as sub volumes measuring 200 x 200 x 200 voxels (thus volumes with approximately 10 mm sides), excised from the neck and equator of each fruit (Fig. 2). Figures 2A-C show the positions where ROI cubes were excised from the virtual fruit. On the equator, the ROI was positioned in the hypanthium next to the ovarium. In the neck, the position was chosen halfway between the core and stalk, and next to the centre line vascular tissue. The 4 samples at each position were taken at 90° angles around the axis of the fruit. Figures 2D-G show tissue structure and the result of the segmentation of the high porosity voxels for a neck and equator sample. The pear fruit scans from day 1 and day 7 of shelf life were registered to allow analysis of the same tissue cubes as affected by shelf life. The cubic samples of registered positions in the neck and equator were used to visualise and quantify differences between the three mealiness conditions as well as examine the progression of mealiness over time.

In order to visualise images in 3-D space, isosurface rendering was done on ROI cubes using Avizo Fire 7.1 (Visualisation Science Group, Bordeaux, France). Volume percentage of high porosity zones was calculated by expressing the total volume of segmented volume as a fraction of the total ROI volume.

2.5 Micro-CT

In order to determine the cell level information and show the pore space distribution at high resolution, micro-CT scanning was done on 24 fruit at KU Leuven, Belgium. Eight fruit were scanned on day 1 of the shelf life when fruit were still firm after being removed from the 11 week cold storage period at -0.5 °C. Sixteen fruit were scanned after 7 days shelf life. Micro-CT scans were done using the high resolution Skyscan- 1172 system (Bruker micro-CT, Kontich, Belgium), fitted with a Hamamatsu 10 Mp camera with a pixel size of 11.72 μm. Sampling was done in the radial direction of the equator and neck of the fruit. A cylindrical tissue sample of diameter 0.55 cm was excised from the radial direction in the neck and equator of each fruit. The cylindrical tissue was cut on either end to about 1 cm long and wrapped in parafilm to avoid desiccation during image acquisition. The X-ray source was set at a voltage of 59 kV and current of 167 μA. Image rotation steps were 0.31° and the pixel size was 4.87 μm. The distance of the object to source was set at 45.57 mm while the distance from the camera to source was set as 219.23 mm. To reduce artefacts resulting from the machine (Wildenschild and Sheppard, 2012), frame averaging was set at 3 and random movement adjusted to 2. The median filtering, flat field correction as well as geometrical correction were also set. The resulting images were 16-bit tiff images. The images were reconstructed using the NRecon software. A total of 937 slices were reconstructed in a total time of 736 seconds, at a rate of 0.807 seconds per slice. To produce quality images during reconstruction, a smoothing operation was set at 2, with a ring artefact correction set at 8 and beam hardening correction set at 35%. The resulting images after reconstruction were 8-bit bitmap images, consisting of 1208 x 1180 x 937 voxels.

On each micro-CT dataset, an ROI cylinder was cropped over 800 slices. The 800 slices long cylinder was analysed quantitatively with CT Analyser Version 1.13.5.1 software (Bruker micro-CT, Kontick, Belgium). A sub-volume cube measuring 200 x 200 x 200 voxels (pixel size = 4.87μm) was cropped and processed in Avizo Fire 8.2 (VSG, Bordeaux, France). A grey value threshold of 73 was chosen as a cut off value for discriminating between pores and cells, using the Otsu method as described by Herremans et al. (2013b). Otsu thresholding is favoured because it maximises interclass variance of the threshold between black and white pixels and is uniform with regards to uniformity and shape measures (Liao et al., 2001). After thresholding for pores and cells, a surface generation module was attached to produce 3D presentations of pores and tissue.

In order to further study mealiness at the cellular level, individual cells were manually segmented in the 200 x 200 x 200 voxels ROI cubes (pixels size = 4.87 μm) (Fig. 10). Wrapping was applied which builds the cell volume from segmentations of three orthogonal views through each cell (Herremans et al., 2013b). The morphometric parameters calculated are presented in Table 1.

2.6 Data analysis

In order to establish differences between the three mealiness conditions from macro-CT images, analysis of variance (ANOVA) was performed on quantitative characters using STATISTICA Version 12 (StatSoft Inc, 1984-2012, Tulsa, OK 74104, USA). To quantify tissue and pore differences within each mealiness category, histograms of pore volume and tissue volume distributions were plotted. ANOVA was also performed on morphometric parameters such as porosity (%), pore count, pore volume, cell volume and cell elongation. Means were separated using the least significant differences ($LSD_{0.05}$).

3. Results

3.1. Fruit quality and mealiness

Mealiness (by a sensory panel) and juiciness (by instrumental analysis) of fruit were measured after storage and shelf life. ANOVA was performed to verify if the instrumental variables mean juice area and juice weight correctly represent the mealiness categories used in the sensory evaluation (Fig 3. A and B). These results showed a clear separation in juice area and weight which confirmed the mealiness classes used by the trained panel. Table 2 presents the quality parameter values of fruit used in the study. Fruit harvested from Koelfontein and La Plaisante had a comparable TA, mass, diameter and firmness at harvest. Results show no differences in fruit diameter and TA after shelf life. However, there were significant differences in TSS and firmness between La Plaisante and Koelfontein. Mealiness incidence at La Plaisante was lower compared to that of Koelfontein. It is unclear why mealiness incidence differs between farms and if it is linked to the observed differences in TSS after shelf life.

3.2 Macro-CT

In order to assess differences in tissue structure between fruit that will eventually become mealy, and those that become partly mealy or non-mealy, non-destructive macro-CT was

performed for 24 fruit using the Phoenix scanner. The same fruit was measured at the start and end of shelf life, in order to evaluate whether fruit tissue that will become mealy after shelf life is different after storage before ripening and softening commenced. Table 3 shows the effects of mealiness and fruit side on the volume fraction of air spaces of 24 fruit at the end of cold storage at -0.5 °C for 8 weeks and again after shelf life for seven days determined from multiple ROIs in the neck and the equator of the fruit. There was a significant mealiness x side interaction on the fraction of defects in the fruit (P = 0.0035) (Table 3). The largest fraction defects (%) were observed in the neck of mealy fruit. Further analysis of the neck data where differences were observed showed differences at the end of cold storage and at the end of shelf life (Fig. 4). Mealy fruit had a significantly higher volume fraction of defects (airspaces) compared to non-mealy fruit. These structural differences were already observed after storage before ripening. On the equator, the differences between mealiness classes were not significant. However, there was a noticeable increase in the volume fraction of defects in shelf life. Mealy fruit had an average increase in the defects during shelf life of 351% in the equator while non mealy fruit defects increased by 60% in the equator.

Differences between mealy and non-mealy fruit are clearly seen in cross sectional images of the fruit with darker (less dense) voxels in the neck region (concentric pattern) of mealy fruit compared with non-mealy fruit (Fig. 5). Non-mealy and partly mealy fruit had a homogenous endocarp and hypanthium, with little grey material indicating low porosity. Figure 6 further shows representative sections of registered grey scale slices taken from mealy, partly mealy and non-mealy 'Forelle' pears taken at the end of cold storage and shelf life. The slices show clear differences in presence of defects between different types of fruit and between neck and equator tissue. Macro-CT therefore seems to already indicate differences in neck tissue structure before ripening and softening, when mealiness is observed by a sensory panel. The largest volume fraction of airspaces was found in the neck of fruit that were scored mealy after ripening. A close to zero volume fraction of defects was observed in non-mealy fruit. Micro-CT scans were performed to investigate the structural changes in more detail.

3.3 Microstructural changes in mealy fruit

To study the microstructural differences between mealy, partly mealy and non-mealy fruit, micro-CT scans of pear fruit tissues taken after shelf life were analysed. Figure 7 shows representative micro-CT sections through non-mealy, partly mealy and mealy fruit as well as

the tissue porosity of that sample. Micro-CT sections of the three mealiness classes exhibited different void shapes, sizes and orientation. In non-mealy fruit, few distinguishable air spaces could be observed in a dense cell stacking. In mealy fruit, individual cells were clearly discernible with a larger pore network around them (Fig. 7). Big cavities in mealy fruit showed evidence of cell separation and disintegration. The cavities could be containing leaked cell content though it is difficult to ascertain as the grey scale for intra- and extracellular liquids is similar. However, cell disintegration (resulting in large pores) is evidenced by fragments of damaged cells seen in tomographic sections of extremely mealy fruit (Fig. 8A).

Morphometric parameters such as number of objects, porosity, Euler number and connectivity were used to compare mealy and non-mealy fruit. Table 4 presents morphometric parameters of the fruit scanned at the end of shelf life. ANOVA was performed on the morphometric parameters of the mealy, partly mealy and non-mealy fruit. Results showed no significant differences in the majority of morphometric parameters between mealy and non-mealy fruits in the equator region, confirming the macro-CT results. However, there were significant differences observed on average porosity in the neck region. Partly mealy fruit did not show substantive structural differences with non-mealy fruit, suggesting that the partly mealy stage is a transition stage in the development of mealiness.

Total porosity percentage was significantly higher in mealy fruit compared to partly mealy and non-mealy fruit in the neck (P=0.031). Although not significantly different, the number of cell clusters/ objects in mealy fruit was appreciably higher in mealy than in non-mealy and partly mealy fruit (Table 4). In the neck, the number of objects in the mealy fruit was more than 10 times larger than in the non-mealy and partly mealy fruit, respectively. In the equator, the difference was smaller (Table 4). Pore connectivity was also higher in mealy compared to non-mealy fruit, although not significantly so. Due to the limited amount of samples with micro-CT these differences at p>0.07 may have been significant, if a larger samples size would have been possible. Number of closed pores, volume of closed pores and closed porosity were not significantly affected by mealiness. Further analyses were done to show the contribution of different pore sizes to total volume of pores in the ROI cube (Fig. 9). There were significant differences between the volumes of voids greater than 53.6 μm^3 (P=0.003). The contribution of the larger voids ($\geq$53.62 μm^3) to total volume of pores was significantly higher in mealy fruit compared to partly mealy and non-mealy fruit (Fig. 9).

To study the effect of mealiness on the cell shape and size, manual segmentation was done to obtain individual cells from the micro-CT images. Volumetric quantification of individual cells was applied for the fruit neck tissue where significant differences were observed between mealy and non-mealy fruit (Table 5). Results showed significant differences in area, volume, length and width to length ratio between mealy and non-mealy fruit cells. Mealy fruit had significantly larger cells, with respect to both cell volume and cell area. The size difference was attributed more to the length of mealy cells (P<0.0001). The average length of cells observed in the neck of mealy fruit was 68.22 µm whereas that of non-mealy fruit was 58.71 µm. Cell width was not significantly affected by the mealiness condition (P=0.18085). The average width of mealy cells was 42.63 µm while that of non-mealy fruit was 40.04 µm. Mealy fruit had ellipsoidal (melon shaped) cells compared to non-mealy fruit (Fig. 10A and B). The width to length ratio which best describes the shape of spherical objects was significantly higher in non-mealy than in mealy fruit cells.

Fig. 11 shows the frequency distributions of volume of manually segmented cells. Mealy fruit had a higher proportion of larger cells compared to non-mealy tissues. The percentage of cells larger than 80000 μm^3 was 0%, 17.2% and 13.8% in three individual non-mealy pears and 50%, 46.7% and 41.4% for individual mealy pears.

4. Discussion

'Forelle' pears develop mealiness during ripening when not stored sufficiently long under cold storage before ripening (Crouch et al., 2005, Crouch and Bergman, 2013). Understanding structural differences between mealy and non-mealy fruit greatly aids our understanding of mealiness. In this study, storing fruit for 8 weeks at -0.5 °C and 11 weeks at -0.5 °C and ripening for 7 days at 20 °C shelf life produced mealy fruit. Computed tomography was applied to establish differences in tissue macrostructure of fruit that would become mealy compared to fruit that would not become mealy (after storage) as well as the tissue microstructure of non-affected versus affected fruit after ripening.

Macro-CT gray scale images showed that mealy tissues have lower density (darker voxels) compared to non-mealy tissues in the neck region. Darker voxels represent material of low X-ray attenuation due to an increased presence of air (Brecht et al., 1991). CT images revealed that affected portions were not uniformly distributed in the fruit, varying according to the

81

extent and progression of mealiness but most concentrated in the mid-cortex of the hypanthium in the neck of the fruit. Examination of the fraction of defects associated with mealiness showed that mealy fruit were more porous compared to partly mealy fruit and non-mealy fruit. Mealy fruit had a higher percentage of larger pores in the neck than in the equator. The pores were evident at harvest, increasing during shelf life. Although in the equator, the values were not significant, results showed an increasing trend in the defects. Increasing void volume in the hypanthium suggests that in this region mealiness could have been progressing at the time of analysis. The increase in mealiness in shelf life has been observed in previous studies (Crouch et al., 2005). However, it has been shown that mealiness tends to disappear with longer shelf life (Carmichael, 2011; Crouch, 2011; Martin, 2002). Our results also showed an increase in mealiness incidence from the neck going downwards confirming earlier observations that mealiness and ripening in 'Forelle' starts in the neck region extending down the fruit, then outwards (Crouch, 2011). Examination of macro-CT scans taken at the end of cold storage show that fruit that eventually become mealy already have the darker voxels in the mesocarp of the neck at the end of cold storage, indicating a high volume fraction of voids. A hypothesis for the observed changes during shelf life could be the following. Mealy fruit have more separated and collapsed cells after storage creating larger defects mainly in the neck.

X-ray CT was shown to be a useful tool to detect tissue changes in relation to mealiness in fruit without destructive sampling. As it was seen that the tissues are already affected after storage, screening for fruit with affected tissue may prove a viable tool as a non-destructive method for sorting mealy fruit before commercialisation. In recent studies the potential of X-ray CT was also demonstrated for detecting watercore and browning disorder of apples (Herremans et al., 2014a & 2014b). To realise such a method, however, the technique must be implemented inline on sorting lines, for which no method exists to date for fruit (Nicolai et al., 2014). The imaging should be faster and cheaper which would require lower resolution systems. This could potentially be achieved if differences in grayscale contrast can be detected also at lower resolution with pixel sizes in the order of 1 mm rather than 50 μm.

In the micro-CT study on ripened fruit, we showed that in addition to differences in void volume, there were significant differences between mealy and non-mealy pears in terms of pore sizes greater than 53.62 μm^3. Mealy fruit had more pores larger than 53.62 μm^3 when compared to non-mealy and partly mealy fruit. Two forms of porosities were evident from

our study: lysigenous intercellular porosity whereby individual cells disintegrated as well as schizogenous intercellular porosity which involves separation of cells resulting from cell-to-cell debonding (Ting et al., 2013). Mealy fruit were characterised by both lysigenous and schizogenous intercellular spaces while non-mealy fruit were dominated by schizogenous intercellular spaces. The larger voids (>53.62 μm^3) are likely lysigenous spaces while smaller voids (≤53.62 μm^3) are of schizogenous origin. This observation is the first indication found for lysigenous pore formation in fruit that has been hypothesized before (Verboven et al., 2008), but was considered only present in apple fruit.

Higher porosity of mealy fruit was already visible after cold storage. It is therefore possible that pore formation may have been influenced pre-harvest rather than by cold storage. Cronje et al. (2015) found that canopy position plays a role in mealiness development which may support such a hypothesis. However ripening or the stage of mealiness also plays a role in perceived mealiness incidence as Martin (2002) found that longer shelf life days reduces the incidence perceived. Longer cold storage periods (>12 weeks at -0.5 °C) for 'Forelle' pears reduces mealiness (Crouch, 2011), probably due to suppression of ethylene regulated cell wall enzymes. The stage of ripening and mealiness development may therefore both play a role in lysigenous pore formation.

These results, together with observations from pore visualisation of mealy fruit showed that mealiness is a result of cells collapsing in addition to extensive cell-to-cell debonding. These results confirm findings by Harker and Hallet (1992) who first postulated that mealiness in apples results from cell separation at the middle lamella. This was supported by findings of mealy 'Forelle' light micrographs as well as earlier depolymerisation of the water soluble polyuronides of mealy tissues (Crouch, 2011). However, contrary to an earlier notion that mealiness results from separation of cells during mastication (Harker and Hallet, 1992), our findings suggest that mealy cells separate during mealiness development, often before mastication. The sensation of flouriness may therefore not necessarily be a result of mechanical activity of the teeth but sliding of already quasi-amorphous material. In addition, the higher number of individual objects shown in mealy fruit by morphometric analysis is an indicator of separated tissues or groups of cells/ individual cells.

In the neck, mealy fruit had a high connectivity of pores compared to partly mealy and non-mealy fruit. This is contrary to other disorders where connectivity falls due to the disorder. A

good example is browning disorder of apples where connectivity was shown to decrease (Herremans et al, 2013b). In mealiness however, there is separation of cells at the middle lamella (Crouch, 2011; Harker and Hallet, 1992,). The pore network is maintained whereas in other disorders there is cell membrane damage (Herremans et al., 2013b). Mealy fruit have been shown to maintain cell wall integrity in scanning electron micrographs (data not shown). The differences in connectivity and Euler number of mealy and non-mealy fruit in the neck suggest microstructural compositional changes which are associated with mealiness. In the equator where mealiness was not as advanced, the differences were not significant.

Our study showed that cells were closely packed in non-mealy tissues with little gaps in between the cells. Mealy fruit showed extensive dissolution with large air spaces. According to Verboven et al., 2008, each pear cell is surrounded by a tight and continuous pore network. The pores were quite evident in mealy fruit where cells had separated. In a study by Ben-Arie and Kislev (1979), similar empty regions were observed after treating pear tissue with commercial preparations of enzymes polygalacturonase and cellulase. Ben-Arie and Kislev (1979) attributed the softening and subsequent loss of middle lamella binding material to the activity of disassembly enzymes.

Examination of individual cells using individual cell measures analysis showed differences in both cell volume and cell shape between mealy and non-mealy fruit. Mealy fruit had longer, ellipsoidal shaped cells while non-mealy fruit tended to be spheroidal. Pear cells radiate from brachyscleroids (stone cells), increasing in size away from the centre. Various theories have emerged to link physiological disorders with cell sizes. It has been argued that larger cells tend to have smaller area of cell-to-cell contact compared to smaller cells (Harpartap et al., 2005). The size of cells has implications on two aspects of mealiness development. The first is that larger cells tend to be loosely connected making them easier to separate during mastication (Harpartap et al., 2005). The second theory is linked to calcium in plant cells. The more contact there is between cells, the more calcium is found in the intercellular spaces (De Freitas et al., 2010). Because of the smaller area of cell contact in larger celled fruit, the amount of available calcium to reinforce cell-to-cell contact may be less compared to where cell-to-cell contact is large. This may in turn result in weaker contact and hence cells readily separate.

5. Conclusion

Mealiness development in 'Forelle' was visualised over the shelf life period using macro- and micro-CT. Further, microstructural differences between mealy and non-mealy fruit were observed. Fruit that would develop mealiness after ripening have a larger volume fraction of airspaces at the end of cold storage in the neck region, possibly already at harvest. Mealiness was also shown to be associated with high fruit porosity. Cells of mealy fruit were also shown to be larger and ellipsoidal in shape while non-mealy cells were smaller and more rounded. Separations of cells in 'Forelle' seem to commence before shelf life and progresses during shelf life. Our results have demonstrated the potential of macro X-ray CT to describe fruit that would become mealy, before mealiness can be detected organoleptically. This study also noted that lysigenous pores are present in mealy pear tissues which open up new avenues for research in understanding the mechanism of action and stages involved in mealiness development of 'Forelle' pears.

Literature Cited

Barreiro, P., Ortiz, C., M. Ruiz-Altisent., De Smedt, V., Schotte, S., Andani, Z., Wakeling, I., Beyts, P.K., 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples. A collaborative test. J. Texture Stud. 29, 509-525.

Barreiro, P., Ruiz-Cabello, J., Fernandez-Valle, M. E., Ortiz, C and Ruiz-Altisent, M., 1999. Mealiness assessment in apples using MRI techniques. Magn. Reson. Imaging. 17 (2), 275-281.

Barreiro, P., Ortiz, C., Ruiz_Altisent M., Ruiz_Cabello, J., Fernandez_Valle, M. E., Recasens, I., Asensio, M., 2000. Mealiness assessment in apples and peaches using MRI techniques. Magn. Reson. Imaging 18, 1175-1181.

Bechar, A., Mizrach, A., Barreiro, P., Landahl, S., 2005. Determination of mealiness in apples using ultrasonic measurements. Biosys. Eng. 91 (3), 329-334.

Ben-Arie, R., Kislev, N., 1979. Ultrastructural changes in the cell walls of ripening apple and pear fruit. Plant Physiol. 64, 197-202.

Brecht, J.K., Robert. L., Joseph, S., Garner, C., Tollner, E.W., 1991. Using X-ray computed tomography to non-destructively determine maturity of green tomatoes. HortSci.26, 45-47.

Cantre, D., East, A., Verboven, P., Araya, X.T., Herremans, E., Nicolai, B., Pranamornkith, T., Loh, M., Mowat, A., Heyes, J., 2014a. Microstructural characterization of commercial kiwifruit cultivars using X-ray micro computed tomography. Postharvest Biol.

Technol. 92, 79-86.

Cantre, D., Herremans, E., Verboven, P., Ampofo-Asiama, J., Nicolai, B.M., 2014b. Characterisation of the 3-D microstructure of mango (*Mangifera indica* L. cv. Carabao) during ripening using X-ray computed microtomography. Innov. Food Sci. Emerg. Technol. 24, 28-39.

Carmichael, P.C., 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc (Agric) Thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, A., Crouch, E.M., Muller, M., Theron, K.I., van der Rijst, M., Steyn, W.J., 2015. Canopy position and cold storage duration affects mealiness incidence and consumer preference for the appearance and eating quality of 'Forelle' pears. Sci. Hortic. 194, 327-336.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L.). PhD (Agric) Thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Crouch, E.M., Holcroft, D.M., Huysamer M., 2005. Mealiness of 'Forelle' pears-quo vadis? Acta Hortic. 671, 369-376.

Crouch, I., Bergman H., 2013.Consumer acceptance study of early marketed 'Forelle' pears in the United Kingdom and Germany. SA Fruit Journal, 11(6), 64-71.

De Freitas, S.T., Do Amarante, C.V.T., Labavitch, J.M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biol. Technol. 57, 6-13.

Fonseca, R.B., Branco, C.A., Haiter-Neto, F., de Souza Gonçalves, L., Soares, C.J., Carlo, H.L., Sinhoreti, M.A.C., Correr-Sobrinho, L., 2009. Radiodensity evaluation of dental impression materials in comparison to tooth structures. J. Appl. Oral. Sci. 18 (5), 467-476.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortSci. 27, 1291-1294.

Harpartap, M., Bedford, D., Luby, J., Vickers, Z., Tong, C., 2005. Relationship of Instrumental and Sensory Texture measurements of fresh and stored apples to cell numbers and size. HortSci. 40, 1815-1820.

Herremans, E., Bongers, E., Estrade, P., Gondek, E., Hertog, M., Jakubczyk, E., Do Trong, N, N., Rizzolo, A., Saeys, W., Spinelli, L., Torricelli, A., Vanoli, M., Verboven, P., Nicolai, B.M., 2013a. Microstructure-texture relationships of aerated sugar gels:

Novel measurement techniques for analysis and control. Innov. Food Sci. Emerg. Technol. 18, 202-211.

Herremans, E., Verboven, P., Bongaers, E., Estrade, P., Verlinden, B.E., Wevers, M., Hertog, M.L.A.T.M., Nicolai, B.M., 2013b. Characterisation on 'Braeburn' browning disorder by means of X-ray micro-CT. Postharvest Biol. Technol. 75, 114-124.

Herremans, E., Melado-Herreros, A., Defraeye, T., Verlinden, B., Hertog, M., Verboven, P., Val, J., Fernández-Valle, M., Bongaers, E., Estrade, P., Wevers, M., Barreiro, P., Nicolai, B. 2014a. Comparison of X-ray CT and MRI of watercore disorder of different apple cultivars. Postharvest Biol. Technol. 87, 42-50.

Herremans, E., Verboven, P., Defraeye, T., Rogge, S., Ho, Q., Hertog, M., Verlinden, B., Bongaers, E., Wevers, M., Nicolai, B. 2014b. X-ray CT for quantitative food microstructure engineering: The apple case. Nucl. Instrum. Meth. B. 324, 88-94.

HORTGRO, 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

Johansson, A., 2004. Magnetic resonance imaging with ultrashot echo time as a substitute for X-ray computed tomography. Print and Media, Umea University. Sweden. ISBN: 978-91-7601-138-6.

Kotwaliwale, N., Singh, K., Kalne, A., Jha, S.N., Seth, N., Kar, A., 2014. X-ray imaging methods for internal quality evaluation of agricultural produce. J. Food. Sci. Technol. 51 (1), 1-15.

Lammertyn, J., Verlinden, B.E., Nicolai, B.M., 2002. Applying advanced instrumental methods: mealiness in fruit. In: Jogen, W. 2002. Fruit and Vegetable processing: Improving quality. Woodhead Publishing in Food Science. ISBN 9781855735484.

Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B., 2003a. Analysis of the time course of core breakdown in 'Conference' pears by means of MRI and X-ray CT. Postharvest Biol. Technol. 29 (1), 19-28.

 Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B., 2003b. MRI and X-ray CT study of spatial distribution of core breakdown in 'Conference' pears. Mag. Reson. Imaging. 21 (7), 805-815.

Liao, P.S, Chen, T.S., Pau-Choo, Chung, P.C., 2001. A fast algorithm for multilevel thresholding. J. Inform. Sci. Eng.17, 713-727.

Magwaza, L.S., Opara, U.L., 2014. Investigating non-destructive quantification and characterisation of pomegranate fruit internal structure using X-ray computed tomography. Postharvest Biol. Technol. 95, 1-6.

Manning, N., 2009. Physical, sensory and consumer analysis of pear genotypes among South

African consumers and preference of appearance among European consumers. MSc Food Science thesis. Department of Food Science, Stellenbosch University, Stellenbosch, South Africa.

Martin, E.M., 2002. Ripening responses of 'Forelle' pear. MSc (Agric) thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Mendoza, F., Verboven, P., Mebatsion, H.K., Kerckhofs, G., Wevers, M., Nicolai, B., 2007. Three-dimensional pore space quantification of apple tissue using X-ray computed microtomography. Planta. 226, 559-570.

Newman, S., 2006. Stonefruit mealiness- the quiet destroyer. Netpro Update. NSW Department of Primary Industries. Australia. www.lowchillaustralia.com (accessed 14.10.2014).

Nicolai, B., Defraeye, T., De Ketelaere, B., Herremans, E., Hertog, M., Saeys, W., Toricelli, A., Vandendriessche, T., Verboven, P., 2014. Nondestructive measurement of fruit and vegetable quality. Annu. Rev. Food Sci. Technol. 5, 285-312.

Sonego, L., Ben-Arie, R., Raynal, J., Pech, J.C., 1995. Biochemical and physical evaluation of textural characteristics of nectarines exhibiting woolly breakdown: NMR imaging, X-ray computed tomography and pectin composition. Postharvest Biol. Technol. 5, 187-198.

Ting, V.J.L., Silcock, P., Bremer, P.J., Biasioli, F., 2013. X-ray computer tomographic method to visualise the microstructure of different apple cultivars. J. Food Sci. 78(11), 1735-1742.

Van Dalen, G., Nootenboom, P., van Vliet, L.J., Voortman, L., and Esveld, E., 2007. 3-D imaging, analysis and modelling of porous cereal products using X-ray microtomography. Image Anal. Stereol. 26, 169-177.

Verboven, P., Kerckhofs, B., Mebatsion, H.K., Ho, Q.T., Temst, K., Wevers, M., Cloetens, P., Nicolai, B.M., 2008. Three-dimensional gas exchange pathways in pome fruit characterised by Synchroton X-ray computed tomography. Plant Physiol. 147, 518-527.

Villalobos-Acuna, M., Mitcham, E.J., 2008. Ripening of European pears: the chilling dilemma. Postharvest Biol. Technol. 49, 187-200.

Wildenschild, D., Sherpard, A.P., 2012. X-ray imaging and analysis techniques for quantifying pore scale structure and processes in subsurface porous medium systems. Adv. Water. Resour. http://dx.i.org/10/1016/j.advwatres.2012.07.018. (accessed 23.03.2015).

Table 1. Morphometric parameters used to describe 3-D micro-CT images, based on Herremans et al. (2013b), with modifications.

Microstructure parameter	Unit	Description
Number of objects	-	Number of individual segmented cells
Porosity	%	Pore volume divided by total volume of the analysed sample (pores and cells)
Closed porosity		The connected assemblage of space (black) voxels that is fully surrounded on all sides in 3D by solid (white) voxels.
Open porosity		Any space located within a solid object or between solid objects with connection in 3D space with outside space.
Volume	mm^3	Volume of object
Area	mm^2	Area of the object boundary
Length	mm	Maximum of the Feret diameters measured over a range of angles
Width	mm	Minimum of the Feret diameters measured over a range of angles
Euler number	-	Indicator of connectedness of a 3-D complex structure, and a global characterisation of topology. Higher values indicate poorly connected structures and lower values better connected structures.
Connectivity	-	The number of connections between matrix structures per unit volume, based on Euler number.

Table 2. Physico-chemical parameters of 'Forelle' pear fruit used in the study, at harvest and after 8 weeks of storage at -0.5 °C plus 7 days shelf life at 20 °C. TSS, TA at harvest represents an average of ten replicates of 10 fruit each from each farm.

	Farm	Mass (g)	Diameter (cm)	Firmness (kg)	TSS (%)	TA (%)	Mealiness (%)*
Harvest	La Plaisante	174.2	64.7	6.4	14.4	0.3	-
	Koelfontein	178.2	65.3	6.3	14.9	0.3	-
	Pr>F	*0.928*	*0.4354*	*0.071*	*0.088*	*0.763*	
Shelf life	La Plaisante	165.9	-	1.5 a	15.6 b	0.26	29
	Koelfontein	171.1	-	1.3 b	17.2 a	0.27	61
	Pr>F	*0.077*		*0.0002*	*<0.0001*	*0.0786*	

*Mealiness percentage was determined by expressing the number of mealy fruit (sensory) as a percentage of the total fruit examined (100 fruit per farm).

Table 3. Effect of mealiness and fruit position on percentage of defects (high density of darker voxels). Means were separated using LSD at 5% level of significance.

	Mealiness	Defects (%)
Equator	Mealy	0.047b
	Partly mealy	0.010b
	Non mealy	0.002b
Neck	Mealy	1.670a
	Partly mealy	0.117b
	Non mealy	0.052b
P-value		
Side		*
Mealiness		**
Side x mealiness		*

[a] Means with different letters differ at P < 0.05, 0.01 (*. ** respectively)

Table 4. Morphometric parameters of 'Forelle' pear fruit tissue analysed with micro-CT. Fruit tissue was analysed after 11 weeks of storage and 7 days shelf life. Values are mean±SD. Means with different letters are significantly different at $p<0.05$.

	Number of cell clusters	Number of pores	Closed porosity (%)	Open porosity (%)	*Total porosity (%)	Connectivity
Neck						
Non-mealy	963.9±897.0	14159.1±4846.2	1.3±0.8	4.5±1.8	5.8±1.1b	11010.7±4667.8
Partly mealy	719.7±774.4	14996.7±5097.6	1.5±1.0	4.3±2.7	5.8±1.8b	9624.0±8079.7
Mealy	9132.0±7269.6	15275.3±8604.9	1.3±1.2	8.0±3.9	9.2±2.9a	22572.0±11517.9
Equator						
Non-mealy	663.6±296.1	10507.8±4905.1	0.6±0.3	7.1±0.6	7.6±0.5	26342.0±7440.6
Partly mealy	798.3±246.7	9720.0±2629.3	0.8±0.4	6.9±1.1	7.6±0.9	19982.3±5468.1
Mealy	2775.8±2600.8	10781.5±3561.0	0.7±0.3	7.4±0.5	8.1±0.4	25521.5±4499.8

* Open porosity + closed porosity = Total porosity.

Table 5. Morphometric parameters of manually segmented individual cells determined using cell measures analysis. Results are for the multiple regions of interest (ROI) taken from the neck of the mealy (n=3) and non-mealy (n=3) fruit.

	Area (3-D) (μm^2)	Volume (3-D) (μm^3)	Length (3-D) (μm)	Width (3-D) (μm)	Width:Length ratio	Sphericity
Neck						
Mealy	8543.88a	63422.47a	68.22a	42.63	0.633b	0.884
Non-mealy	6983.69b	48900.34b	58.71b	40.04	0.700a	0.910
	Pr>F					
	0.00001	*0.00035*	*<0.00001*	*0.18085*	*<0.00001*	*0.12838ns*
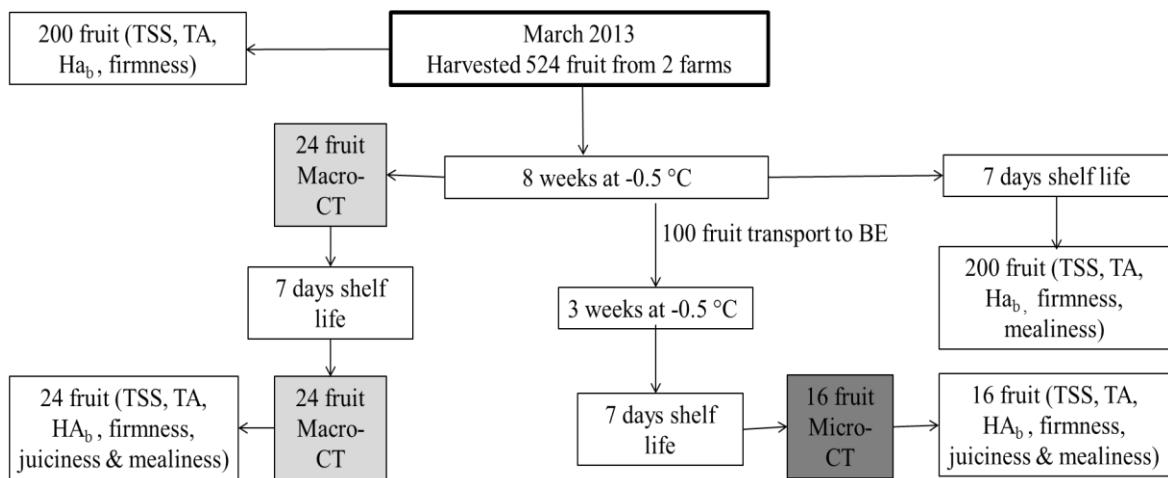
Fig. 1. Schematic presentation of fruit collection, quality measurements, mealiness assessment and macro- and micro-CT scanning of 'Forelle' pear fruit. (TSS- total soluble solids, TA- titratable acidity, HA_b- hue angle at blush side, BE- Belgium).
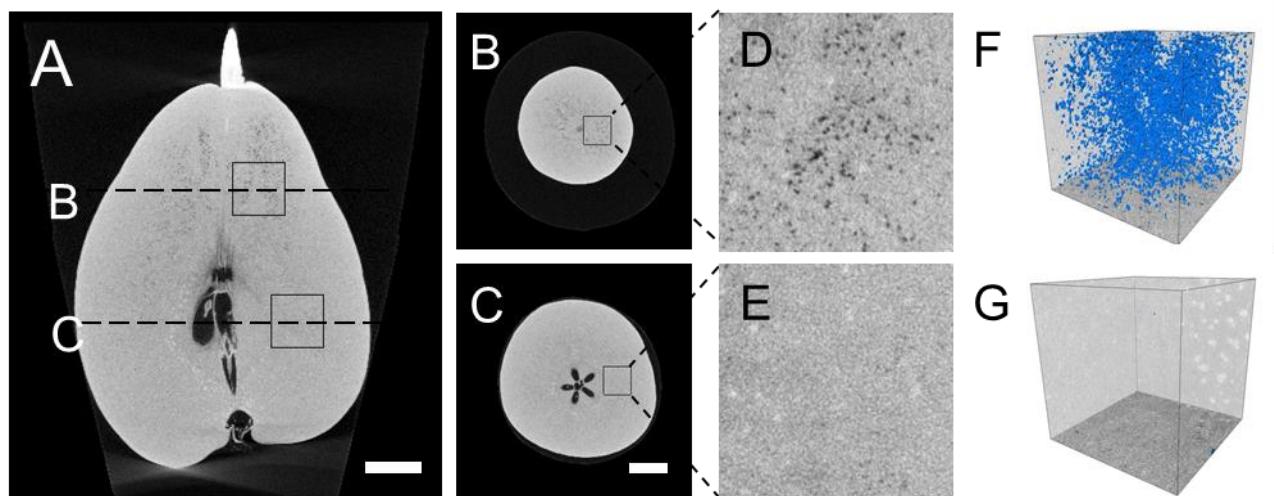
Fig. 2. X-ray CT of a mealy Forelle pear after 7 days shelf life. Plot A shows a saggital CT slice of the fruit. Plots B and C are transverse sections through the neck and equator of the fruit, respectively. Black squares identify cubical tissue samples (with sides of 200 pixels or 10.8 mm) that were analysed in detail for increased porosity (dark grey spots). High porosity regions were present in the neck region tissue in plot D but not in the equatorial tissue in plot E. In plots F and G, 3D images are given with the segmented high porosity regions coloured in blue. The scale bar measures 10 mm. Reconstructed CT images had a pixel resolution of 54 μm.
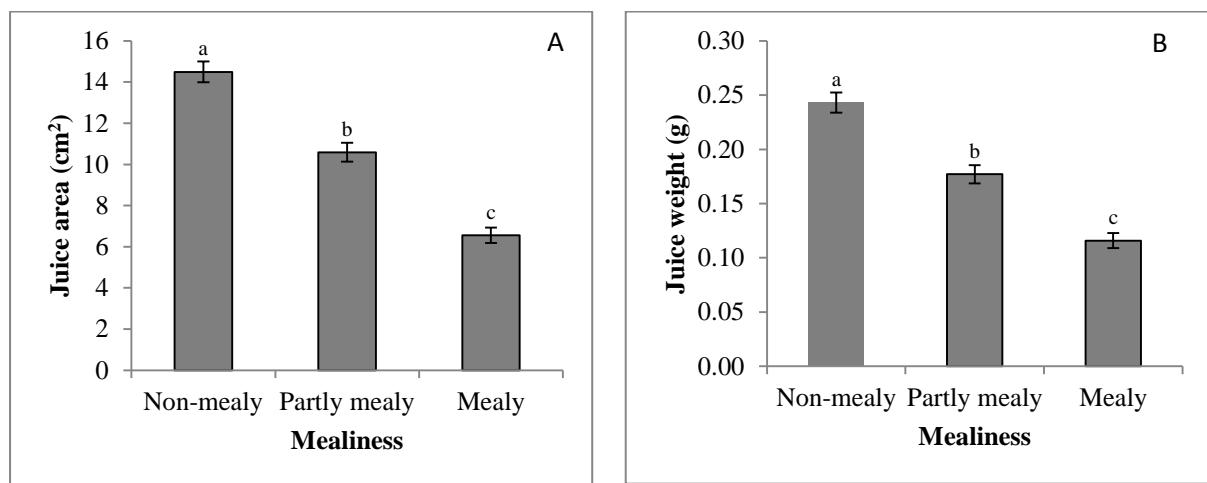
Fig. 3. (A) Area (cm^2) covered by confined compression juice on filter paper, B) Weight (g) of released juice on compression of a 1 cm high x 1 cm diameter 'Forelle' pear tissue of 100 fruit after 8 weeks at -0.5 °C and 7 days at 20 °C. Letters show significant differences at p≤0.05.
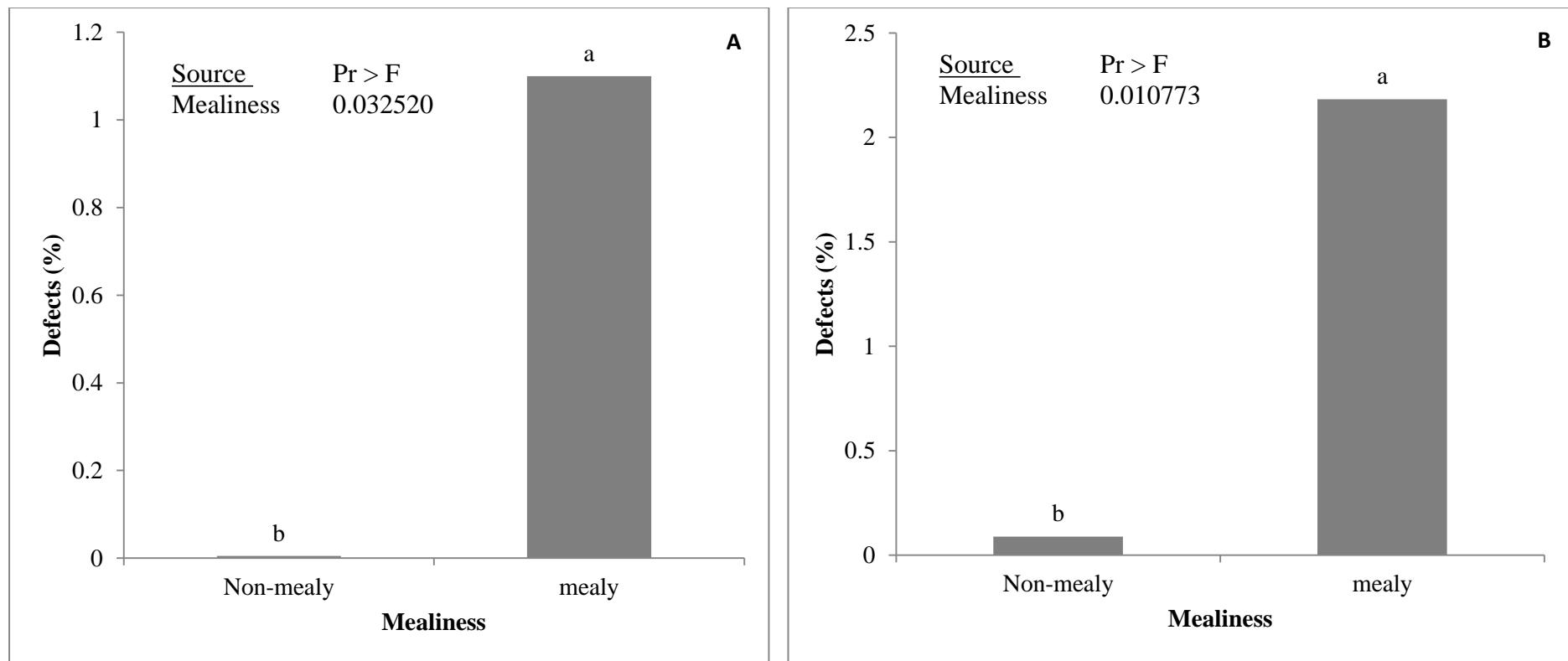
Fig. 4. Effect of mealiness on the fraction defects (%) (structural disorders with high porosity voxels) measured on multiple region of interest (ROI) cubes excised in the neck 24 'Forelle' pear images. (**A**) at the end of cold storage of 8 weeks at -0.5 °C, and **B**) at the end of cold storage of 8 weeks at -0.5 °C plus seven days of shelf life at 20 °C. Images were measured using the Phoenix macro CT scanner at a pixel resolution of 54 µm.
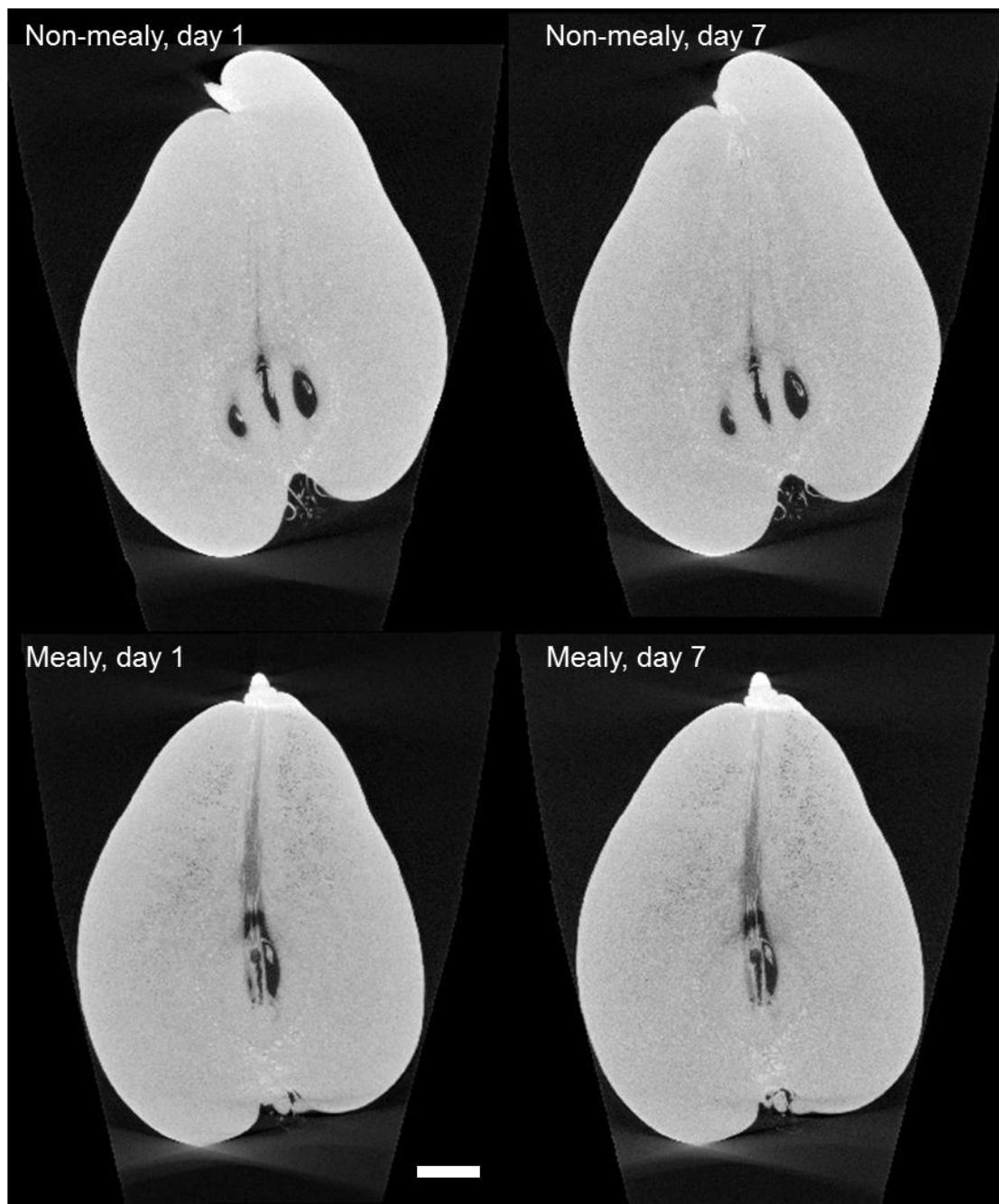
Fig. 5. Registered X-ray CT saggital slices of 'Forelle' pears at day 1 and day 7 of shelf life after 8 weeks of cold storage at -0.5 °C. The top row is a non-mealy pear, the bottom is a mealy fruit. The scale bar is 10 mm. Images have a pixel resolution of 54 μm. The darker voxels in the image show the region of low X-ray attenuation which indicates a higher air volume fraction.
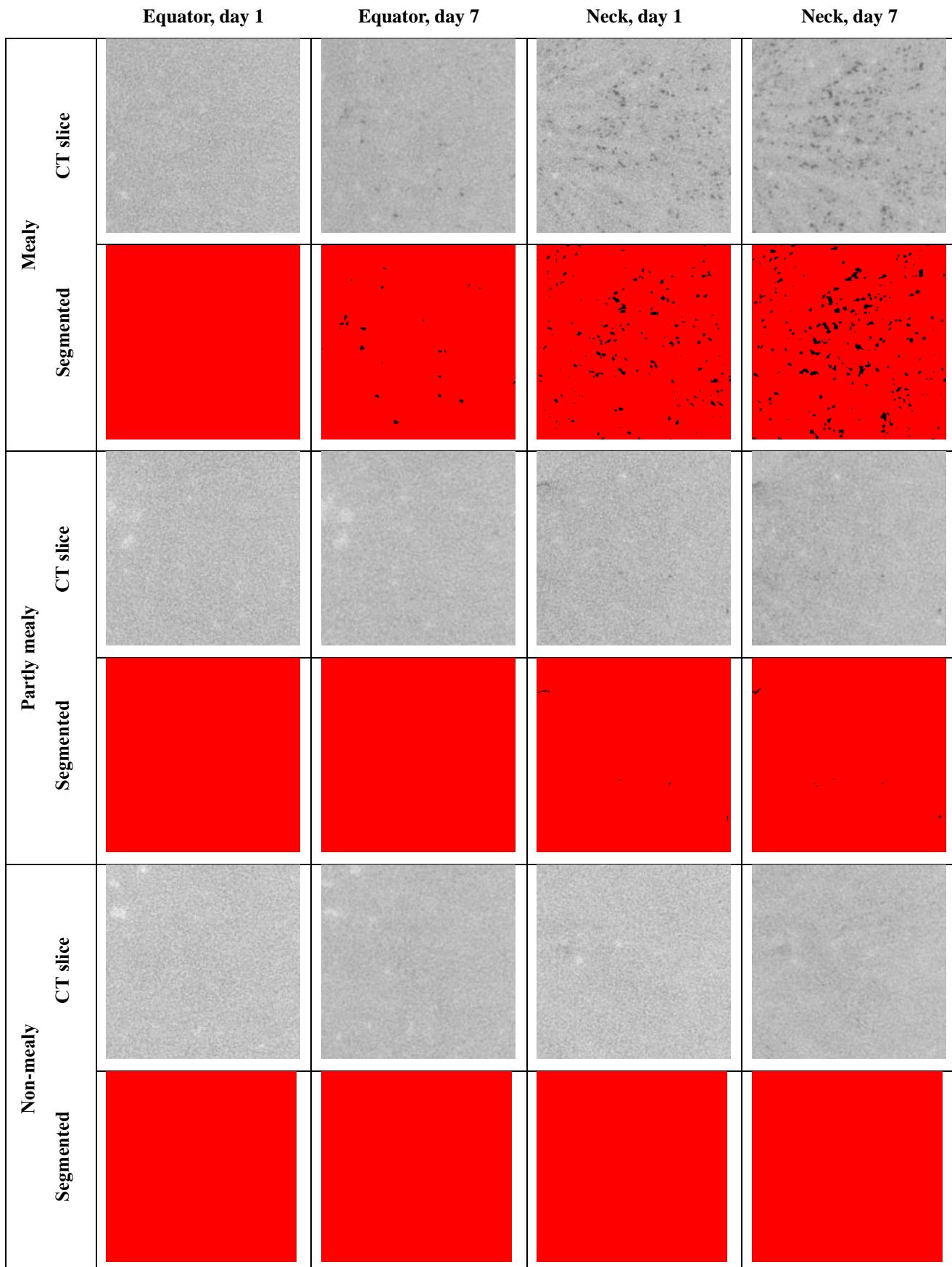
Fig. 6. Representative sections through mealy, partly mealy and non-mealy fruit taken at the end of cold storage of 8 weeks at -0.5 °C and at the end of shelf life of 7 days. Slices were

taken in the equator and neck of the fruit. Images show grey scale slices on the top row and slice after segmentation with entropy algorithm in second row (red). Black spots in the slices represent defects (darker voxels) which result from mealiness.

Mealiness class	2-D slice	Tissue (3-D)	Pores (3-D)	Porosity (%)
Mealy				12.4
Partly mealy				5.2
Non-mealy				4.4
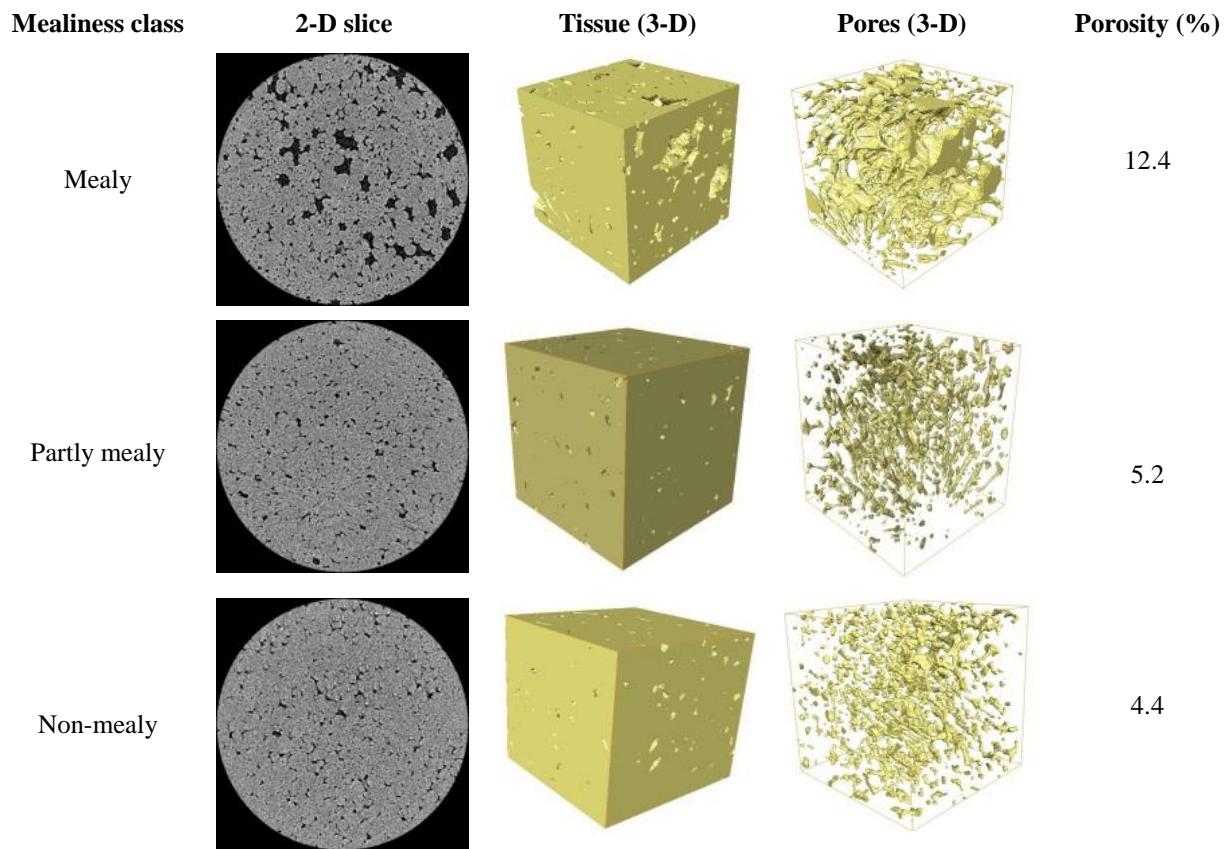
Fig. 7. Micro-CT of 'Forelle' pear samples with different mealiness manifestations (column 2). Samples were taken in the neck after 11 weeks of storage at -0.5 °C plus 7 days of shelf life. Segmentation and rendering was performed in Avizo Fire 8.2. Side length of cubes is 200 pixels or 0.97 mm.
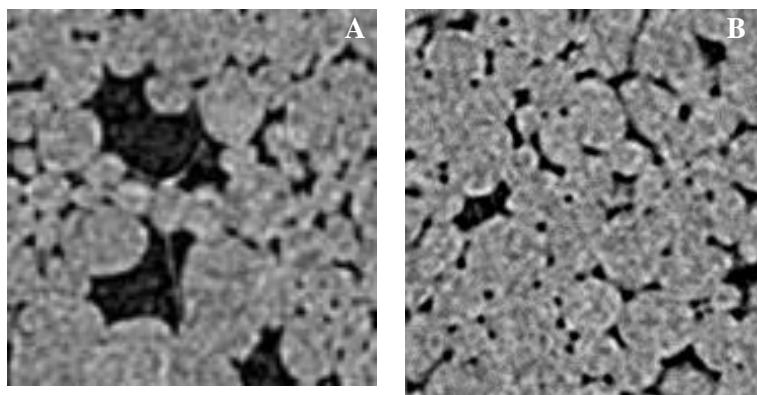
Fig. 8. Micro-CT cross section of mealy fruit tissue, showing (A) lysigenous pore formation evidenced by large pores and the remainders of fragments of cell walls and (B) schizogenous pore formation due to cell separation evidenced by the intact convex shapes of turgid cells surrounded by smaller air spaces.
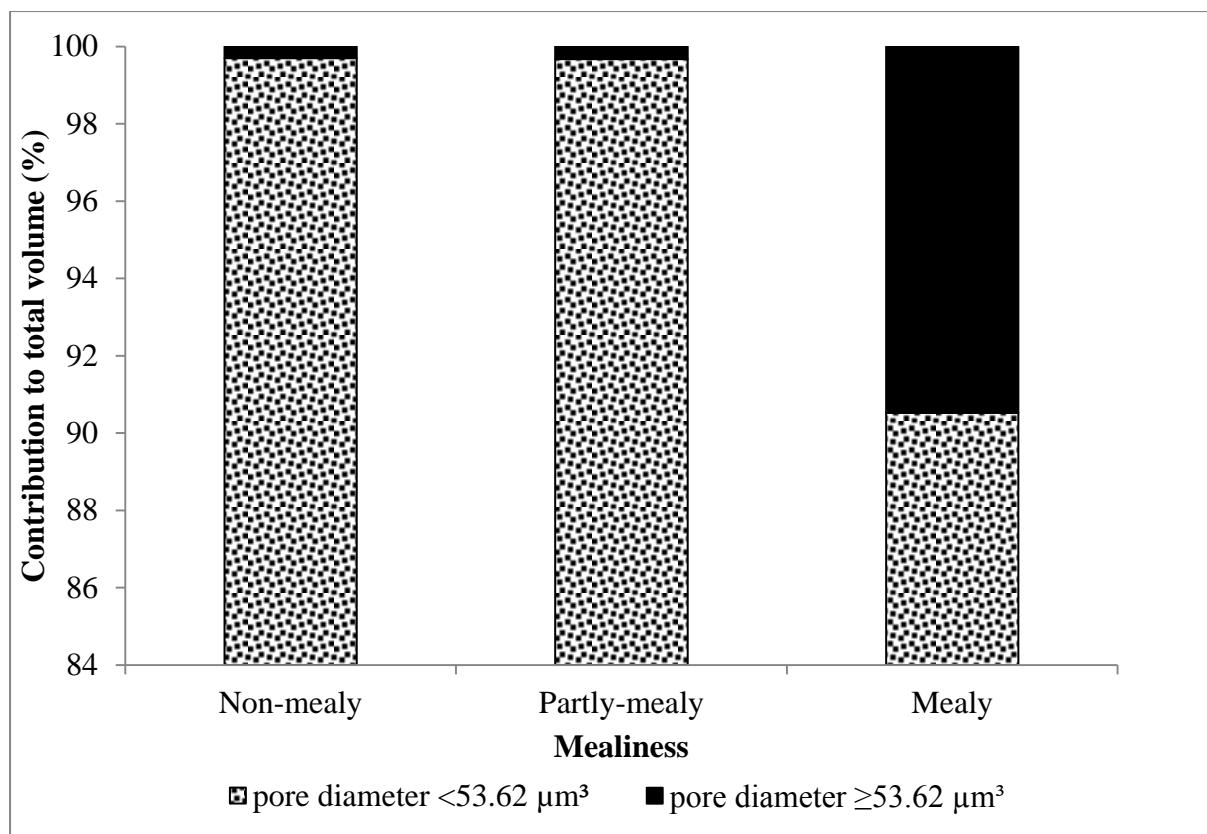
Fig. 9. Relative contribution of pores with volume larger than 53.62 μm^3 to the total pore volume for different mealiness stages of 'Forelle' pear, analysed after 11 weeks of storage and 7 days shelf life.
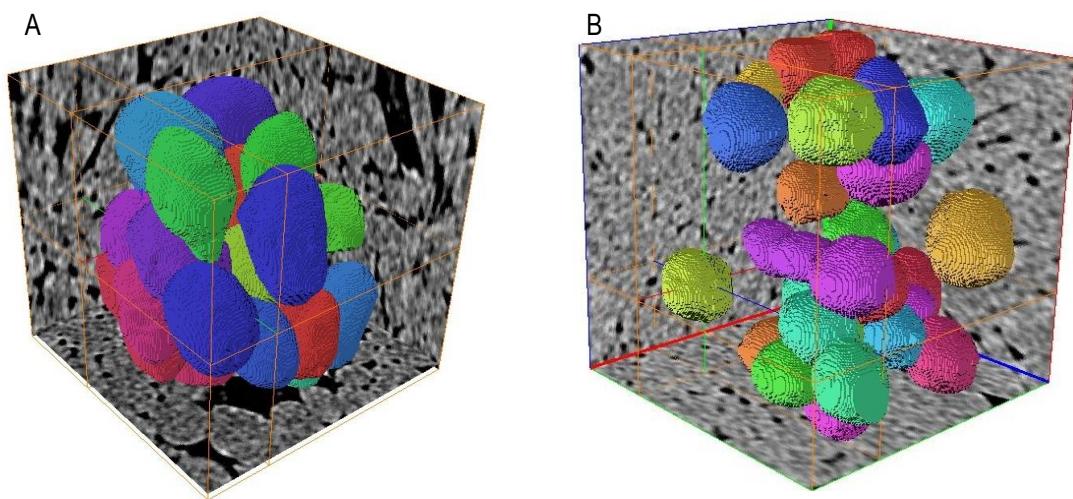
Fig. 10. Segmentation of individual cells in mealy fruit (A) and non-mealy fruit (B). Images were taken from scans in the neck of the fruit after 11 weeks of cold storage at -0.5 °C plus 7 days of shelf life. Side length of cubes is 200 pixels or 0.97 mm.
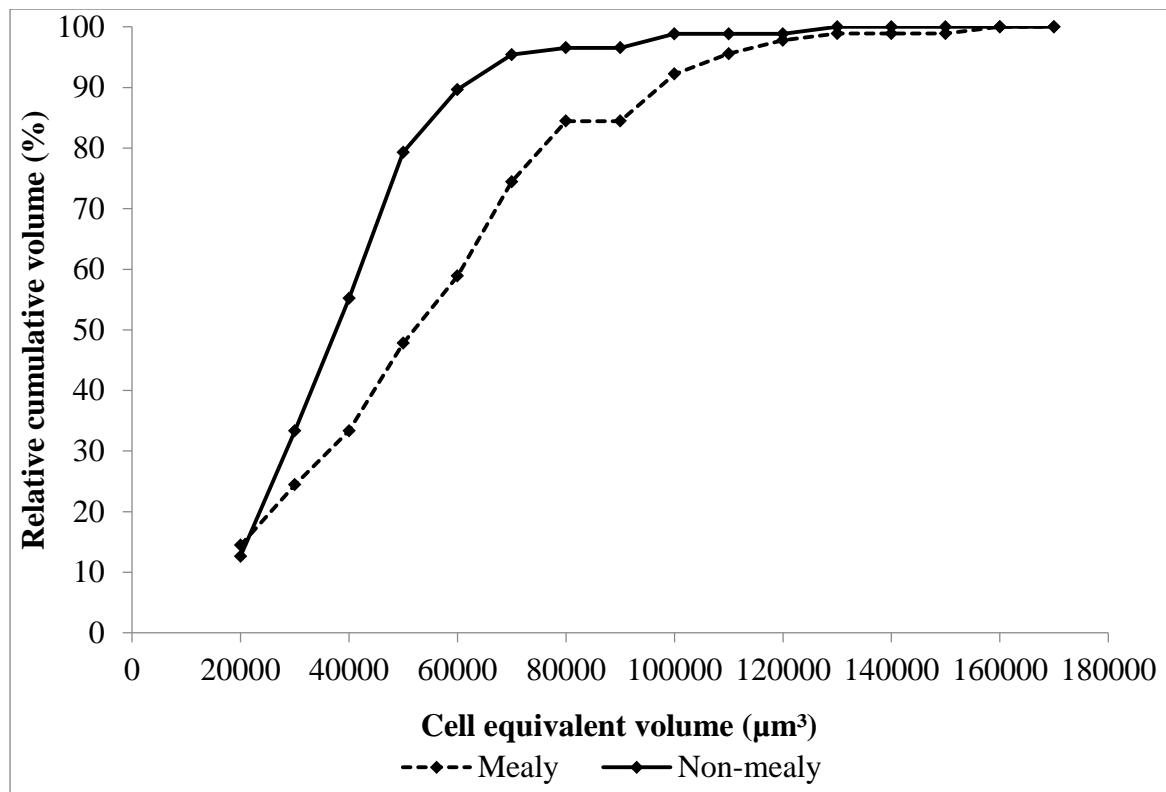
Fig. 11. Pore volume distribution expressed as cumulative volume fraction of individual cells in function of its equivalent volume (µm³).

PAPER 4

The relationship between instrumental and sensory measurements with mealiness and the detection of mealiness in 'Forelle' pears (*Pyrus communis* l.) using FT-NIR spectroscopy

Abstract

Mealiness in 'Forelle' pears is a dry textural disorder of fruit, which is characterised by loss of free juice and development of a floury texture when fruit softens. Mealiness is only detected destructively when fruit is ripe and soft. There is currently no known technique that can detect mealiness non-destructively. Fourier transform-near infrared (FT-NIR) spectroscopy was evaluated to determine whether it can detect mealiness in 'Forelle' pears. FT-NIR absorbance spectra were acquired on 400 fruit harvested from four farms over two seasons in the Western Cape Province of South Africa. Spectra were acquired at four positions on each fruit (two on the neck and two equatorially on the blushed and unblushed sides) in order to take into account the spatial variation of chemical attributes within fruit. Maturity indices were determined using standard destructive techniques. Thereafter, mealiness was determined through sensory analysis by a trained panel of three judges, as well as by measuring juice weight and juice area released after compression of a 1 cm x 1 cm cube of fruit tissue. Principal component analysis (PCA) was done on maturity indices of individual fruit to identify parameters that closely relate to mealiness. Mealy fruit closely associated with total soluble solids (TSS) and hue angle at the blushed side of the equator. Fruit were placed into three mealiness classes (non-mealy, partly mealy and mealy) based on the mealiness score and TSS. Spectra data were divided into calibration and validation data sets with proportionate numbers of mealy and non-mealy fruit. Two-class and three-class classifications were done using orthogonal partial least squares discriminant analysis (OPLS-DA). Two–class discriminant analysis produced sound models, with accuracies ranging from 51% to 95%. The best models were found at the equator-blushed side using TSS based mealiness classes. The results demonstrated the potential of NIR for detection of mealiness in 'Forelle' pears. Spectral analysis indicated that mealiness caused an increase in transmittance in the overtone and combination regions associated with water. Results from this study suggest that NIR technology together with OPLS-DA analysis has potential to detect mealiness in 'Forelle' pears.

Keywords: *Pyrus communis* L., mealiness, total soluble solids, PLS-DA.

1. Introduction

Pears are an important deciduous crop in South Africa, in terms of employment creation and foreign exchange earnings. In 2012/13 season, the pear industry contributed about 16% (R2 billion) of the total gross income of deciduous fruits (Department of Agriculture, Forestry and Fisheries, 2014). Forelle is now the second most exported pear cultivar from South Africa, accounting for 26% of the total area planted (HORTGRO, 2014). 'Forelle' has a pleasant aroma, sweet taste, crunchy texture and buttery, melting texture when ripe which is favoured by the majority of traditional pear consumers (Manning, 2009). However, unlike other pears, the cultivar develops mealiness when not properly stored after harvest (Martin et al., 2003). Mealiness in 'Forelle' pears is characterised by loss of the juicy, melting, buttery texture, which is associated with ripe pears of good eating quality (Crouch, 2011; Lammertyn et al., 2002). The disorder causes consumers to dislike fruit with mealiness (Manning, 2009; Newman, 2006).

Research over the past two decades aimed to determine factors causing mealiness (Crouch, 2011). The major challenge observed when studying mealiness is that the disorder cannot be identified prior to ripening. The condition is detected when fruit is cut, as no prior prediction or detection is possible with available instrumental methods. Sensory panels are the most commonly used for mealiness assessments (Andani et al., 2006; Barreiro et al., 2000; Crisosto and Labavitch, 2002). However, sensory panels are time consuming, destructive and not appropriate for large quantities of fruit (Barreiro et al., 2000). The most suitable method therefore would be a non-destructive method that is capable of discriminating mealy from non-mealy fruit prior to ripening or purchase. Recently, non-destructive techniques to determine produce quality were evaluated on a number of products. Reviews and studies by López et al. (2013), Louw and Theron (2010), Magwaza et al. (2012), Nicolai et al., (2007), and recently by Wang et al. (2015) identified near-infrared spectroscopy as a potential technique to use in quality grading of fruit.

Near infrared reflectance (NIR) spectroscopy is a rapid, precise and non-destructive technique based on the ability of electromagnetic radiation to interact with matter (Alander et al., 2013; Givens et al., 1997; Huang et al., 2008). The use of near infrared has grown in various fields of applied and natural sciences (Teyer et al., 2013). NIR is suitable for use in

fruit due to the fact that the NIR spectra interacts with overtones and combinations of chemical bonds such as C-H, O-H and N-H, which are associated with most foods (Alander et al., 2013, Louw and Theron, 2010). Combined with chemometrics, near infrared spectroscopy is capable of providing real time data that can be linked readily with wet chemistry data (Blanco and Villarroya, 2002; Heil, 2007). The use of near infrared spectroscopy comes with a number of advantages over previously used physical and wet chemistry based techniques. The advantages include speed of execution, absence of sample preparation and ease of use in process control and grading (Alander et al., 2013; Lammertyn et al., 2000). Further, the technique allows for repeated measurement of the same object (Magwaza et al., 2014; Teyer et al., 2013). Most NIR research in fruit has concentrated on quantitative predictions of physical and chemical compounds such as total soluble solids (TSS), titratable acidity (TA) and firmness (Li et al., 2013; Louw and Theron, 2010; Yande et al., 2010; Nicolai et al., 2007). Very little research has been done on qualitative aspects or parameters that do not have quantifiable measurements on internal disorders such as mealiness. Only two studies were available from the literature -Mehinagic et al., (2003) and Huang and Lu (2010).The former used NIR to discriminate mealiness in apple while the latter performed mealiness detection using a hyperspectral scattering technique, also in apple fruit.

Mealiness results in changes of the tissue structure due to cell-to-cell debonding plus accompanying biochemical changes in the fruit (Crouch, 2011; Harker and Hallet, 1992; McAtee et al., 2009). Tissue microstructural and macrostructural changes resulting from cell-to-cell debonding lead to light scattering, which has a bearing on NIR spectra (Rinnan et al., 2009), and also have an influence on absorption properties. However, no physical or chemical parameter closely related to mealiness has been found. This study therefore aimed to, firstly determine parameters that relate with mealiness and secondly, to determine if near infrared spectroscopy can be used as a non-destructive tool to identify mealiness in 'Forelle' pear.

2. Materials and methods

2.1 Fruit material

Fruit used in this study were harvested in 2013 from Koelfontein (lat. 33.00°S, long. 19.33°E) farm in Ceres and La Plaisante (33.41°S, long 19.19°E) farm in Wolseley. In 2014, fruit were harvested from Fairfield (lat 33.32°S, long. 19.31°E) farm in Ceres and Oak Valley (lat 34.15°S, long. 19.05°E) farm in Elgin. All farms are situated in the Western Cape Province, South Africa. Trees were randomly selected in the middle of the orchard. Twenty fruit were

harvested at shoulder height from each tree, ten on either side of the tree row. Representative samples were collected from within and outside the canopy. A total of 800 fruit were harvested from 40 trees in four blocks. A subsample of 100 fruit was taken from the harvested fruit for each farm. Harvested fruit were packed in polyethylene (37.5µm) lined pear cartons and stored for 8 weeks at -0.5 °C in order for this winter pear to sufficiently ripen during the shelf-life period (20 °C) after 7 days and assess mealiness (Crouch, 2011). Maturity indexing and other evaluations, such as mealiness were done at the end of shelf life after spectral acquisition.

2.2 Near infrared spectral acquisition

NIR spectral acquisitions of fruit were taken at the end of 8 weeks storage at -0.5 °C plus seven days shelf life at 20 °C (8w + 7d). On the evaluation day, individual fruit were numbered and placed in the laboratory for three hours to equilibrate to room temperature. FT-NIR spectra were acquired on each fruit using a multi-purpose analyser (MPA) spectrometer (Bruker Optics, Ettlingen, Germany), fitted with an internal TE-InGaAs detector, and a tungsten lamp as the NIR source. The solid probe was used to acquire absorbance spectra on four positions of each fruit. Two spectra were acquired in the neck region and two more on the equatorial region, one on the blushed and the other on green side of the fruit. NIR spectra were taken in the 800.19 – 2500.11 nm absorbance regions. For each position, 16 scans with a resolution of 4 nm were taken at a scanner velocity of 10 kHz. The resulting spectra were an average of the 16 spectra. Two background correction spectra were taken after every 2 h during acquisition and a clean blue lid was scanned periodically to correct for detector drifts.

2.3 Determination of maturity indices

Maturity indices were determined on each individual fruit using standard destructive procedures. Background colour, fruit weight, diameter, firmness, titratable acidity (TA) and TSS were determined as physicochemical measurements. Hue angle, weight and diameter were measured a day after harvest and again after 8 weeks at -0.5 °C plus 7 days of shelf life at 20 °C. TA, TSS and firmness of individual fruit were determined after shelf life. Fruit firmness was measured on opposite pared sides of the fruit using a universal fruit texture analyzer (FTA 2007, Güss Manufacturing, Strand, South Africa), fitted with a 7.9 mm diameter probe. Fruit diameter was determined using a Cranston gauge, fitted onto the fruit texture analyser. Fruit weight was determined using an electronic balance (Güss, Strand, South Africa). Hue angle was measured using a hand held digital colorimeter (Chroma Meter

CR-400, Minolta Co, Ltd, Oska 590-855, Japan). TSS was measured on a drop of expressed juice from each individual fruit using a hand held digital refractometer (PR-32, Atago, Tokyo, Japan). TA was measured by titration of individual fruit juice using an automated titrator (Metrohm AG 760. Harison, Switzerland). Ten grams of expressed juice from each individual fruit (pulped and allowed to settle) were titrated with 0.1 M NaOH to an end-point of pH 8.2. In 2013, expressible juice was measured from each individual fruit using the confined compression test (Barreiro et al., 1998). Briefly, a cube of tissue (1 cm^3) was randomly excised from the equator region of each fruit and was compressed using a Texture Analyser (model TA. xTPlus, Stable Micro Systems Inc. Surrey, UK). The instrument was set to move at 1 mm s^{-1}, and compress tissue to a distance of 2 mm from the surface, and return at 10 mm s^{-1}. The machine was calibrated using a 10 kg steel block. A Bechkote protector filter paper (Whatman No. 2300 916, GE Healthcare, Burkingharmshire, UK) was used as sample holding paper, in order to collect juice released upon compression. After reaching the maximum deformation, the probe returned to the original position. The filter paper was weighed and then air-dried for 48 h. Thereafter the filter paper was dried in an oven at 40 °C for 24 h to develop the colour on the area covered by juice. The surface area covered by the released juice was measured using the ImageJ programme (Wayne Rasband, National Institute of Health, USA), after scanning the filter paper.

2.4 Mealiness evaluation

Mealiness was evaluated on each individual fruit at the end of shelf life. A sensory panel comprising three trained evaluators scored fruit for mealiness. Three judges from the Department of Horticultural Science laboratory, who each have a minimum of 8 years' experience of mealiness determination, tested each individual fruit for mealiness. Two wedges of tissue from the same fruit were cut and tested both organoleptically and by looking for free juice after squeezing the second wedge between the thumb and index finger. Periodically during testing, the evaluators were given water biscuits and water to rinse their palate.

2.5 Physicochemical attributes and farm groups

Analysis of variance (ANOVA) was carried out on ground colour (green to yellow), fruit weight, diameter, firmness, TA, TSS and mealiness using STATISTICA Version 12 (StatSoft Inc, 1984-2012, Tulsa, OK 74104, USA). Principal component analysis (PCA) was performed on the physicochemical attributes to identify factors that relate to mealiness and to establish

110

correlation between individual factors using XLSTAT (XLSTAT.exe, 2014. Addinsoft, NY, USA). In order to increase fruit numbers of mealy versus non-mealy tissues for discriminant analyses, farms were grouped by combining orchards. PCA was done on spectra from the four different farms using SIMCA (version 13.0.3.0, Umetrics AB, Umea, Sweden) in order to identify farms with similar spectral patterns. Farms used in the 2013 season were grouped together and referred to as group 1 while farms harvested in 2014 were referred to as group 2, based on the similarity of spectra (Fig. 1).

2.6 Orthogonal Partial Least Squares Discriminant Analysis

Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA software (SIMCA version 13.0.3.0, Umetrics AB, Umea, Sweden). In brief, fruit were placed into proportional groups for classification based on farm groups for a season and fruit positions / sides. In each scheme, a proportional number of calibration samples and validation samples were selected. Outliers were detected by examining the distance to the model in X- space (DModX) plots and Hottelings T^2 (Fig. 2). The DModX shows the variation of individuals from the established model. Based on the residual variance, observations with DmodX more than twice the maximum tolerable deviation distance (2×Dcrit) were considered outliers with structure that cannot be captured in the model, and therefore were discarded (Trygg et al., 2006). Spectra from 2014 readings had noise in the 2300-2500 nm spectral regions, attributable to the detector, hence all classifications were done using the 800-2300 nm spectral range. A calibration sample with proportional mealy and non-mealy samples was loaded into SIMCA and an OPLS-DA model created and fitted. Afterwards, the validation data set was added and the model fitted. Thereafter, the calibration model was used to predict the validation model. Miscalculation results were recorded for each validated classification.

OPLS-DA, like Partial Least Squares Discriminant Analysis (PLS-DA) is a supervised classification method based on partial least squares (PLS) regression. It explains data structure by separating it into predictive and orthogonal (unrelated) components (Bylesjö et al., 2006; Trygg et al., 2006,). In a previous study where mealiness in apples was classified using PLS-DA, hardness and juiciness were used to place fruit in mealiness groups (Huang and Lu, 2010). Currently, there are no physicochemical parameters that define mealiness in 'Forelle' pears. We first had to come up with factors that relate to mealiness for use in grouping fruit into mealiness classes. In addition to the mealiness classes, an analysis of

variance of physicochemical attributes and principal component analysis (PCA) was used to determine the second class categories of mealiness (refer to 2.5). Fruit were grouped according to farm groups (group 1 and group 2) and side of spectral acquisition for classification. Two classification schemes were used. The first only included mealy and non-mealy fruit. In the second scheme, partly mealy fruit were pooled together with mealy fruit in one group and compared to non-mealy fruit.

3. Results

3.1   Relationship between destructive instrumental measurements and mealiness

The heaviest fruit were partly mealy fruit from La Plaisante (178.8 g) which were comparable to mealy fruit from the same orchard (161.2 g), mealy and partly mealy fruit from Koelfontein (175.3 g and 174.0 g, respectively), partly mealy fruit from Oak Valley (163.6 g) and mealy fruit from Fairfield (163.6 g) (Table 1). The lightest fruit were non-mealy fruit at Fairfield (120.9 g). There was a significant farm x mealiness interaction on hue angle on the blushed side of the fruit (Table 1). Fruit from Koelfontein had significantly higher hue angles compared to other farms. Hue angle on the blushed side of mealy and non-mealy fruit did not differ significantly from each other at the majority of locations except for Oak Valley where non-mealy fruit had a significantly higher hue angle. However, partly mealy fruit and mealy fruit hue angle did not differ at Oak Valley. TSS: TA ratio was significantly affected by the interaction of farm and mealiness (Table 1). TSS: TA ratio was the highest in mealy fruit at Oak Valley (149.1). At Koelfontein and La Plaisante, mealy fruit had a higher TSS:TA ratio compared to partly mealy and non-mealy fruit. At Fairfield, mealy fruit also had a higher TSS:TA ratio and differed significantly from non-mealy fruit, but not partly mealy fruit. Koelfontein had the highest mealiness percentage of 61% (Table 2), while La Plaisante, Oak Valley and Fairfield had 29%, 29% and 11% mealy fruit, respectively (Table 2).

 No interactions between orchard and mealiness level were found for firmness, TSS, TA and hue angle on the equator green side (Table 2). The softest fruit with highest TSS, TA hue angle on the green side and highest mealiness were harvested from Koelfontein. Hue angle on the green side was lowest at Oak Valley and Fairfield, suggesting that fruit at these locations were yellower compared to fruit harvested at Koelfontein. Fruit firmness was the highest at Oak Valley with the lowest TSS and TA and a moderate mealiness incidence (29%), comparable with mealiness observed at La Plaisante (Table 2). Fairfield had the second highest firmness, TSS and TA and the lowest hue angle (but was comparable to Oak

112

Valley) and the lowest mealiness incidence (11%). The softest fruit were found in the mealy category while non-mealy fruit were the firmest (Table 2). Mealy fruit had the highest TSS followed by partly mealy fruit and the lowest TSS was recorded for non-mealy fruit (Table 2). Partly mealy fruit had slightly higher TA levels compared to mealy and non-mealy fruit. Mealy fruit had the lowest hue angle on the green side (P<0.0001) and were therefore yellower compared to non-mealy fruit. There were significant farm x mealiness interactions for area and weight of juice released by confined compression at Koelfontein and La Plaisante (Table 3). Non-mealy fruit had the highest juice weight and juice area. However, partly mealy fruit at Koelfontein had comparable juice weight with mealy fruit at La Plaisante. Results showed although orchard had an influence on juiciness, mealy classes determined by sensory tests were significantly different in terms of juice released. Mealy fruit from Koelfontein had the overall lowest juice weight and area.

3.2 PCA of maturity indices

A PCA was done to establish relationships between mealiness and physiochemical characters. The first (F1) and second (F2) principal components of farm group 1 dataset explained 74.1% and 25.9% of the variation in the data, respectively (Fig. 3). Mealy and non-mealy fruit segregated on the first principal component (F1). TSS and TSS:TA ratio associated with mealy fruit whereas partly-mealy fruit associated with fruit mass and TSS. Firmness, hue angle on the equator green side (G), juice weight, and juice area were closely associated with non-mealy fruit, both at the end of cold storage (8w+0d) and after shelf life (8w+7d). Hue angle on the blushed side (B) after storage (8w+0d) and after ripening (8w+7d), fruit mass and TA caused variation on the second (F2) principal component.

In 2014 (farm group 2), juice area and juice weight were not measured on fruit that was assessed and therefore PCA was done without juice area and juice weight. Results from the PCA showed that the first (F1) and second (F2) principal components of the farm group 2 explained 92.6% and 7.4% of the variation in the data (Fig. 4). Non-mealy pears grouped with hue angle on the equator green side (G) (8w+7d), firmness and TA on the first principal component (F1). Mealy fruit grouped with TSS:TA ratio on F1.

To establish correlations of mealiness with physico-chemical attributes, PCA was done using means of ten replicates of 10 fruit each (Fig. 5) for fruit from Koelfontein and La Plaisante. The first two principal components (F1 and F2) contributed 64.51% of the variation in the data. TSS, hue angle on the blushed side (B) (8w+7d), firmness, juice weight, juice area and

113

hue angle on the equator blushed side (B) (8w + 0d) caused the variation on the first principal component (F1) (Fig. 6). Mealy fruit clustered with TSS and hue angle on the equator blush side (B) on F1. There were positive correlations between mealy fruit and hue angle on the blushed side (B) (8w+0d and 8w+7d r = 0.66 and r = 0.67, respectively) and TSS (r = 0.70) (Table 4). Non-mealy fruit on the other hand correlated positively with firmness (r = 0.55), juice weight (r = 0.78) and juice area (r = 0.76) (Table 4). At Fairfield, mealy fruit positively correlated with fruit size (r = 0.72) and negatively with firmness (r = -0.72) (Table 5). At Oak Valley, mealy fruit correlated positively with TSS (r = 0.72) and negatively with hue angle on the blushed side (B) (8w+7d) (r = -0.66) (Table 6).

3.3 'Forelle' pear near infrared spectra

'Forelle' pear spectra from this study depicted a pattern typical of fruit and other vegetable NIR spectra, with peaks in the regions where the overtone and combination regions of water occur (arrows in Fig. 6). The mealy fruit spectra showed an increase in transmittance with only a few small regions where transmittance did not increase (approximately at 1150 nm, 1350 nm-1400 nm, 1850 nm-1880 nm, 1920 nm- 2010 nm) (Fig. 8).

3.4 Mealiness classes used in OPLS-DA

ANOVA results showed that TSS and TSS:TA were significantly higher in mealy fruit compared to non-mealy fruit (Tables 1 and 2). In addition, PCA showed that TSS and TSS:TA was related to mealy fruit at the studied locations. In addition, previous NIR studies have shown TSS as the single most used physicochemical attribute that readily relates to NIR spectra (Bobelyn et al., 2010; Liu et al., 2008; Liu et al., 2007; Nicolai et al., 2007). For the current study, TSS was considered as the parameter for further OPLS-DA. Based on analysis of variance for all fruit used in the study and the three classes of mealiness, TSS ranges were determined per mealiness class. OPLS-DA was therefore carried out based on two mealiness groupings, one based on the sensory scores and the second based on TSS ranges (Table 7).

3.4 Discriminant models for the classification of sensory based mealiness

Firstly, OPLS-discriminant analysis was done using farm group as a grouping variable. Farm group 2 produced the highest classification accuracy, with 80.6% of the samples correctly classified while 19.4% were misclassified (Table 8). Mealy fruit in farm group 1 and 2 were correctly classified with high accuracy (75.6% and 78.1%, respectively). The overall accuracy of classifying mealy fruit for the combined farm groups was 80%. The model

proved to be more sensitive to mealy fruit in farm group 1 (75.6%) than for non-mealy fruit (66.3%). Conversely, with farm group 2 data, the model had better classification accuracy for non-mealy (83.1%) than for mealy fruit (78.1%). The difference in model performance was, however, very small. OPLS-DA with a pooled data set for mealiness, containing proportional mealy and partly mealy fruit, produced good classification results (Table 9). The classification results were slightly lower when compared to the classification model where only mealy and non-mealy fruit were used. Farm group 1 had an overall classification accuracy of 66.7% while farm group 2 was 79.1% correctly classified when the pooled mealy dataset was used. The overall classification accuracy for the combined dataset was 70.6%. The model was more sensitive to mealy fruit in farm group 1. In farm group 2, the model had comparable classification accuracies for the non-mealy and "pooled" mealy fruit. Three-class classification of mealy, partly mealy and non-mealy fruit yielded poor classification results for both farm groups (Table 10). The overall classification accuracy for all the farm groups was below 60%.

In the second scheme, fruit side was used as a grouping variable (Table 11). The best classification was on the equator green side followed by the neck and finally the equator blush side. The average classification accuracy for the equator blush side was 51.3%. The equator green sides had an average classification accuracy of 80.6% whilst the neck had 72.1% accuracy. Non-mealy fruit were better classified with the models for the equator green side while the neck had comparable classification of the mealy and non-mealy fruit. The equator blush side had a higher classification accuracy (67.4%) for mealy fruit compared to non-mealy fruit (37.9%). However, mealy fruit were still better classified by the equator green side (78.1%). Non-mealy and mealy spectra from the equator green side showed a good separation in the scores plots (Fig. 8).

3.5 Discriminant models for the classification of TSS based mealiness

Classification using TSS mealiness categories produced high classification accuracies for both mealy and non-mealy fruit. The overall classification for farm group 1, farm group 2 and the combined dataset were 88%, 80% and 81%, respectively (Table 12). The models with TSS based classes proved most sensitive to the mealy fruit. In farm group 1, 93% of the mealy fruit were correctly classified whilst in farm group 2, 88% mealy fruit were classified correctly. When the two farm groups were combined, 89% mealy fruit were correctly classified. When non-mealy and "pooled" mealy fruit were used for discriminant analysis,

high classification accuracies were recorded (Table 13). The average classification accuracy of farm group 1 dataset was 80.1% with 83.3% mealy fruit correctly classified. In farm group 2, the percentage of mealy fruit correctly classified was 73.7%. When the all farms data set was used, 84% of the mealy fruit were correctly classified with an overall classification accuracy of 80%. Farm group 1 and the combined dataset models were not affected by pooling of partly mealy fruit with mealy fruit. Three-class classification of the non-mealy, partly mealy and mealy classes produced poor discrimination results (Table 14). The highest accuracy was observed using farm group 1 (56.0%).

OPLS-DA was also done using fruit position on which spectra were acquired as the grouping variable (Table 15). The classification accuracies for mealy fruit were $\geq$ 89.1% for each of the sides. Mealy fruit were discriminated with 98% accuracy while > 73.3% non-mealy fruit were correctly classified for all the sides. The equator blush side produced the best separation (Fig. 9).

4. Discussion

4.1 Effect of mealiness on near infrared spectra of 'Forelle'

Spectra from other fruits with high water content such as plums (Louw and Theron, 2010), and apples (Bobelyn et al., 2010), have peaks around 1450 nm, 1790 nm, 1930 nm and 2380 nm regions which are absorption bands for water (Workman, 2014). Peaks around 976 nm and 1180 nm are associated with C-H overtone regions associated with sugar solutions (Heil, 2007). Ripe pears comprise predominantly of water and sugars, including fructose, sucrose, glucose and sorbitol (Pasquariello et al., 2013). Examination of spectra showed that mealiness has an effect on 'Forelle' spectral characteristics. The mealy fruit spectra showed an increase in transmittance with only a few small regions where transmittance did not increase (approximately at 1150 nm, 1350 nm-1400 nm, 1850 nm-1880 nm, 1920 nm- 2010 nm). A hypochromic shift is evident in the region of the combination and first overtone for water, but also in regions associated with sugar solutions (976 nm – 1180 nm) (Sandorf et al., 2007, Kradjel et al., 2008). Shifts in spectra are usually caused by changes in the internal fruit tissue absorption and scattering that are normally associated with changes in physicochemical attributes (Peirs et al., 2000). Nicolai et al. (2007) noted that fruit NIR spectra are dominated by the water absorption spectrum. Spectral analysis and loadings showed that the most discriminating wavelengths were found in the 800-1400 nm, 1500-1800nm and 2200-2400 nm.

116

4.2 Relationship between instrumental measurements and mealiness

Physicochemical parameters that best describe mealiness in 'Forelle' have not been studied. In order to identify parameters that describe mealiness, we explored the relationship between mealiness and various physicochemical and maturity indices of 'Forelle' pear using ANOVA and PCA. TSS was positively correlated with mealy fruit, and showed a strong negative correlation with non-mealy fruit at La Plaisante and Koelfontein in 2013 and Oak Valley in 2014. TSS was significantly higher in mealy fruit when compared to non-mealy fruit. TSS mainly reflects the sugars dissolved in the fruit, mostly sucrose, glucose, sorbitol and fructose (Jackson, 2003; Pasquariello et al., 2013). These are generally products of hydrolysis of starch and other polysaccharides and tend to increase with ripening. Simple sugars become predominant as the fruit matures and ripens (Jackson, 2003). In 'Forelle', outside canopy fruit accumulate more TSS than inside canopy fruit (Cronje et al., 2015). However, in this study redder blush (lower hue angle) was only correlated to a higher TSS at Fairfield and Oak Valley in 2014, but not at Koelfontein and La Plaisante (2013). In this study, the relationship between the red blush to TSS was not that clear although the correlation of TSS to mealiness was a lot clearer.

Titratable acidity did not vary between mealy and non-mealy fruit. Our results were congruent with previous reports, which have shown that TA does not change considerably during ripening (Nicolai et al., 2007). 'Forelle' mealiness, however, was associated with loss in fruit firmness. Locations with a high mealiness incidence had softer fruit. This was not expected, as previous studies have not found differences in firmness between mealy and non-mealy fruit (Crouch, 2011; Martin, 2002). The difference observed in this study could have been caused by the slightly higher firmness observed at Oak Valley and Fairfield. Further investigations need to be done to confirm and describe this finding. The area of juice and weight of juice released by confined compression test were found to be related to non-mealy fruit. It is known that mealiness causes a dry texture and this confirms previous findings on 'Forelle' where expressible juice was measured by a Chylofel (Crouch, 2011).

Overall, mealiness seems to be associated with larger, softer fruit with higher TSS. Mealy fruit could be fruit that are riper at harvest or simply fruit that ripen faster during shelf life. Whether such fruit are of advanced ripening stage at harvest remains speculative, as there is no method that can show this in intact fruit and allow fruit to go into ripening for mealiness to occur. Cronje et al., (2015) showed that 'Forelle' fruit from the outer canopy had elevated

117

mealiness and TSS. Although they did not show the link between mealiness and TSS, our results point to the growing hypothesis that outer canopy fruit could be predisposed to conditions promoting higher TSS. Outer fruit are closer to leaves that are exposed to full sunlight, so they are photosynthesising at maximum rates in comparison to inner leaves that are limited by inadequate photo active radiation levels (Rosati et al., 1998). Translocation of photosynthates is largely confined to the sinks in the immediate vicinity of the source (Fischer et al. 2012). Outer fruit therefore tend to have greater source of photosynthate and, hence, will have higher TSS levels, which in turn could be linked to higher mealiness levels. Further studies are needed to explore the role of fruit position and TSS on mealiness development.

4.3 FT-NIR classification of mealy and non-mealy fruit based on sensory scores

Results showed good discrimination of mealy and non-mealy fruit based on the sensory scores. Farm group 1 data produced an average classification accuracy of 71%, while farm group 2 data produced 81% accuracy. When data from both seasons were combined in one model, the classification accuracy remained relatively high (78%). When partly mealy fruit were pooled together with the mealy fruit, there was a decline in model sensitivity. Farm group 1 was mostly affected, with the percentage classification falling from 71% to 67%. However, there was no effect on model sensitivity on the farm group 2 data set. Classifications done using spectra from the equator green side had the best results (81%), followed by the neck (72%). The lowest sensitivity was observed with spectra from the equator blushed side. The current results therefore show potential for successful screening of non-mealy fruit from a batch containing mealy fruit.

Three-way classification (using non-mealy, partly mealy and mealy) of the sensory based classes yielded poor classification results. The overall accuracy of classification was in the order of 56%, 38% and 41% for farm group 1, farm group 2 and the combined data for all farms, respectively. Although our sensory panel consisted of highly experienced judges, the current results showed the pitfalls of transferring sensory scores to more discrete class structures, reinforcing the need for an objective non-destructive method for mealiness determination. Mealiness is a sensory perception resulting from a complex interaction of chemical and structural / textural components (Rusik, 1996). Class overlaps are inevitable when sensory panels are used to group fruit in categories based on opinion of taste. Secondly, partly mealy fruit could be a stage in the progression of mealiness, which is difficult to

differentiate using the sensory panels. There was also no significant difference in TSS between mealy and partly mealy fruit. On a commercial scale, partly mealy fruit would therefore have to be pooled with mealy fruit, as they would eventually become mealy. Their storage regime to reduce mealiness after storage and ripening would therefore be the same. Thirdly, this study used two wedges for sensory analysis, which may not have been representative of the spatial variation in mealiness of the fruit. This should be addressed in future studies.

4.4 FT-NIR classification of 'mealy' and 'non-mealy' fruit based on TSS

In the two-class classification using mealy and non-mealy fruit based on TSS, an average classification accuracy of 81% was observed when data from all farms were used. Farm group 1 and farm group 2 data produced 88% and 80% accuracies, respectively. When the partly mealy fruit was pooled with the mealy fruit, the classification accuracies were subdued, particularly in farm group 2 (67% as opposed to 80% using only mealy fruit). The combined dataset of all farms remained with high classification accuracies (80%). When position on the fruit was used as the grouping variable, classification accuracies for the sides were exceptionally good. An average classification accuracy of 95% was recorded when spectra from the equator blushed side was used. In addition, mealy fruit were classified with a 98% accuracy when spectra from the blushed side were used. Classification models using spectra from the neck side produced an average classification accuracy of 87%. The lowest classification efficiency was recorded with spectra from the equator green side (81%). However, differences in model sensitivity across sides were not significant implying that side of the fruit had little influence on classification accuracy. This has implications for the practical on-line grading of fruit where fruit tend to sit randomly on the grading lines.

In summary, the OPLS-DA classification showed that there is potential for discriminating between mealy and non-mealy fruit using NIR. Classification accuracy was superior when fruits were categorised based on their TSS compared to when fruits were categorised using the sensory panel. The sensory panel OPLS-DA was less sensitive to mealy fruit in both seasons, although results were acceptable (> 70% accuracy in most cases). Pooling mealy and partly mealy fruit resulted in a decline in the classification accuracies in both schemes (farm group and spatial fruit position). Three-class classification produced the lowest classification accuracies indicating that the OPLS-DA technique is effective for classifying extreme mealiness conditions but not efficient in discriminating intermediate conditions. Huang and

Lu (2010) obtained a similar trend when they classified mealy, partly mealy and non-mealy apples using hyperspectral imaging spectra. The overall best classification was observed when spectra taken from the equator blush were used.

5. Conclusion

Analysis of spectra showed that mealiness caused an increase in transmittance in specific regions of spectra. Mealy fruit were characterised by low firmness, high TSS, low juiciness and high hue angle on the green side of the fruit. Orthogonal partial least squares discriminant (OPLS-DA) analysis showed the possibility of using NIR to discriminate between mealy and non-mealy 'Forelle' pears based on the sensory and TSS classes. The study showed the potential of NIR for detection of mealiness in 'Forelle' pear. Further studies are recommended using specific sides of the fruit such as the equator blushed side. Studies are also recommended to establish the ability of NIR to detect TSS in 'Forelle' pears and to establish the use of juice area, juice weight and hue angle at the blushed side in mealiness determination.

Literature cited

Alander, J.T., Bochko, V., Martinkauppi, B., Saranwong, S., Mantere, T., 2013. A review of optical nondestructive visual and near infrared methods for food quality and safety. Int. J. Spectrosc. 10, 155. Hindawi Publishing Corporation. http://dx.doi.org/10.155/2013/341402.

Andani, Z., Jaeger, S.R., Wakeling, I., Macfie, H.J.H., 2006. Mealiness in apples: Towards a multilingual consumer vocabulary. J. Food Sci. 66 (2), 872-879.

Barreiro, P., Ortiz, C., M. Ruiz-Altisent., De Smedt, V., Schotte, S., Andani, Z., Wakeling, I., Beyts, P.K., 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples. A collaborative test. J. Texture Stud. 29, 509-525.

Barreiro, P., Ortiz, C., Ruiz_Altisent M., Ruiz_Cabello, J., Fernandez_Valle, M.E., Recasens, I., Asensio, M., 2000. Mealiness assessment in apples and peaches using MRI techniques. Magn. Reson. Imaging. 18, 1175-1181.

Blanco, M., Villarroya, I., 2002. NIR spectroscopy: a rapid-response analytical tool. Trends Anal. Chem. 21, 240-250.

Bobelyn, E., Serban, A.S., Nicu, M., Lammertyn, J., Nicolai, B.N., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of

biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133-143.

Bylesjö, M., Rantalainen, M., Nicholson, J.K., Holmes, E., Trygg, J., 2006. OPLS discriminant analysis: combining the strength of PLS-DA and SIMCA classification. J. Chemom. 20, 341-351.

Crisosto, C.H., Labavitch, J.M., 2002. Developing a quantitative method to evaluate peach (*Prunus persica*) flesh mealiness. Postharvest Biol. Technol. 25 (2), 151-158.

Cronje, A., Crouch, E.M., Muller, M., Theron, K.I., van der Rijst, M., Steyn, W.J., 2015. Canopy position and cold storage duration affects mealiness incidence and consumer preference for the appearance and eating quality of 'Forelle' pears. Sci. Hortic. 194, 327-336.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L.). PhD(Agric) Dissertation. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Department of Agriculture Forestry and Fisheries. 2014. A profile of the South African Pear Market Value Chain. Directorate Marketing, Arcadia. South Africa. www.webapps.daff.gov.za/mis. (accessed 02-12-2014).

Fischer, G., Jose Almanza-Merchán, P., Ramirez, F., 2012. Source-sink relationships in fruit species. A review. Revista Colombiana de Ciencias Horticolas. Vol 6 (2), 238-253.

Givens, D.I., Boever, J.L., Deaville, E.R., 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. Nutr. Res. Rev., 10, 83-114.

Harker, F.R., Hallett I,C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortSci. 27(12), 1291-1294.

Heil, C., 2007. Rapid in process moisture determination of a fluid bed drier. Application note #51543. Thermo Fischer Scientific, Madison, WI, USA.

HORTGRO, 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

Huang, H., Yu, H., Xu, H., Ying, Y., 2008. Near infrared spectroscopy for on/ in-line monitoring of quality foods and beverages. A review. J. Food Eng. 87, 303-313.

Huang, M., Lu, R., 2010. Apple mealiness detection using hyperspectral scattering technique. Postharvest Biol. Technol. 58, 168-175.

Jackson, J.E., 2003. Biology of Horticultural Crops. The Biology of Apples and Pears. Cambridge University Press, Cambridge, UK. p 343.

Kradjel, C., Lee, K.A., 2008. NIR analysis of polymers. In Burns, D.A., Ciurczak, E.W., (eds). Handbook of near infrared analysis (3rd edn). CRS Press. London, UK. Pp 539-563.

Lammertyn, J., Peirs, A., De Baerdemaeker, J., Nicolai, B., 2000. Light penetration properties of NIRs radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121-132.

Lammertyn, J., Verlinden, B.E., Nicolai, B.M., 2002. Applying advanced instrumental methods: mealiness in fruit. In: Jogen, W. 2002. Fruit and Vegetable processing: Improving quality. Woodhead Publishing in Food Science. Cornwall, England. Pp 170-187.

Li, J., Huang, W., Zhao, C., Zhang, B., 2013. A comparative study for the quantitative determination of soluble solids content, pH and firmness by Vis/NIR spectroscopy. J. Food. Eng. 116, 324-332.

Liu, Y., Chen, X., Dong, S.X., Ying, Y., 2007. Non-destructive measurement of pear internal quality indices by visible and near infrared spectrometric techniques. N.Z. J. Agric. Res. 50, 1051-1057.

Liu, Y., Yinying, P., Rongjie, G., Xudong, S., Yong, H., 2010. Online detection of soluble solids content and size of crystal pear based on LEDs light source detector. Trans. Chinese Soc. Agric. Engin. 26 (11), 338-343.

López, A., Arazuri, S., García, I., Mangado, J., Jaŕen, C., 2013. A review of the application of near infrared spectroscopy for the analysis of potatoes. J. Agric. Food Chem. 61, 5413-5424.

Louw, D.E and Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina*), using NIR spectroscopy. Postharvest Biol. Technol. 58, 176-184.

Magwaza, L.S., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., Mouazem, A.M., Nicolai, B.M., Terry, L.A., Opara, U.L., 2014. The use of Vis/NIR and chemometric analysis to predict fruit defects and postharvest behaviour of 'Nules Clementine' mandarin fruit. Food Chem. 163, 267-274.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolai, B., 2012. NIR spectroscopy Applications for internal and external quality analysis of citrus fruit-A review. Food Bioprocess Technol. 5(2), 425-444.

Manning, N. 2009. Physical, sensory and consumer analysis of pear genotypes among South African consumers and preference of appearance among European consumers. MSc.

Food Science. Thesis, University of Stellenbosch, Department of Food Science. Stellenbosch. South Africa.

Martin, E.M., 2002. Ripening responses of 'Forelle' pears. MSc (Agric) Thesis Department of Horticultural Sciences, Stellenbosch University, South Africa.

Martin, E.M., Crouch, I.J., and Holcroft, D.M., 2003. Ripening and mealiness of 'Forelle' pears. Acta Hort. 600, 449-452.

McAtee, P.A., Hallet, I.C., Johnston, J.W., Schaffer, R.J., 2009. A rapid method of fruit cell isolation for cell size and shape measurements. Plant Methods. 5 (5), 1-7.

Mehinagic, E., Royer, G., Bertrand, D., Symoneaux, R., Laurens, F., Jourjon, F., 2003. Relationship between sensory analysis, penetrometry and visible–NIR spectroscopy of apples belonging to different cultivars. Food Qual. Pref. 14, 473-484.

Newman, S., 2006. Stonefruit mealiness- the quiet destroyer. Netpro Update. NSW Department of Primary Industries. Australia

Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99-118.

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 early ripening pear cultivars. Sci. Hortic. 162, 341-350.

Peirs, A., Lammertyn, J., Ooms, K., Nicolai, B.M., 2000. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. Postharvest. Biol. Technol. 21, 189-199.

Rinnan, A., van de Berg, F., Engelsen, S.B., 2009. Review of the most common pre-processing techniques for near-infrared spectra. Trends Anal. Chem. 28 (10), 1201-1222.

Rosati, A., Espaarza, G., DeJong, TM., Pearcy, RW., 1999. Influence of canopy light environment and nitrogen availability on leaf photosynthetic characteristics and photosynthetic nitrogen-use efficiency of field grown nectrarine trees. Tree Physiology, 19, 173-180.

Rusik, E., 1996. Understanding latent structure. In: Naes, T and Risik, E. 1996. (eds). Data Handling in Science and Technology. 16. Multivariate analysis of data in sensory science. Elsevier Science. B.V. The Netherlands. Pp 5-18.

Sandorf, C., Buchet, R., Lachenal, B., 2007. Principles of molecular vibrations for near infrared spectroscopy. In: Ozaki, Y., McClure, W.F., Christy, A.A., 2007. Near

infrared spectroscopy in food science and technology. John Wiley and Sons. New Jersey, USA. Pp 11-46.

Teyer, E., Huang, S., Afoakwa, N., 2013. Review on the potential of near infrared spectroscopy (NIRs) for the measurement of chemical residues in food. Am. J. Food Sci. Technol. 1, 1-8.

Trygg, J., Holmes, E., Lundstedt, T., 2006. Chemometrics in Metabonomics. J. Proteome Res. 2006, 469-479.

Wang, H., Peng, J., Xie, C., Bao, Y., He, Y., 2015. Fruit quality evaluation using spectroscopy technology: A review. Sensors, 15, 11889-11926, doi 10.3390/s 150511889.

Workman, J. (Jr)., 2014. An introduction to near infrared spectroscopy. John Wiley and Sons. www.spectroscopy.com [accessed on16/03/2015].

Yande, L., Yinying, P., Rongjie, G., Xudong, S., Yong, H., 2010. Online detection of soluble solids content and size of crystal pear based on LEDs light source detector. Trans. Chinese Soc. Agric. Engin. 26 (11), 338-343.

Table 1. The effect of farm and mealiness on fruit weight, hue angle on the blush side and TSS:TA ratio at the end of 8 week cold storage at -0.5 °C plus shelf life (7 days at 20 °C) of 'Forelle' pears that were harvested in the Western Cape, South Africa from Koelfontein and La Plaisante in Ceres, in March 2013 and from Fairfield (Ceres) and Oak Valley (Elgin) in 2014.

Farm	Mealiness score	Fruit weight (g)	Hue angle (°) Blush side	TSS:TA	
Koelfontein	Non-mealy	159.3 bc	80.9 a	56.2	f
	Partly mealy	174.0 ab	86.3 a	61.9	f
	Mealy	175.3 a	90.9 a	72.9	e
La Plaisante	Non-mealy	159.3 cd	60.0 cd	58.0	f
	Partly mealy	178.8 a	58.4 cd	61.0	f
	Mealy	161.2 ab	64.6 cd	66.6	e
Oak Valley	Non-mealy	155.6 d	70.0 b	109.2	c
	Partly mealy	163.6 ab	68.3 bc	126.9	b
	Mealy	154.9 d	52.5 cde	149.1	a
Fairfield	Non-mealy	120.9 e	61.9 cd	71.7	e
	Partly mealy	147.4 d	60.0 cd	87.5	d
	Mealy	163.6 abc	55.3 cd	92.8	d
Farm		*<0.0001*	*<0.0001*	*<0.0001*	
Mealiness		*<0.0001*	*0.0651*	*<0.0001*	
Farm × Mealiness		*0.0093*	*0.0044*	*0.0010*	

Means followed by the same letter are not significantly different at p<0.05.

Table 2. The effect of farm and mealiness on fruit firmness, TSS, TA and hue angle of 'Forelle' pears harvested in 2013 from Koelfontein and La Plaisante in Ceres, South Africa in March 2013 and in March 2014 from Fairfield (Ceres) and Oak Valley in Elgin, South Africa in 2014 and stored for 8 weeks at -0.5°C plus ripened for 7 days at 20 °C.

Farm	Firmness (kg)	TSS	TA	Hue angle (°) Green side	Mealiness (%)
Farm					
Koelfontein	1.30d	16.99a	0.27a	102.92a	61a
La Plaisante	1.51c	15.70c	0.26a	102.15b	29b
Oak Valley	2.22a	14.45d	0.12c	98.89c	29b
Fairfield	1.70b	16.47b	0.22b	99.47c	11c
Mealiness					
Non-mealy	1.85a	15.24c	0.22b	102.36a	
Partly mealy	1.67b	16.11b	0.23a	101.03a	
Mealy	1.55c	16.36a	0.21b	99.70b	
Farm	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	0.0072
Mealiness	*0.014*	*<0.0001*	*<0.0001*	*<0.0001*	--
Farm × Mealiness	*0.068*	*0.696*	*0.146*	*0.064*	--

Table 3. Juice area and weight from the confined compression test of fruit from Koelfontein and La Plaisante for fruit classified as mealy, partly mealy and non-mealy by a trained panel. Letters show significant differences at p≤0.05.

		Juice weight (g)	Juice area (cm²)
Koelfontein	Mealy	0.10d	5.50d
	Partly mealy	0.16c	9.54c
	Non-mealy	0.26a	15.79a
La Plaisante	Mealy	0.16c	8.78c
	Partly mealy	0.20b	11.56b
	Non-mealy	0.24a	14.16a
Farm		*0.0052*	*0.0288*
Mealiness		*<0.0001*	*<0.0001*
Farm x mealiness		*0.0097*	*0.0030*

Means with different letters in the same column differ at p<0.05

Table 4. Correlation matrix for principal component analysis of physicochemical attributes of 'Forelle' pears from two farms (Koelfontein and La Plaisante) harvested in 2013.

Variables	Mass (g)	Firmness (kg)	Hue angle (°) (G)(8w+0d)	Hue angle (°) (G) (8w+7d)	Hue angle (°) (B) (8w+0d)	Hue angle (°) (B) (8w+7d)	Juice weight (g)	Juice area (cm²)	TSS (° Brix)	TA (%)	TSS:TA	Mealy	Partly mealy	Non-mealy
Mass (g)	1	-0.418	0.369	0.161	**0.498**	**0.469**	**-0.486**	-0.265	0.197	0.079	0.098	0.254	-0.069	-0.232
Firmness (kg)	-0.418	1	**-0.456**	0.231	**-0.711**	**-0.736**	**0.703**	**0.706**	**-0.684**	-0.101	-0.340	**-0.666**	0.298	**0.545**
Hue angle (°) (G)(8w+0d)	0.369	**-0.456**	1	0.148	**0.558**	**0.598**	**-0.562**	**-0.590**	**0.673**	0.328	0.121	0.424	-0.116	-0.389
Hue angle (°) (G)(8w+7d)	0.161	0.231	0.148	1	-0.080	-0.132	0.207	0.199	-0.250	0.063	-0.281	-0.282	-0.091	0.354
Hue angle (°) (B)(8w+0d)	**0.498**	**-0.711**	**0.558**	-0.080	1	**0.992**	**-0.617**	**-0.555**	**0.671**	0.130	0.312	**0.656**	-0.238	**-0.568**
Hue angle (°) (B)(8w+7d)	**0.469**	**-0.736**	**0.598**	-0.132	**0.992**	1	**-0.634**	**-0.576**	**0.719**	0.173	0.308	**0.666**	-0.207	**-0.597**
Juice weight (g)	**-0.486**	**0.703**	**-0.562**	0.207	**-0.617**	**-0.634**	1	**0.923**	**-0.743**	0.167	**-0.604**	**-0.788**	0.123	**0.775**
Juice area (cm²)	-0.265	**0.706**	**-0.590**	0.199	**-0.555**	**-0.576**	**0.923**	1	**-0.751**	0.028	**-0.492**	**-0.811**	0.191	**0.761**
TSS (° Brix)	0.197	**-0.684**	**0.673**	-0.250	**0.671**	**0.719**	**-0.743**	**-0.751**	1	-0.041	**0.608**	**0.697**	-0.024	**-0.734**
TA (%)	0.079	-0.101	0.328	0.063	0.130	0.173	0.167	0.028	-0.041	1	**-0.794**	-0.057	0.079	0.016
TSS:TA	0.098	-0.340	0.121	-0.281	0.312	0.308	**-0.604**	**-0.492**	**0.608**	**-0.794**	1	**0.460**	-0.049	**-0.465**
Mealy	0.254	**-0.666**	0.424	-0.282	**0.656**	**0.666**	**-0.788**	**-0.811**	**0.697**	-0.057	**0.460**	1	-0.388	**-0.852**
Partly mealy	-0.069	0.298	-0.116	-0.091	-0.238	-0.207	0.123	0.191	-0.024	0.079	-0.049	-0.388	1	-0.151
Non-mealy	-0.232	**0.545**	-0.389	0.354	**-0.568**	**-0.597**	**0.775**	**0.761**	**-0.734**	0.016	**-0.465**	**-0.852**	-0.151	1

Values in bold are different from 0 with a significance level alpha=0.05

 G= green side, B= blush side, 8w+7d =storage at -0.5°C for 8 weeks; 8w+7d =storage at -0.5°C for 8 weeks and shelf life at 20 °C for 7 days.

Table 5. Correlation matrix for principal component analysis of physicochemical attributes of 'Forelle' pears from Fairfield harvested in 2014.

Variables	Mass (g)	Firmness (kg)	Hue angle (°) (G) (8w+0d)	Hue angle (°) (B) (8w+0d)	Hue angle (°) (G) (8w+7d)	Hue angle (°) (B) (8w+7d)	TSS (° Brix)	TA (%)	TSS:TA	Mealy	Partly mealy	Non-mealy
Mass (g)	1	**-0.750**	-0.460	-0.167	-0.417	-0.274	0.373	0.220	-0.039	**0.723**	0.104	-0.351
Firmness (kg)	**-0.750**	1	0.285	0.438	0.340	0.406	-0.598	-0.005	-0.291	**-0.724**	0.052	0.256
Hue angle (°) (G) (8w+0d)	-0.460	0.285	1	-0.517	**0.835**	-0.279	-0.049	-0.073	0.112	-0.481	0.068	0.163
Hue angle (°) (B) (8w+0d)	-0.167	0.438	-0.517	1	-0.298	**0.801**	**-0.665**	-0.184	-0.055	-0.209	-0.202	0.253
Hue angle (°) (G) (8w+7d)	-0.417	0.340	**0.835**	-0.298	1	-0.135	-0.195	0.025	0.074	-0.389	-0.311	0.560
Hue angle (°) (B) (8w+7d)	-0.274	0.406	-0.279	**0.801**	-0.135	1	-0.541	-0.117	0.106	-0.392	0.071	0.062
TSS (° Brix)	0.373	-0.598	-0.049	**-0.665**	-0.195	-0.541	1	**0.634**	-0.397	0.262	0.276	-0.360
TA (%)	0.220	-0.005	-0.073	-0.184	0.025	-0.117	**0.634**	1	**-0.844**	-0.114	0.252	-0.079
TSS:TA	-0.039	-0.291	0.112	-0.055	0.074	0.106	-0.397	**-0.844**	1	0.272	-0.267	0.094
Mealy	**0.723**	**-0.724**	-0.481	-0.209	-0.389	-0.392	0.262	-0.114	0.272	1	-0.376	0.000
Partly mealy	0.104	0.052	0.068	-0.202	-0.311	0.071	0.276	0.252	-0.267	-0.376	1	**-0.905**
Non-mealy	-0.351	0.256	0.163	0.253	0.560	0.062	-0.360	-0.079	0.094	0.000	**-0.905**	1

Values in bold are different from 0 with a significance level alpha=0.05

G= green side, B= blush side, 8w+7d =storage at -0.5°C for 8 weeks; 8w+7d =storage at -0.5°C for 8 weeks and shelf life at 20 °C for 7 days.

Table 6. Correlation matrix for principal component analysis of physicochemical attributes of 'Forelle' pears from Oak Valley harvested in 2014.

Variables	Mass (g)	Firmness (kg)	Hue angle (°) (G) (8w+0d)	Hue angle (°) (B) (8w+0d)	Hue angle (°) (G) (8w+7d)	Hue angle (°) (B) (8w+7d)	TSS (° Brix)	TA (%)	TSS:TA	Mealy	Partly mealy	Non-mealy
Mass (g)	1	0.412	-0.546	0.249	0.117	0.222	0.270	0.447	-0.294	0.082	0.116	-0.173
Firmness (kg)	0.412	1	0.056	0.163	0.476	0.098	0.021	0.401	-0.291	-0.229	0.128	0.291
Hue angle (°) (G) (8w+0d)	-0.546	0.056	1	-0.517	0.304	-0.144	0.311	-0.553	0.605	0.203	-0.005	-0.176
Hue angle (°) (B) (8w+0d)	0.249	0.163	-0.517	1	-0.434	-0.047	**-0.732**	0.040	-0.206	**-0.659**	0.262	**0.667**
Hue angle (°) (G) (8w+7d)	0.117	0.476	0.304	-0.434	1	0.073	0.292	0.497	-0.415	-0.111	0.249	-0.093
Hue angle (°) (B) (8w+7d)	0.222	0.098	-0.144	-0.047	0.073	1	0.125	0.427	-0.379	-0.106	-0.109	0.174
TSS (° Brix)	0.270	0.021	0.311	**-0.732**	0.292	0.125	1	-0.007	0.283	**0.718**	-0.128	**-0.806**
TA (%)	0.447	0.401	-0.553	0.040	0.497	0.427	-0.007	1	**-0.951**	-0.154	-0.096	0.197
TSS:TA	-0.294	-0.291	0.605	-0.206	-0.415	-0.379	0.283	**-0.951**	1	0.371	0.034	-0.411
Mealy	0.082	-0.229	0.203	**-0.659**	-0.111	-0.106	**0.718**	-0.154	0.371	1	**-0.656**	**-0.698**
Partly mealy	0.116	0.128	-0.005	0.262	0.249	-0.109	-0.128	-0.096	0.034	**-0.656**	1	-0.060
Non-mealy	-0.173	0.291	-0.176	**0.667**	-0.093	0.174	**-0.806**	0.197	-0.411	**-0.698**	-0.060	1

Values in bold are different from 0 with a significance level alpha=0.05

G= green side, B= blush side, 8w+7d =storage at -0.5°C for 8 weeks; 8w+7d =storage at -0.5°C for 8 weeks and shelf life at 20 °C for 7 days.

Table 7. Classification of 'Forelle' into mealiness classes based on soluble solids content (TSS). Fruit were harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014.

	Three classes		
	Non-mealy	Partly mealy	Mealy
TSS range (%)	<15.24	15.24-16.36	>16.36

*The TSS ranges were based on analysis of variance of all data from the two seasons and four locations (**Table 2**).*

Table 8. Two-class classification for the validation/calibration sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the sensory score by a laboratory trained panel with 8 years of experience tasting for mealiness. Only fruit categorised as mealy and non-mealy were used in this classification.

Sample origin	Sensory panel classification		Model classification		Classification accuracy (%)
		Members	Mealy	Non-mealy	OPLS-DA
Farm group 1	Mealy	205	155	50	75.6
	Non-mealy	196	66	130	66.3
	Overall	401	221	180	71.1
Farm group 2	Mealy	160	125	35	78.1
	Non-mealy	160	27	133	83.1
	Overall	320	152	168	80.6
All farms	Mealy	365	292	73	80.0
	Non-mealy	356	85	271	76.1
	Overall	721	377	344	78.1

Rows: classified by Sensory panel
Columns: predicted by near infrared spectroscopy (NIR)

Table 9. Two-class classification for the validation/calibration sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Partly mealy fruit were pooled with mealy fruit in one group as mealy. Fruits were put into mealiness classes based on the sensory score by a laboratory trained panel with 8 years of experience tasting for mealiness.

Sample origin	Sensory panel classification		Model classification		Classification accuracy (%)
		Members	Mealy (pooled)	Non-mealy	OPLS-DA
Farm group 1	Mealy (pooled)	203	152	51	74.9
	Non-mealy	196	82	114	58.2
	Overall	399	234	165	**66.7**
Farm group 2	Mealy (pooled)	160	126	34	78.8
	Non-mealy	160	33	127	79.4
	Overall	320	159	161	**79.1**
All farms	Mealy (pooled)	363	266	97	73.3
	Non-mealy	356	114	242	68.0
	Overall	719	380	339	**70.6**

Rows: classified by Sensory panel
Columns: predicted by near infrared spectroscopy (NIR)

Table 10. Three class classification for the validation sets of 'Forelle' pears from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the sensory score by a laboratory trained panel with 8 years of experience tasting for mealiness.

Sample origin	Panel classification		Model classification			Classification accuracy (%)
		Members	Mealy	Partly mealy	Non-mealy	OPLS-DA
Farm group 1	Mealy	205	117	27	61	57.1
	Partly mealy	204	89	36	79	17.7
	Non-mealy	196	74	35	87	44.4
	Overall	605	280	98	227	**39.7**
Farm group 2	Mealy	160	98	31	31	61.3
	Partly mealy	168	72	39	57	23.2
	Non-mealy	160	62	38	60	37.5
	Overall	488	232	148	108	**40.4**
All farms	Mealy	402	295	41	66	73.4
	Partly mealy	402	188	71	143	17.7
	Non-mealy	402	52	19	331	82.3
	Overall	1206	535	131	540	**57.8**

Rows: classified by Sensory panel
Columns: predicted by near infrared spectroscopy (NIR)

Table 11. Two-class classification for the validation/calibration sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the sensory score by a laboratory trained panel with 8 years of experience tasting for mealiness. Only fruit categorised as true mealy and true non-mealy were used in this classification.

Sample origin	Panel classification		Model classification		Classification accuracy (%)
		Members	Mealy	Non-mealy	OPLS-DA
Equator blush	Mealy	84	58	28	67.4
	Non-mealy	103	64	39	37.9
	Overall	189	122	67	**51.3**
Equator green	Mealy	160	125	35	78.1
	Non-mealy	160	27	133	83.1
	Overall	320	152	168	**80.6**
All neck	Mealy	182	132	50	72.5
	Non-mealy	176	50	126	71.6
	Overall	358	182	176	**72.1**

Rows: classified by Sensory panel
Columns: predicted by near infrared spectroscopy (NIR)

Table 12. Two-class classification for the validation/calibration sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the average total soluble solids (TSS). Only fruit categorised as mealy and non-mealy were used in this classification.

Sample origin	TSS classification		Model classification		Classification accuracy (%)
		Members	Mealy	Non-mealy	OPLS-DA
Farm group 1	Mealy	136	127	9	93.38
	Non-mealy	105	20	85	80.95
	Overall	241	147	94	**87.97**
Farm group 2	Mealy	76	67	9	88.2
	Non-mealy	76	22	54	71.1
	Overall	152	89	63	**79.6**
All farms	Mealy	211	187	24	88.6
	Non-mealy	187	53	134	71.7
	Overall	398	240	158	**80.7**

Rows: classified by TSS.
Columns: predicted by near infrared spectroscopy (NIR).

Table 13. Two class classification for the validation sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the total soluble solids (TSS) (Table 7). The partly mealy fruit were pooled with mealy fruit and termed 'pooled' mealy.

Sample origin	TSS classification	Model classification		Classification accuracy %
		Mealy (pooled)	Non-mealy	OPLS-DA
Farm group 1	Mealy (pooled)	90	18	83.3
	Non-mealy	25	83	76.9
	Overall	115	101	**80.1**
Farm group 2	Mealy (pooled)	56	20	73.7
	Non-mealy	27	49	64.5
	Overall	84	69	**69.1**
All farms	Mealy (pooled)	155	29	84.2
	Non-mealy	43	141	76.6
	Overall	198	170	**80.4**

Rows: classified by TSS.
Columns: predicted by near infrared spectroscopy (NIR).

Table 14. Three class classification for the validation sets of 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Fruits were put into mealiness classes based on the total soluble solids (TSS) (Table 7).

Sample origin	TSS classification	Model classification			Classification accuracy (%)
		Mealy	Partly mealy	Non-mealy	OPLS-DA
Farm group 1	Mealy	68	58	8	50.8
	Partly mealy	10	34	63	58.9
	Non-mealy	44	99	27	58.2
	Overall	122	191	98	**56**
Farm group 2	Mealy	40	36	0	52.6
	Partly mealy	29	47	0	61.8
	Non-mealy	33	43	0	0
	Overall	102	126	0	**38.2**
All farms	Mealy	79	22	109	37.6
	Partly mealy	29	63	91	34.4
	Non-mealy	67	49	130	52.9
	Overall	175	134	330	**42.6**

Rows: classified by TSS.

Columns: predicted by near infrared spectroscopy (NIR).

138

Table 15. Two-class classification for 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Position on the fruit as grouping variable for all the test dates. Fruits were put into mealiness classes based on the total soluble solids (TSS) (Table 2 and 7). Only fruit categorised as mealy and non-mealy were used in this classification.

Sample origin	TSS classification	Model classification		Classification accuracy (%)
		Mealy	Non-mealy	OPLS-DA
Equator blush side	Mealy	45	1	97.8
	Non-mealy	4	42	91.3
	Overall	49	43	**94.6**
Equator green side	Mealy	41	5	89.1
	Non-mealy	12	33	73.3
	Overall	53	38	**81.3**
Neck (both sides)	Mealy	83	9	90.2
	Non-mealy	15	77	83.7
	Overall	98	86	**86.9**

Rows: classified by TSS.
Columns: predicted by near infrared spectroscopy (NIR).
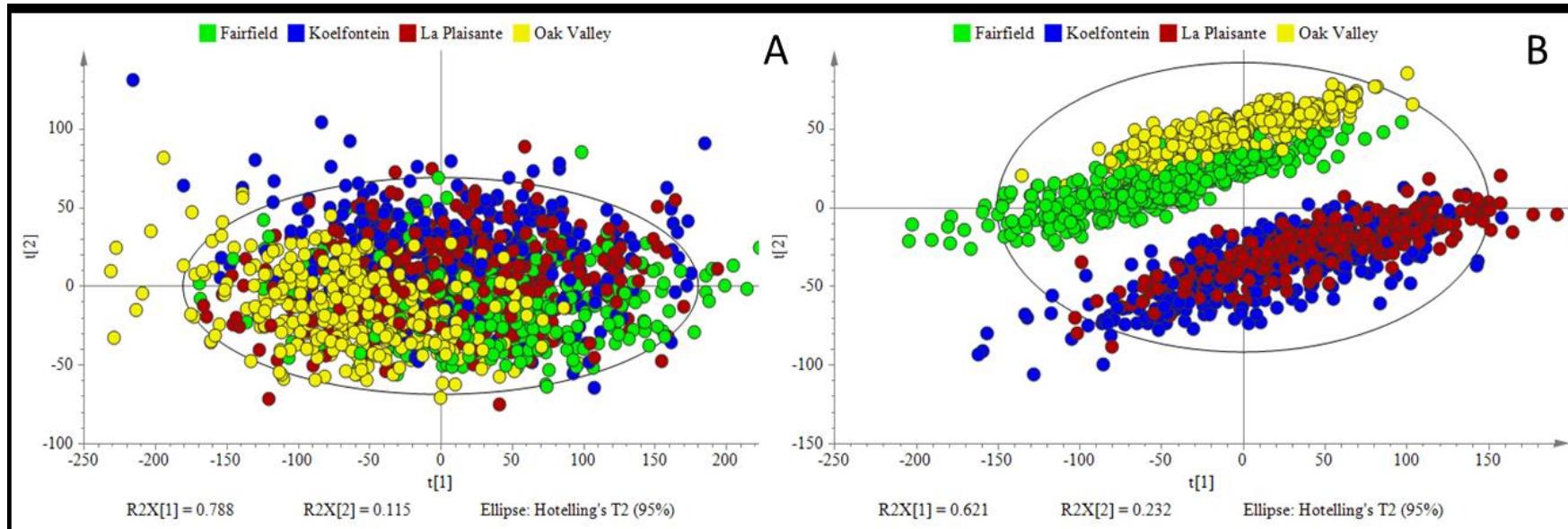
Fig 1. Principal component analysis (PCA) scores plots for all the Fourier transformed near infrared (FT-NIR) absorbance spectra measured on 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. A) Unprocessed spectra PCA showing the four farms. B) Pre-processed spectra (Standard Normal Variate (SNV) showing clustering of Koelfontein and La Plaisante together and Fairfield and Oak Valley together.

140
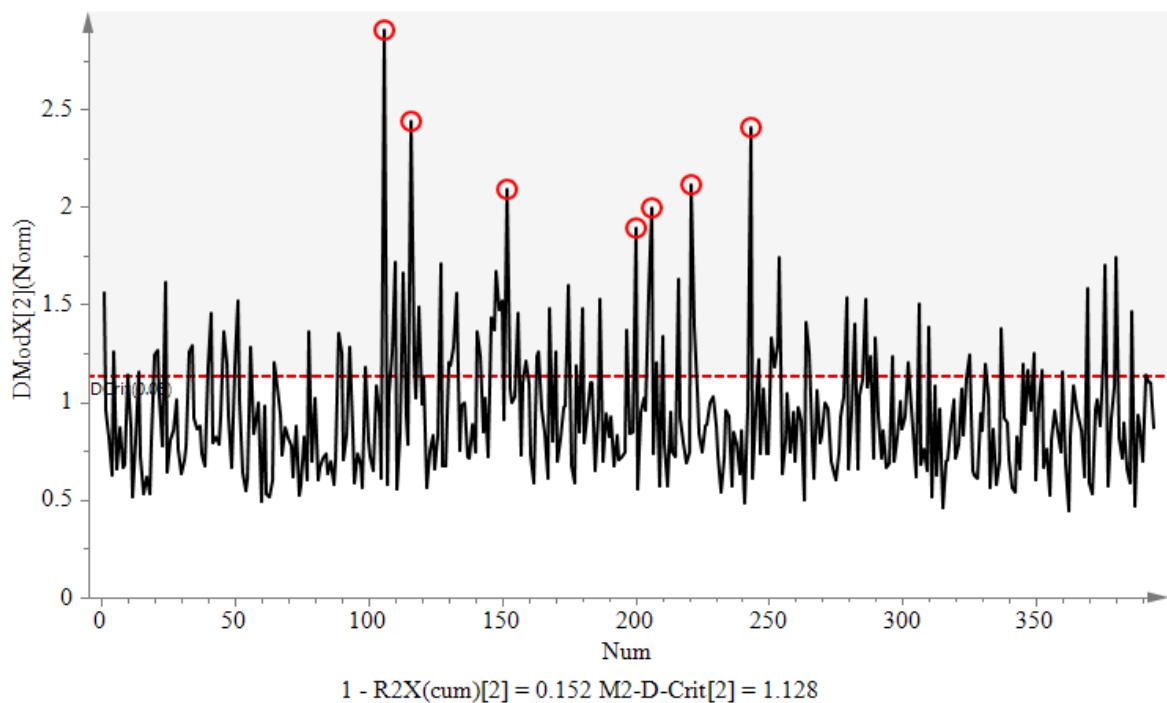
Fig. 2. DmodX plot showing outliers (circled). Observations with DmodX in excess of 2 X Dcrit were considered outliers with structure that cannot be captured in the model. Outliers within the 2 X Dcrit limit were considered after review. This presentation is based on spectra of fruit harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014.
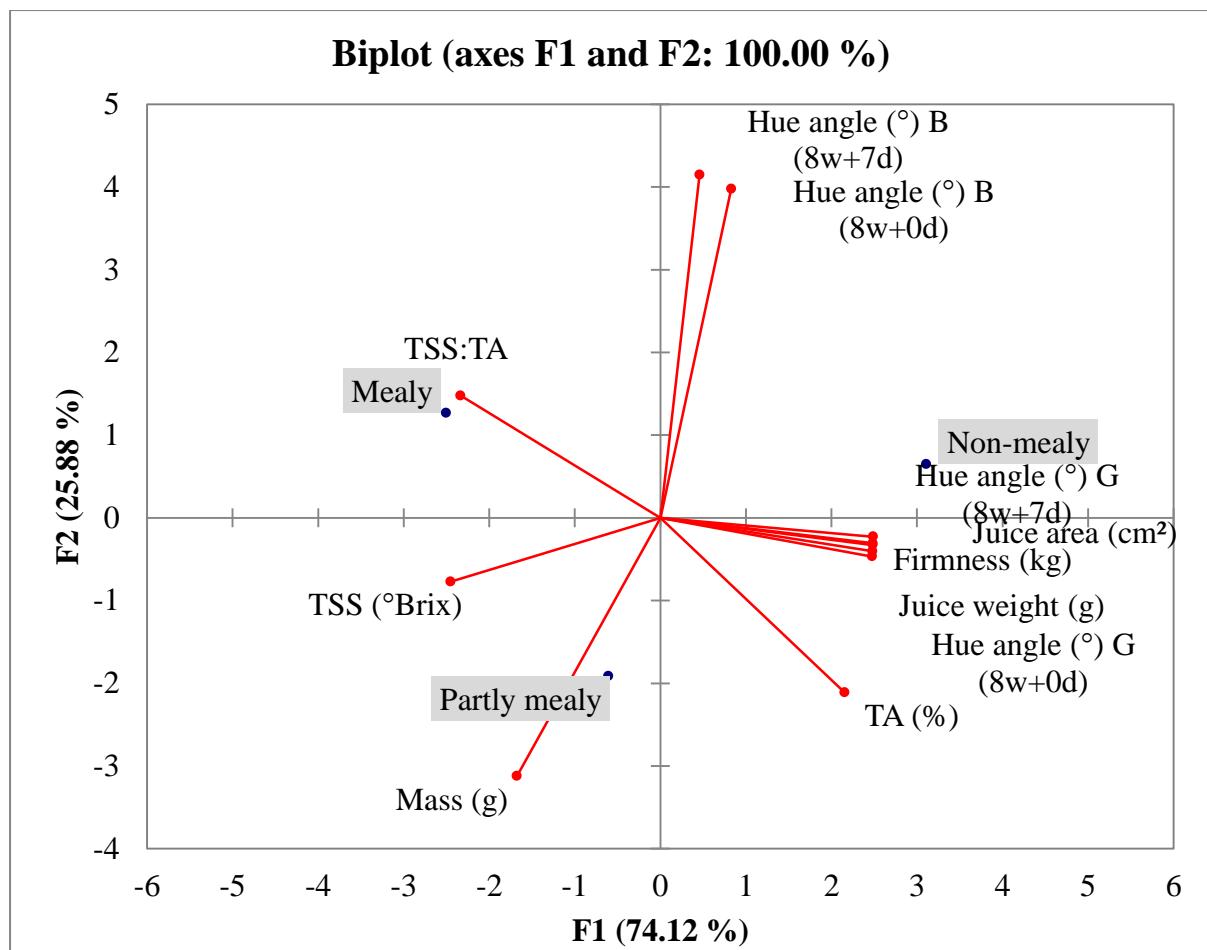
Fig 3. Principal component analysis biplot indicating mealiness score in relation to physicochemical attributes of 'Forelle' pears from region 1 (Koelfontein and La Plaisante) harvested in 2013. TA- titratable acidity, TSS- total soluble solids, G- equator green side, B- equator blushed side, 8w+0d- after 8 weeks of storage at -0.5 °C, 8w+7d- after 8 weeks of storage at -0.5 °C plus 7 days shelf life at 20 °C. F1 = principal component 1; whereas F2 = principal component 2.
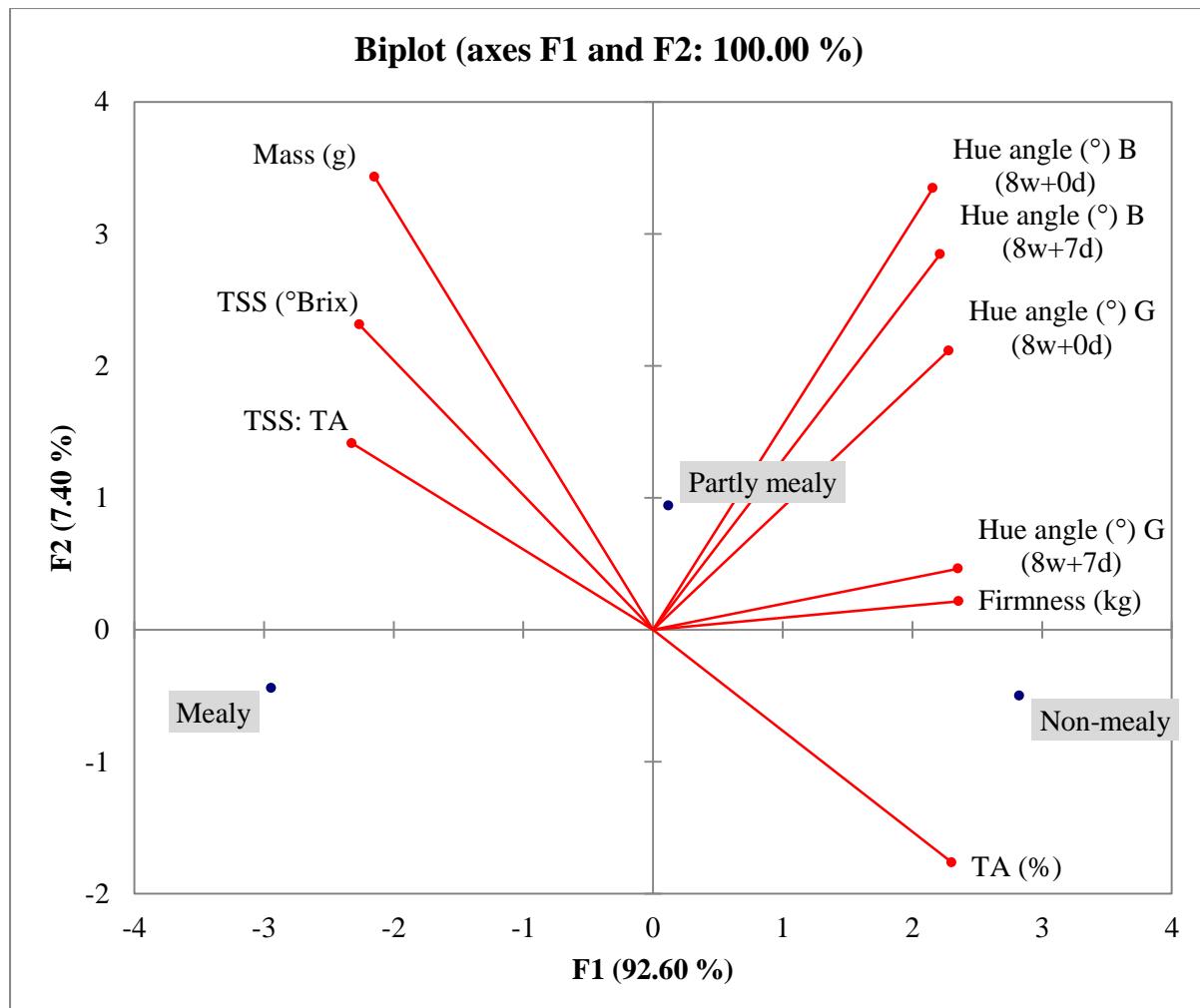
Fig 4. Principal component analysis biplot indicating mealiness score in relation to physicochemical attributes of 'Forelle' pears from farm group 2 (Fairfield and Oak Valley) harvested in 2014. TA- titratable acidity, TSS- total soluble solids, G- equator green side, B- equator blushed side, 8w+0d- after 8 weeks of storage at -0.5 °C, 8w+7d- after 8 weeks of storage at -0.5 °C plus 7 days shelf life at 20 °C. F1 = principal component 1; whereas F2 = principal component 2.

Biplot (axes F1 and F2: 64.51 %)
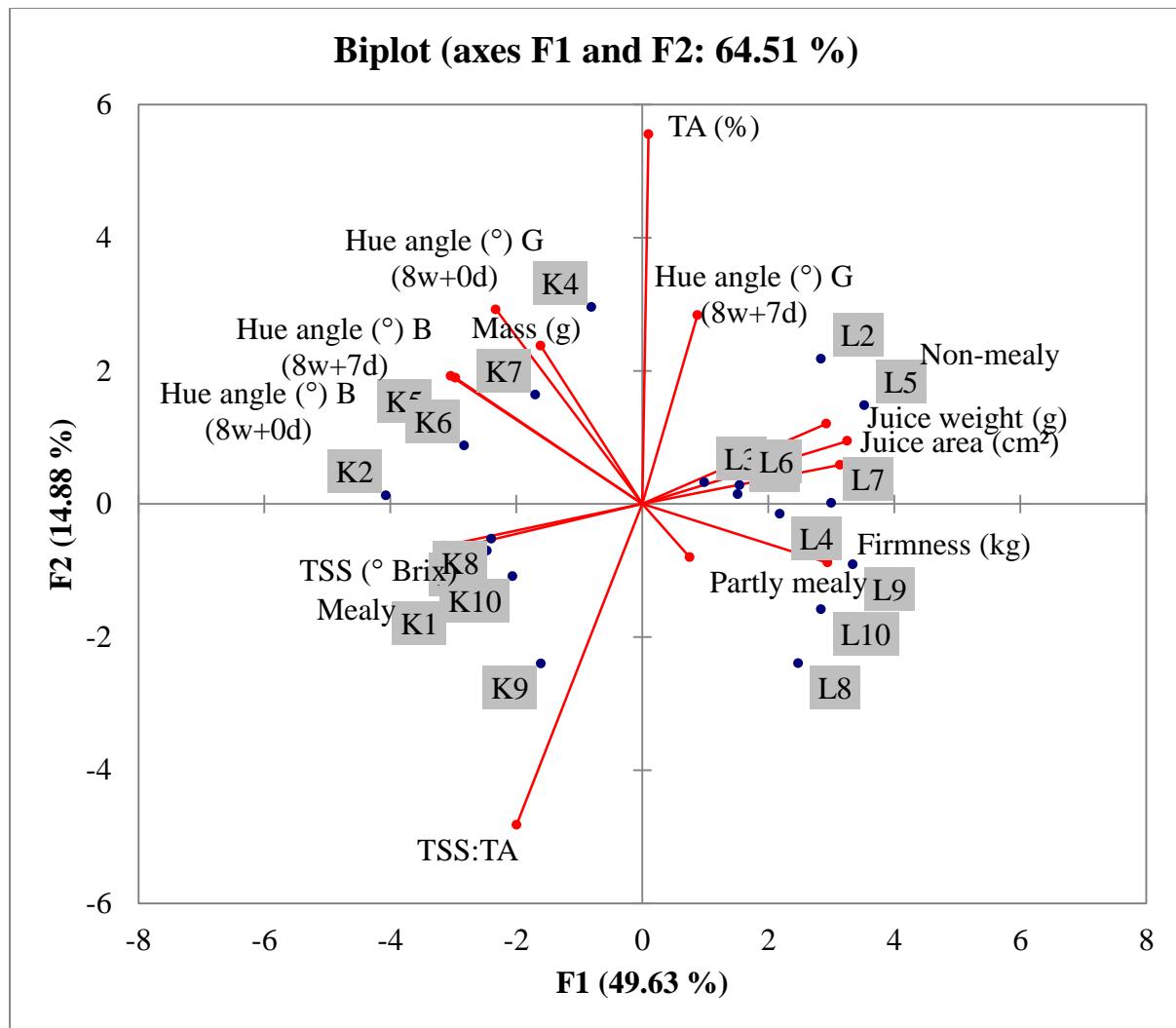
Fig 5. Principal component analysis biplot indicating farm in relation to mealiness score and physicochemical attributes of 'Forelle' pears from Koelfontein (K) and La Plaisante (L) harvested in 2013. TA- titratable acidity, TSS- total soluble solids, EG- equator green side, EB- equator blushed side, 8w+0d- after 8 weeks of storage at -0.5 °C, 8w+7d- after 8 weeks of storage at -0.5 °C plus 7 days shelf life at 20 °C. F1 = principal component 1; whereas F2 = principal component 2.
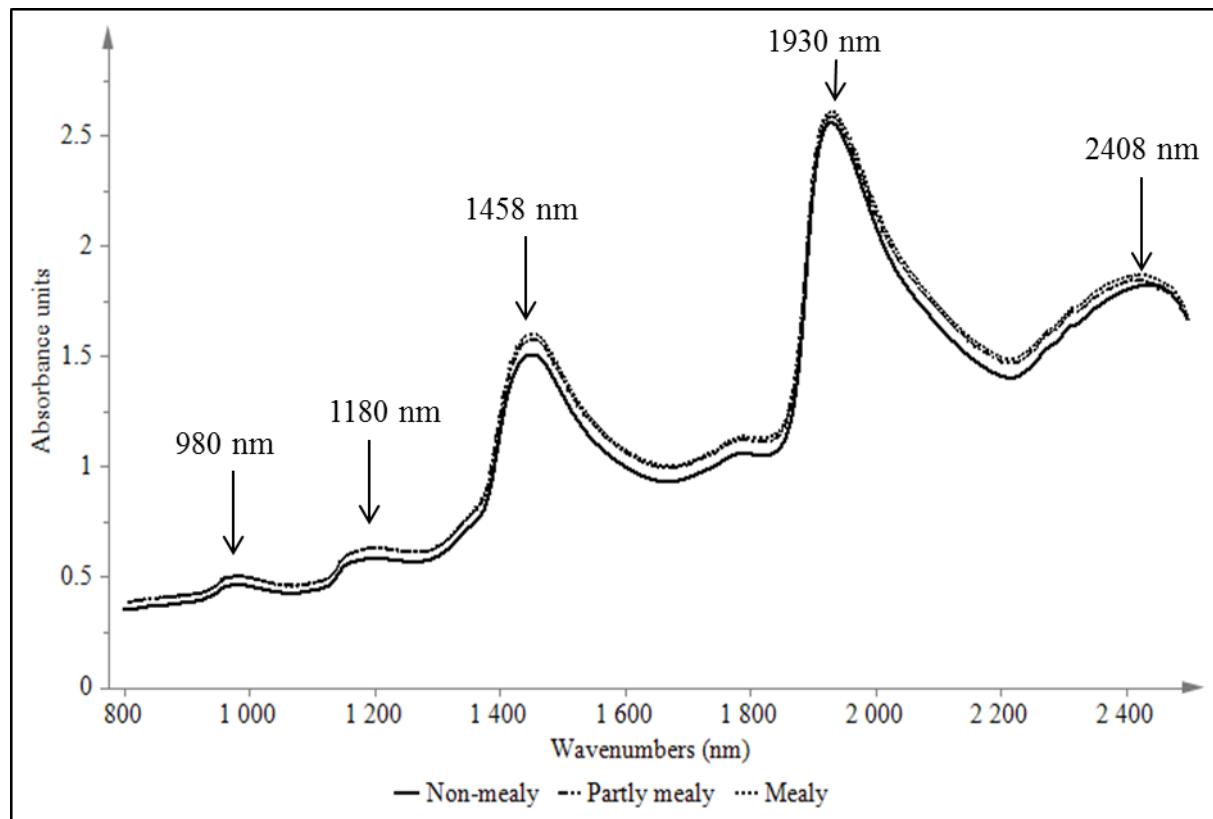
Fig. 6. Typical absorbance spectra for 'Forelle' pear taken in this study. Spectra show the complete spectral band between 800 and 2500 nm. Each spectrum presented is an average of spectra for 15 mealy fruit, 15 partly mealy fruit and 15 non-mealy fruit.
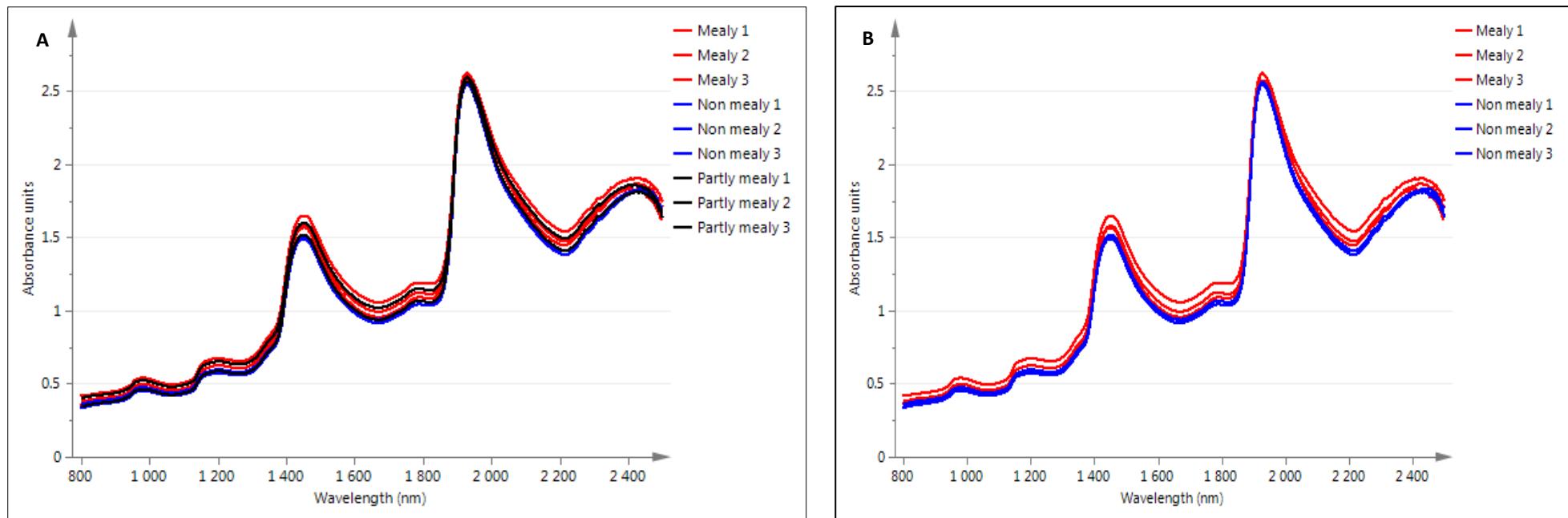
Fig. 7. Effect of mealiness on spectral characteristics of 'Forelle' pear. Spectra were taken from the equatorial blush side of the La Plaisante fruit. For each single spectrum, 15 spectra were averaged to get the resultant spectra. A) Using raw spectra with the three mealiness conditions B) using raw spectra with only true mealy and true non-mealy spectra.
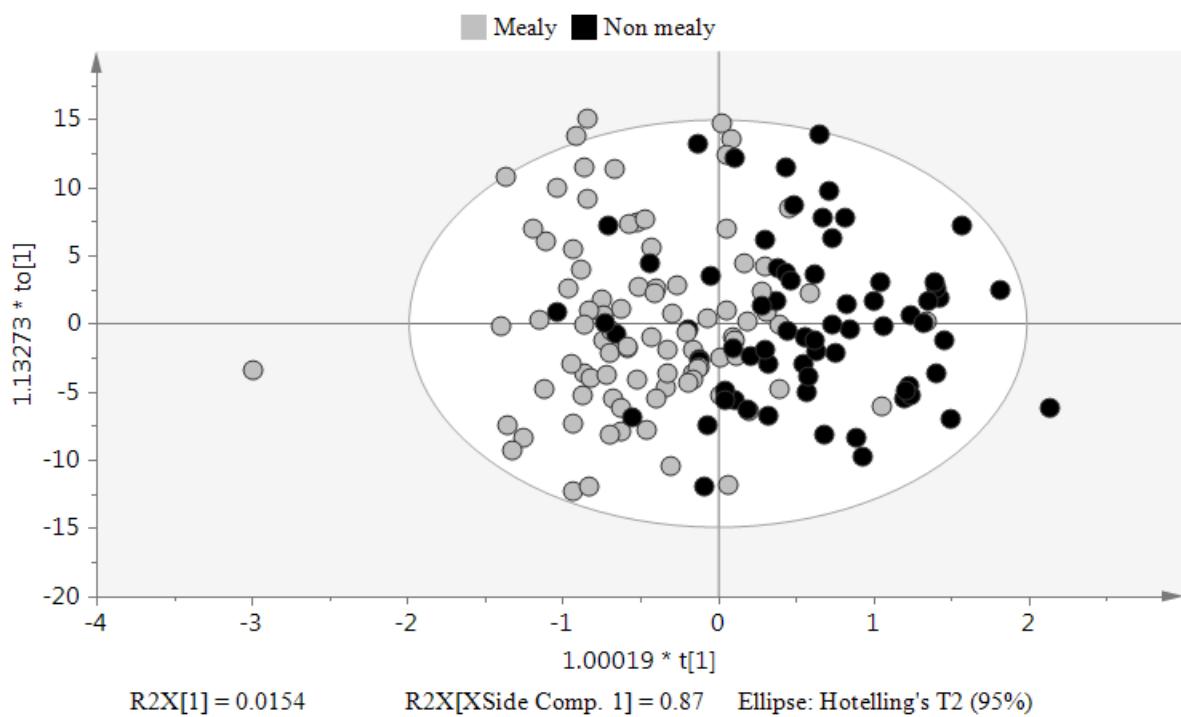
Fig. 8. OPLS-DA scores plot for mealy (pooled) and non-mealy spectra taken from equator green of 'Forelle' pears harvested from Koelfontein, La Plaisante, Oak Valley farms harvested in 2013 and 2014. The fruit were scored using the sensory classification. The plot shows separation of mealy and non-mealy fruit.
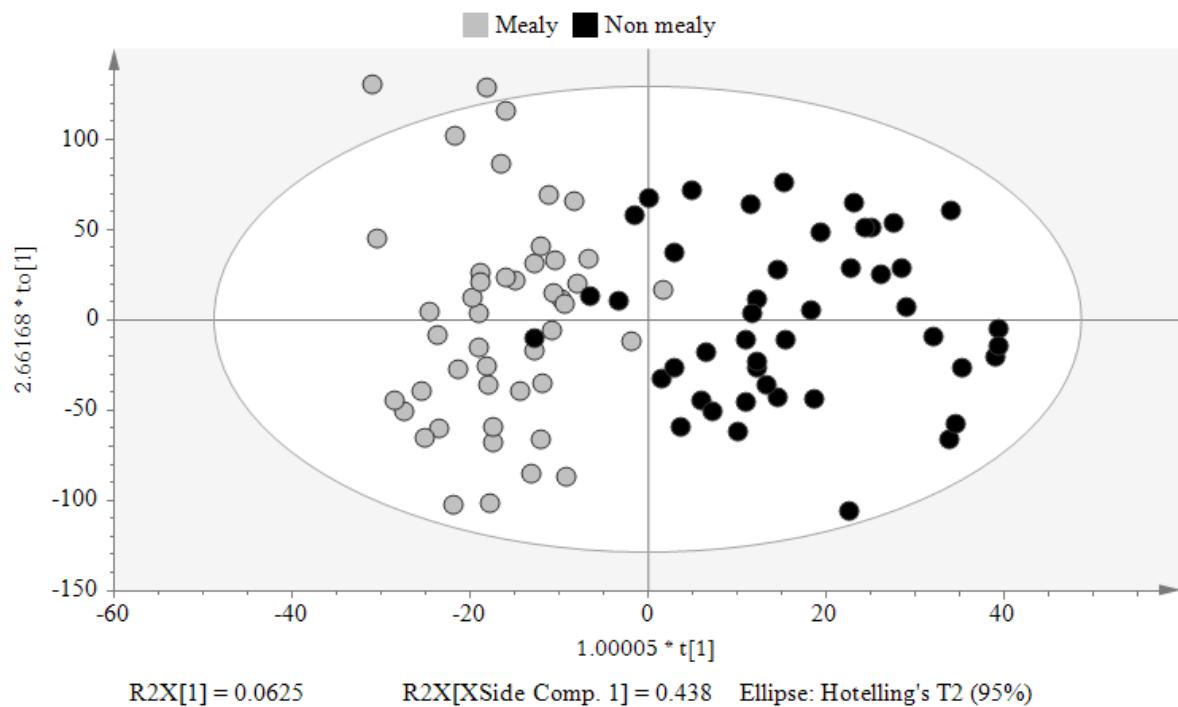
Fig. 9. OPLS-DA scores plot for mealy and non-mealy spectra taken from the equator blush of 'Forelle' pears harvested from Koelfontein, La Plaisante, in March 2013 and from Oak Valley farms harvested in March 2014. Fruit were put in mealiness classes based on their TSS (Table 2 and 7). The plot shows clear separation of mealy and non-mealy fruit.

148

PAPER 5

_____

Quantitative determination of total soluble solids content of 'Forelle' pears using FTR-NIR spectroscopy.

Effect of location and fruit acquisition position on model performance.

_____

Abstract

Fourier transform near infrared (FT-NIR) spectroscopy was evaluated for the quantification of total soluble solids (TSS) in 'Forelle' pears (*Pyrus communis* L.). Fruit were harvested from four farms (Western Cape Province of South Africa) from two groups during the 2013 and 2014 seasons and evaluated after storage (8 weeks at -0.5 °C) and ripening (20 °C). Partial least squares (PLS) calibration models were built using spectra of fruit from each farm group/ season and again from combined farms. Independent TSS calibration models were made for spectra measured on the equator blush, the equator green and two neck positions, on either side of the fruit, in order to capture spatial variation within the fruit. A segmented cross validation was used with 70% of the samples as calibration samples while 30% were test samples. TSS calibration models for group 2 farms (Fairfield and Oak Valley) had the best calibration performance (R^2 = 0.70-0.82, RMSECV=0.63-0.82) compared to the farm group 1 (Koelfontein and La Plaisante) or all farms. Koelfontein (in group 1) had a small range of high TSS values compared to other farms. Farm group 1 models were externally validated using spectra from farm group 2 and vice versa. Models from farm group 1 predicted TSS from Oak Valley, albeit with low precision (RPD 1.28-1.62) and Farm group 2 spectra predicted TSS from La Plaisante with RPD=1.07-1.70). Models made with spectra taken in the equator blush side had higher calibration performance (R^2=0.56-0.82 and RMSECV=0.63-0.91) compared to models of spectra from the other sides or all sides. Validation model results had RMSEP=0.76-0.94 and RPD=1.53-2.17 with the equator blush consistently giving better performance for three farms making them ideal for hand held FT-NIR models. External validation results of farm location showed reduced model robustness. The decrease in prediction performance was attributed to TSS ranges differing in locations and season. Future studies on calibrations with 'Forelle' are recommended with fruit from wide origins and TSS ranges over different seasons.

Key words

Pyrus communis L., total soluble solids, PLS, classification.

2.   Introduction

'Forelle' pear (*Pyrus communis* L.) is one of the most important export crops in South Africa. The cultivar has grown in plantings and export volumes over the past decades (HORTGRO, 2014). The area under 'Forelle' has grown from 2895 ha in 2005 to 3195 ha in 2014, the largest increase of all pear varieties during the period (Theron et al., 2008; HORTGRO, 2014). 'Forelle' is now the second most exported pear cultivar from South Africa, accounting for 26% of the total area planted (HORTGRO, 2014). Fruit destined for export is harvested at commercial maturity which is well before the fruit goes into climacteric ethylene production (Pasquariello et al., 2013). Standard maturity indices such as total soluble solids (TSS) and firmness are defined for the harvesting of commercial fruit, and are usually stipulated in harvesting protocols (Hurndall, 2011). These indices are determined using destructive methods, since there are currently no published non-destructive methods of determining them in 'Forelle'. However, TSS of 'Forelle' pears tends to vary according to location, orchard or even position in canopy (Cronje et al., 2015). This variability has a bearing on subsequent ripening and sensory perception, especially in 'Forelle' where ripening is associated with problems of mealiness and astringency (Crouch, 2011). Furthermore TSS has been linked to mealiness after ripening (Paper 1). To improve efficiency in postharvest handling of 'Forelle' and maintain a good product during shelf life, efficient and non-destructive methods of determining internal attributes of fruit are crucial.

Non-destructive techniques for determination of produce quality parameters have been tested on a number of fruits (Bobelyn et al., 2010; Lammertyn et al., 2002; Magwaza et al., 2012a; Magwaza et al., 2014a; Nicolai et al., 2007; Van Dalen et al., 2007). Over the last three decades, the number of research publications on NIR spectroscopy on food has dramatically increased (Shao and He, 2007). Reviews and studies by Magwaza et al. (2012b), Louw and Theron (2010), and Nicolai et al. (2007), have identified NIR spectroscopy as one of the techniques with potential utility in quality grading of fruit. NIR spectroscopy is a rapid, precise technique based on the ability of electromagnetic radiation to interact with matter (Givens et al., 1997; Omar and MatJafri, 2009). The NIR spectrum (750-2500 nm) is related to absorbances resulting from overtones and combinations of chemical bonds such as C-H, O-H, and N-H (Alander et al., 2013; Teyer et al., 2013), which are contained in many foods. Acquisition of NIR spectra is done using a spectrometer which releases electromagnetic radiation onto a piece of matter such as fruit. As near infrared light is irradiated on fruit, some

of it is scattered, some reflected while some is absorbed. NIR spectra are generated from the coupled computer, reflecting biochemical and physical components of the fruit.

Once NIR spectra have been acquired, the next step is to extract information embedded in the spectra. This is achieved through the use of prediction equations or models, generated by advanced multivariate statistical techniques which relate information perceived through spectra to the true chemical or physical values obtained through traditional wet chemistry methods. Principal component analysis (PCA) and partial least squares regression (PLS) methods are two techniques commonly used to explore data and find relationships between spectra and physicochemical attributes. PCA is an exploratory method that works by reducing dimensionality in datasets to few composite variables or principal components (Pasquariello et al., 2013), which allow for identifying patterns or variations existing in the dataset. PLS regression on the other hand is used for making quantitative predictions of physicochemical parameters by relating spectra to known measurements of the ingredient of interest (Alander et al., 2013). PLS is considered for building predictive models, whereas PCA is used when the factors are many and highly collinear, as is the case with spectroscopic data (Nicolai et al., 2007; Louw and Theron, 2010).

Numerous researchers have reported prediction models for TSS, titratable acidity (TA) and firmness on tomatoes (Jha and Matsuoka, 2004; Peiris et al., 1998; Slaughter et al., 1996), apples (Jha and Garg, 2010), orange (Cayuela, 2008; Cayuela and Weiland, 2010), mango (Saranwong et al., 2004), kiwifruit (McGlone and Kawano, 1998; Moghimi et al., 2010), prunes (Slaughter et al., 2003), Japanese plums (Louw and Theron, 2010) and peach (Shao et al., 2011). In pears, recent NIR spectroscopy research focused on soluble solids content in 'Crystal' pear (Liu et at., 2010); firmness and soluble solids content in 'Conference' pear (Nicolai et al., 2007) and internal quality (Liu, et al., 2008). TSS together with firmness and ground colour are parameters normally used for determination of harvest maturity and fruit quality in 'Forelle' pear (Carmichael, 2011; Hurndall, 2011). NIR technology may provide a technique for the prediction of TSS in 'Forelle' pears. This study therefore aimed to determine if NIRs could be used as a non-destructive tool to determine TSS in 'Forelle' pears. The study was done with 'Forelle' pears from South Africa, produced from a wide geographical area in the Western Cape. In blushed cultivars such as 'Forelle', different sides of the fruit may exhibit differences in fruit development and biochemical characteristics; hence the effect of side of fruit was examined. In addition, microclimatic differences in

temperatures, orchard and other factors tend to cause variations in ripening behaviour of fruit (Martin, 2002). The study also examined the effects of orchard on NIR spectra and TSS calibration model performance.

2. Materials and methods

2.1 Fruit samples

Fruit were harvested from four farms in the Western Cape, South Africa. In 2013, fruit were harvested from the farms Koelfontein (lat. 33.00°S, long. 19.33°E) and La Plaisante (lat. 33.41°S, long 19.19°E). Koelfontein is located in Ceres while La Plaisante is located in the Wolseley area. These farms were broadly grouped as farm group 1 (refer to Paper 4 Section 2.5). In 2014, fruit were harvested from Fairfield (lat 33.32°S, long. 19.31°E) in Ceres and Oak Valley (lat 34.15°S, long. 19.05°E) in Elgin, also in the Western Cape, South Africa. Combined these data were referred to farm group 2 (refer to Paper 4 Section 2.5). Fruit were randomly harvested at shoulder height from either side of the tree canopy from 40 trees per orchard per farm. Twenty fruit were harvested from shoulder height, 10 on either side of the canopy. A subsample of 100 fruit was taken from the harvested fruit for each farm and packed in polyethylene (37.5 μm) lined pear boxes and stored for a period of 8 weeks at -0.5 °C, followed by 7 days of shelf life (ca. 20 °C).

2.2 Near infrared spectral acquisition

NIR measurements were taken on each fruit after storing fruit for 8 weeks at -0.5 °C followed by 7 days at ca. 20 °C. One hundred fruit from each farm were used for near infrared spectral acquisition. FT-NIR spectra were acquired on each fruit in reflectance mode using a multi-purpose analyser (MPA) spectrometer (Bruker Optics, Ettlingen, Germany), fitted with an internal TE-InGaAs detector, and a tungsten lamp as the NIR source. NIR spectra were taken from 800.19 – 2500.11 nm. The solid probe was used to acquire absorbance spectra on four positions of each fruit viz. the equator blush, the equator green and two neck positions opposite each other. The four fruit positions were used in order to capture spatial variations in 'Forelle' pears. For each fruit position, 16 scans with a resolution of 4 nm were taken at a scanner velocity of 10 kHz. A clean blue lid was scanned at the beginning of acquisition and every 2h thereafter to correct for detector drifts. Two background correction scans were taken every 2 h for the background correction of the spectrum.

2.3 Determination of TSS and other maturity indices

TSS was determined on each individual fruit after NIR measurements. A disk of the fruit was cut from the equator of each fruit and crushed in a blender (Model JE-107, PRC, AEG Electrolux, China). A drop of juice released was collected and used for TSS determination. TSS concentration was measured on a drop of expressed juice from each disk of each individual fruit using a hand held digital refractometer (PR-32, Atago, Tokyo, Japan). Fruit firmness was measured on opposite green and blushed sides of the fruit using a universal fruit texture analyzer (FTA 2007, Güss Manufacturing, Strand, South Africa), fitted with an 8 mm diameter probe. Fruit diameter was determined using a Cranston gauge, fitted onto the fruit texture analyser. Maturity indices at harvest were determined on a subset of 100 fruit per farm. Titratable acidity was determined by titration of juice from individual fruit using an automated titrator (Metrohm AG 760. Harison, Switzerland). Ten grams of expressed juice was titrated with 0.1 M NaOH to an end-point of pH 8.2.

2.4 Analysis of physicochemical attributes and spectral inspection

TSS and other fruit physical and chemical attribute data were analysed using analysis of variance (ANOVA). The statistical programme STATISTICA Version 12 (StatSoft Inc, 1984-2012, Tulsa, OK 74104, USA) was used for ANOVA. PCA was performed on NIR spectra and TSS in order to show spectral patterns across orchards using SIMCA (version 13.0.3.0, Umetrics AB, Umea, Sweden).

2.5. Chemometric data analysis

Calibration models were built using partial least squares (PLS) regression in OPUS 7.0 for Word (Bruker Optics, Ettlingen, Germany). Firstly, independent calibration models were made for spectra from the equator blush, the equator green and the pooled data from the two neck positions. A segmented cross validation was done on spectra from individual farms; each segment having 10% of the fruit i.e. 10 segments for 100 spectra. Secondly, farms were combined and test set validation performed by splitting datasets into calibration (70% of spectra) and test set spectra (30%). A proportionate number of calibration and test samples was selected from each of the farms. Model calibration performance was expressed as root mean square error of estimation (RMSEE), root mean square error of cross validation (RMSECV), as well as the coefficient of determination (R^2) of measured and predicted TSS values. RMSEE is a measure of the difference between predicted and measured values which

153

shows the degree of fit of the model. RMSECV shows the uncertainty expected in future predictions using the model (Nicolai et al., 2007; Peirs et al., 2000). R^2 value refers to the coefficient of determination of the predictive models (Zhang et al., 2011). Model predictive performance was first internally tested using the test set validation. The root mean squared error of prediction (RMSEP) and the relative prediction deviation or relative percent difference (RPD) were used to measure the accuracy of model performance. RMSEP shows the quality of the model when tested with an external dataset or average prediction error as estimated by the validations set (Esbesen et al., 2006; Nicolai et al., 2007; Louw and Theron, 2010). RPD is calculated by dividing the standard deviation of reference values with the standard error of cross validation or prediction. Robust models are characterised by high RPD, normally larger than 1.5 and low RMSEP (<1) (Bobelyn et al., 2010; Magwaza et al., 2012b). To establish the effect of location and regions on model calibration performance, calibration models made for group 1 were validated using spectra from orchards harvested in group 2 and vice versa.

$$RMSEE = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{obs} - y_{pred}\right)^2}{N-1}} \quad ⍰$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{pred} - y_{act}\right)^2}{n}} \quad ⍰$$

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{pred} - y_{act}\right)^2}{N}} \quad ⍰$$

$$RPD = \frac{SD}{RMSEP} \quad ⍰$$

Where:

n	number of spectra.(also equivalent to N)
y_{act}	actual value of measured attributes
y_{obs}	actual observed values
y_{pred}	predicted value of the attribute.

SD   standard deviation of reference values

3. Results and discussion

3.1. Physicochemical attributes of fruit used in the study

The biggest fruit in terms of fruit mass were recorded at Koelfontein and La Plaisante while the smallest fruit were from Fairfield (Table 1). Fruit equatorial diameter did not differ across farms. Fruit firmness at harvest was high at Fairfield where the smallest fruit were recorded. After shelf life, Oak Valley had the firmest fruit while the softest fruit were found at Koelfontein. Koelfontein had the highest TSS, followed by La Plaisante. TSS at Koelfontein was characterised by a narrow range (Table 1), skewed towards high values. At La Plaisante and Oak Valley, the TSS range was wide and its distribution near normal. Fairfield had fewer fruit with TSS in the lower ranges (12.7 – 18.5). The low range in TSS at Koelfontein could have been because the majority of the fruit were riper, however fruit firmness after harvest and after shelf-life was similar to La Plaisante.

3.2 'Forelle' near infrared spectra

'Forelle' pear spectra are characterised by broad peaks at 980 nm and around 1180 nm (Fig. 1). It also had peaks at 1458 nm, 1930 nm and at 2408 nm. 'Forelle' pear spectra from this study were comparable with spectra from other fruit with high water content such as plums (Louw and Theron, 2010), and apples (Bobelyn et al., 2010), with peaks around 1450 nm, 1790 nm, 1930 nm and 2380 nm regions which are water absorption bands (Workman, 2014). Peaks around 976 nm and 1180 nm are associated with C-H overtone regions associated with sugar solutions (Delwiche et al., 2008; Workman and Weyer, 2012). Ripe pears comprise of predominantly water and sugars, including fructose, sucrose, glucose and sorbitol (Pasquariello et al., 2013).

3.3 Calibration model performance

The best calibration performance for individual farms was recorded at La Plaisante on the equator blush (R^2=0.78, and RMSECV=0.70). A similar performance was observed at Oak Valley where the model at the equator blush gave an R^2=0.72 and RMSECV=0.66. When models were made with spectra from the equator blush side, performance was weaker at Koelfontein and Fairfield (Table 2). The correlation between TSS predicted by NIR spectra and measured TSS ranged from 0.56 to 0.82 while RMSECV values ranged between 0.63 and 0.91 (Table 3). The highest calibration performance was found on the equator blush when

155

Fairfield was combined with Oak Valley (R^2=0.82 and RMSECV=0.63), followed by the equator blush for La Plaisante + Oak Valley + Fairfield (R^2=0.78, RMSECV=0.68). The models for the equator green side for combined data from Fairfield and Oak Valley performed well with R^2 and RMSECV of 0.70 and 0.79, respectively. Models made with data from the neck performed equally well (R^2 = 0.72 and RMSECV =0.79). The least calibration model performance was obtained when Koelfontein was combined with La Plaisante, where the R^2 ranged between 0.56 at the equator to 0.61 at the equator green side.

Combining farms in farm group 1 was affected by the presence of atypical spectra from Koelfontein (Table 3). The poorer calibration performance observed with models using spectra from Koelfontein fruit could be attributed to the narrow range of TSS at this site (Table 1). Koelfontein had fruit with the highest TSS compared to other farms with a narrow range (Figs. 2 and 3). Secondly, Koelfontein was unimodal with very few fruit with low TSS fruit compared to other orchards (Fig. 3A). Conversely, at La Plaisante and Oak Valley there was a balanced distribution of fruit with low TSS and higher TSS fruit (Figs. 3C and D). Prediction of TSS at these two locations was good. However, the effect of spectra from Koelfontein was subdued when all spectra were combined in the all farms model. When all the farms were pooled together, the model calibration performance improved considerably on the blushed side of the equator (R^2=0.75, RMSECV=0.78) (Fig. 4). These results confirmed that more data are required in order to build robust calibrations models (Bobelyn et al., 2010). There was higher correlation between TSS predicted by NIR and measured TSS at the equator blush side (Fig. 4 A) than the other sides (Fig. 4B-D). The poorest performance was observed when spectra from the green equator side and all combined sides was used (Fig. 4B and D). PCA of spectra showed that indeed there was some variation in spectra measured on different sides of the fruit (Fig. 5). Overall, results showed differences in model performances due to orchard differences in TSS ranges and fruit position of spectral acquisition.

The prediction performance of combined farm spectra was tested using test set validation, involving 70% calibration samples and 30% validation samples. Models made with spectra from combined farms had higher prediction performance as evidenced by low RMSEP and high RPD values (Table 3). The equator blush at La Plaisante + Oak Valley + Fairfield had RMSEP=0.76 and RPD=2.12 while the blush of the four combined farms had RMSEP=0.77 and RPD=2.17. The other farm combinations (La Plaisante+Koelfontein; Fairfield+Oak Valley) and the different sides produced RMSEP ranges of 0.82-0.97. Generally, superior

156

prediction performance was observed on the equator blush side while the neck had comparable results with the equator green side having poorer results (Table 3). Combining spectra from all farms improved the prediction performance on all sides, although the performance did not significantly differ from the combination of La Plaisante + Oak Valley + Fairfield, again probably due to the depressing effects of spectra from Koelfontein. However, all the sides had RMSEP less than 1 and RPD values greater than 1.5, values that are considered to describe models with potential for further screening (Saeys et al., 2005). In addition, the prediction performance in this study was comparable with the model calibration results, which is a sign of a robust model. Further, calibration model performance improved with the inclusion of more samples from different locations.

Calibration model performance observed in this study was comparable with that found in other studies where TSS was studied (Bobelyn et al., 2010, Magwaza et al., 2012a). However, there was a notable variation in model performance across the four sides of the fruit where spectra were acquired, although in general all the sides produced good models. The equator blush side in most cases produced the best calibration models, followed by the equator green side and the neck. Variations in fruit internal soluble solids composition could be the probable cause of these observed variations in model performance (Alander et al., 2013). Products of biochemical activities associated with ripening tend to vary spatially within the fruit following ripening. The blush side is exposed to higher irradiance and temperatures during fruit development, hence the side is characterised by elevated TSS levels (Wawrzyńczak et al., 2006, Cronje et al., 2015). In this study an equatorial disk was juiced per fruit which included all equatorial sides, but not the neck. The neck of the fruit generally ripens first (Crouch, 2011) and may therefore have a TSS comparable to the sunny blushed side of the fruit. In some studies, spectra from different positions on the fruit have been averaged to level out differences due to physicochemical gradients within the fruit (Louw and Theron, 2010; Magwaza et al., 2012a). Magwaza et al. (2012a) established better calibration and validation models when they averaged spectra. Results from this study show that although averaging spectra produced good calibration performances, it did not significantly improve calibration performance (Table 2). However, the good prediction performance at the blush side provides opportunity for hand held spectrometers for the determination of TSS.

3.4 Effect of location

After all model calibration work has been done, the most important thing to do is to test the model for ability to predict future samples and samples from other locations (Esbensen, 2006). In the current study, the ability of the models to predict an external dataset was examined using spectra from four different farms. Models calibrated with spectra from broad farm group 1 were able to predict TSS from Oak Valley, albeit with low robustness, with RMSEP ranging from 0.96-1.49 and RPD =1.28-1.62 (Table 4). Models made with farm group 2 were poor in predicting La Plaisante spectra. The RMSEP for the prediction of La Plaisante ranged between 1.23-1.43 with RPD=1.07-1.7. RMSEP values were higher than cross validation results while RPD values were lower than those from cross validation results. RMSEP is considered to be the most important measure in model robustness (Nicolai et al., 2007) while RPD helps in describing the fitness of the model. Instances where low robustness were observed in this study can be ascribed to differences in TSS between the validation samples and the calibration samples. TSS for farm group 2 (Oak Valley + Fairfield) was lower than that of farm group 1 (Koelfontein + La Plaisante).

Results from this study are consistent with results found in earlier studies (Bobelyn et al., 2010, Nicolai et al., 2008). Bobelyn et al. (2010) working on the effect of biological variability on spectra of apples showed that using a small data set containing fruit with different TSS ranges would result in poor models. Louw and Theron (2010) also noted that when a validation sample with values that lie outside the ranges of those from the calibration samples is used, the resulting predictability will be very low as the model will be forced to predict values outside those from the calibration data set. This is probably what caused the poor prediction of La Plaisante by farm group 2 fruit. In other studies where biological materials, particularly fruit have been studied using NIR spectroscopy, questions of biological variability have always arisen (Bobelyn et al., 2010; Peirs et al., 2003; Peirs et al., 2005). In order to correct for these, it has been recommended that a large data set from a wide geographical origin be used for both calibration and validation. A study is therefore recommended that will take into account these differences in farms from different regions or alternatively perhaps create a model for a specific region but with more farms from that region. Attempts at pre-processing of spectra did not yield improved performance (see Supplementary Tables 1 and 2 showing results obtained using selected pre-processed spectra). All calibration models were therefore done without spectral pre-processing. Spectra were only subjected to mean centering prior to calibration and validation.

4.0 Conclusion

The study has shown that NIRs can be used to predict TSS in 'Forelle' pears. The equator blush side produced better models compared to other sides. Model performance also varied with farm group, attributable to different spectra from individual farms. External validation results showed reduced model robustness. The decrease in prediction performance was attributed to biological variability due to TSS ranges originating from location and perhaps season. This is expected when an external dataset is introduced, especially if it has values that fall outside the ranges of the calibration set. PCA of NIR spectra showed that spectra from different locations and from different seasons exhibit dissimilar patterns. Future studies are recommended on calibrations with 'Forelle' from as wide origins as there are cultivated in South Africa and over different seasons.

Literature cited

Alander, J.T., Bochko, V., Martinkauppi, B., Saranwong, S., Mantere, T., 2013. A review of optical nondestructive visual and near infrared methods for food quality and safety. Int. J. Spectroscopy. Hindawi Publishing Corporation. http://dx.doi.org/10.155/2013/341402.

Bobelyn, E., Serban, A.S., Nicu, M., Lammertyn, J., Nicolai, B.N., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133-143.

Carmichael, P.C., 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Cayuela, J.A., 2008. Vis/NIR soluble solids prediction in intact oranges (Citrus sinensis L.) cv. Valencia Late by reflectance. Postharvest Biol. Technol. 47, 75-80.

Cayuela, J.A., Weiland, C., 2010. Intact orange quality prediction with two portable NIR spectrometers. Postharvest Biol. Technol. 58, 113-120.

Cronje, A., Crouch, E.M., Muller, M., Theron, K.I., van der Rijst, M., Steyn, W. J., 2015. Canopy position and cold storage duration affects mealiness incidence and consumer preference for the appearance and eating quality of 'Forelle' pears. Sci. Hortic. 194, 327-336.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L.). PhD (Agric) Dissertation. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Delwiche, S.R., Mekwatanakarn, W., Wang, C.Y., 2008. Soluble solids and simple sugars measurement in intact mango using near infrared spectroscopy. Hort. Technol. 18(30), 410-416.

Esbensen, K.M., 2006. Multivariate data analysis-in practice: An introduction to multivariate data analysis and experimental design. 5th Ed. CAMO Software AS. Nedre Vollgate 8. Oslo. Norway.

Givens, D.I., Boever, J.L., Deaville, E.R., 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. Nutr. Res. Rev. 10, 83-114.

HORTGRO, 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

Hurndall, R.F., 2011. 'Forelle' dispensation procedures for 2011 season. http://www.decidous.co.za (accessed 10-09-2014).

Jha, S.N., Garg, R., 2010. Non-destructive prediction of quality of intact apple using near infrared spectroscopy. J. Food Sci. Technol. 47(2), 207-213.

Jha, S.N., Matsuoka, T., 2004. Non-destructive determination of acid-brix ratio of tomato juice using near infrared spectroscopy. Int. J. Food Sci. Technol. 39, 425-430.

Lammertyn, J., Priers, A., De Baerdemaeker, J., Nicolai, B., 2000. Light penetration properties of NIRs radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121-132.

Liu, Y., Chen, X., Dong, S.X., Ying, Y., 2007. Non-destructive measurement of pear internal quality indices by visible and near infrared spectrometric techniques. N.Z. J. Agric. Res. 50, 1051-1057.

Liu, Y., Yinying, P., Rongjie, G., Xudong, S., Yong, H., 2010. Online detection of soluble solids content and size of crystal pear based on LEDs light source detector. Trans. Chinese Soc. Agric. Engin. 26 (11), 338-343.

Louw, D.E., Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina*), using NIR spectroscopy. Postharvest Biol. Technol. 58, 176-184.

Magwaza, L.M., Opara, L.O., Terry, L.A., Landahl, S., Cronje, P.L., Nieuwoudt, H., Mouazen, A.M., Saeys, W. Nicolai, B.M., 2012a. Prediction of 'Nules Clementine'

mandarin susceptibility to rind breakdown disorder using Vis/ NIR spectroscopy. Postharvest Biol. Technol. 74, 1-10.

Magwaza, L.M., Opara, L.U., Nieuwoudt, H., Cronje, P.J., Saeys, W. Nicolai, B. 2012b. NIR Spectroscopy applications for internal and external quality analysis of citrus fruit. Food Bioprocess Technol. 5, 425-444.

Magwaza, L.M., Opara, L.U., Cronje, P.J.R., Landahl., Nieuwoudt, H.H., Mouazen, A.M., Nicolai, B.M. Terry, L.A., 2014. Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. Sci. Hortic. 165, 421-432.

Martin, E.M., 2002. Ripening responses of 'Forelle' pears. MSc (Agric) Thesis. Department of Horticultural Sciences, Stellenbosch University, South Africa.

McGlone, V.A., Kawano, S., 1998. Firmness, dry-matter and soluble-solids assessment of postharvest kiwifruit by NIR spectroscopy. Postharvest Biol. Technol. 13, 131-141.

Moghimi, A., Aghkani, M.H., Sazgarnia, A., Sarmad, M., 2010. Vis/ NIR spectroscopy and chemometrics for the prediction of soluble solids content and acidity (pH) of kiwifruit. Biosyst. Eng. 106, 295-302.

Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol., 46, 99-118.

Nicolai, B.M., Verlinden, B.E., Desmet, M., Saevels, S., Saeys, W., Theron, K., Cubeddu, R., Pifferi, A. Torricelli, A., 2008. Time resolved and continuous wave NIR reflectance spectroscopy to predict soluble solids content and firmness of pear. Postharvest Biol. Technol. 47, 68-74

Omar, B.A.F., MatJafri, B.M.Z., 2009. Optical Sensor in the measurement of fruit quality: A review on an Innovative Approach. Int. J. Comp. Elec. Eng. 1 (5), 1793-8163.

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 early ripening pear cultivars. Sci. Hortic. 162, 341-350.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1998. Near infrared (NIR) spectrometric technique for nondestructive determination of soluble solids content in processing tomatoes. J. Amer. Soc. Hort. Sci. 123 (6), 1089-1093.

Peirs, A., Lammertyn, J., Ooms, K., Nicolai, B.M., 2000. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. Postharvest Biol. Technol. 21, 189-199.

Peirs, A., Terry, J., Verlinden, B., Darius, P., Nicolai, B.M., 2003. Effect of biological variability on the robustness of NIRS models for soluble solids content of apples. Postharvest Biol. Technol. 28, 269-280.

Peirs, A., Schenk, A., Nicolai, B.M., 2005. Effect of natural variability among apples on the accuracy of VIS-NIRS calibration models for optimal harvest date predictions. Postharvest Biol. Technol. 35, 1-13

Saeys, W., Mouazen, A.M., Ramon, H., 2005. Potential onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst. Eng. 91, 393-402.

Saranwong, S., Sornsrivichai, J., Kawano, S., 2004. Prediction of ripe-stage eating quality of mango fruit from its harvest quality measured nondestructively by near infrared spectroscopy. Postharvest Biol. Technol. 31, 137-145.

Shao, Y., He, H., 2007. Nondestructive measurements of internal quality of bayberry juice using Vis/NIR spectroscopy. J. Food Eng.79, 1015-1019.

Shao, Y., Bao, Y., He, Y., 2011. Visible/ Near-Infrared spectra for linear and nonlinear calibrations: A case to predict soluble solids contents and pH value in peach. Food Bioprocess Technol. 4, 1376-1383.

Slaughter, D.C., Barrett, D., Boersig, M., 1996. Nondestructive determination of soluble solids in tomatoes using near infrared spectroscopy. J. Food Sci. 61 (4), 695-697.

Slaughter, D.C., Thompson, J.F., Tan, E.S., 2003. Nondestructive determination of total and soluble solids in fresh prune using near infrared spectrocscopy. Postharvest Biol. Technol. 28, 437- 444.

Teyer, E., Huang, S., Afoakwa, N., 2013. Review on the potential of near infrared spectroscopy (NIRS) for the measurement of chemical residues in food. Am. J. Food Sci. Technol. 1, 1-8.

Theron, K.I., van der Merwe, P.W., Ferrandi, C.H., 2008. Pear production in South Africa. Acta Hort. 800, 89-94.

Van Dalen, G., Nootenboom, P., Van Vliet, L.J., Voortman, L., Esveld, E., 2007. 3-D imaging, analysis and modelling of porous cereal products using x-ray microtomography. Image Anal. Stereol. 26, 169-177.

Wawrzyńczak, A., Krzysztof, P.R., Kruczyńska, D.E. 2006. Changes in fruit quality in pears during CA storage. J. Fruit Ornam. Plant Res. 14(2), 77-84.

Workman, J (Jr), Weyer, L., 2012. Practical guide and special atlas of Interpretive near infrared spectroscopy. CRC Press. Taylor and Francis. pp 80.

Workman, J (Jr)., 2014. An introduction to near infrared spectroscopy. John Wiley and Sons. www.spectroscopy.com (accessed on 16.03.2015).

Zhang, H., Song, S., Lang, Q., Zhang, J., Pu, J., 2011. Rapid predictive models for minimally destructive Kappa number and pulp yield of Acacia spp. with near infrared reflectance (NIR) spectroscopy. BioResources. 7 (1), 616-623.

Table 1. Physicochemical parameters of 'Forelle' pears harvested on 9 and 13 March 2013 from La Plaisante and Koelfontein, and from Fairfield and Oak Valley in March 2014, in the Western Cape Province, South Africa

Farm	Mass (g)	Diameter (mm)	Firmness (kg)	Firmness (kg)	[b]TA (%)	[a]TSS (°Brix)	TSS range	SD of TSS
		After Harvest					Shelf life	
La Plaisante	177.4a	64.6	6.4ab	1.5c	0.26a	15.6ab	11.2 – 18.8	1.57
Koelfontein	178.2a	65.3	6.3ab	1.4d	0.27a	17.2a	14.5 – 18.7	0.88
Oak Valley	157.2ab	61.7	6.1b	2.2a	0.12c	14.3b	10.8 – 16.9	1.23
Fairfield	130.7b	64.6	6.8a	1.8b	0.22b	16.2ab	12.7 – 18.5	1.19
		F>Pr						
P-value	<0.001	0.5593	<0.0001	<0.001	<0.001	<0.001		

a=Total soluble solids, b=Titratable acidity,. Means followed by the same letter in the same column are not significantly different. ns= not significant, SD = standard deviation.

Table 2. Performance of Fourier transformed near infrared (FT-NIR) total soluble solids (TSS) calibration models developed using spectra of 'Forelle' pear fruit from La Plaisante, Koelfontein, Fairfield and Oak Valley farms, in the Western Cape Province, South Africa for fruit side (equator blush, equator green, neck pooled for two sides and all sides). Models were done using cross validation. No test set validation was done due to limited number of spectra.

Farm	Fruit side	# of samples	Number of factors	R^2	RMSECV	RPD_c
La Plaisante	Equator blush	92	6	0.78	0.70	2.12
	Equator green	97	6	0.66	0.86	1.71
	Neck	189	9	0.65	0.88	1.7
	All	387	10	0.71	0.81	1.85
Koelfontein	Equator blush	93	1	-0.04	0.72	0.98
	Equator green	92	1	-0.02	0.71	1.00
	Neck	184	1	-0.02	0.71	0.99
	All	362	1	-0.04	0.71	0.98
Fairfield	Equator blush	97	7	0.50	0.78	1.42
	Equator green	67	4	0.38	0.88	1.27
	Neck	190	9	0.60	0.69	1.58
	All	379	10	0.56	0.72	1.52
Oak Valley	Equator blush	100	9	0.72	0.66	1.86
	Equator green	98	4	0.57	0.77	1.53
	Neck	191	10	0.68	0.68	1.78
	All	391	10	0.65	0.73	1.68

RPD_c – relative prediction deviation (calibration)

Table 3. Performance of Fourier transformed near infrared (FT-NIR) total soluble solids (TSS) calibration models of 'Forelle' pear fruit developed using combined data from the four farms (La Plaisante, Koelfontein (2013), Fairfield and Oak Valley (2014), in the Western Cape Province, South Africa) for fruit sides (equator blush, equator green, neck pooled for two sides and all sides). Segmented cross validation (SCV) was used with 70% of spectra as calibration set and 30% as validation set.

Farm	Fruit side	# of samples (cal/test)	Calibration			Validation		
			R^2	RMSECV	RMSEE	Bias	RMSEP	RPD
La Plaisante + Koelfontein (2013)	Equator blush	(133/57)	0.56	0.91	0.71	0.032	0.87	1.73
	Equator green	(133/57)	0.61	0.87	0.87	0.0043	0.88	1.53
	Neck	(262/116)	0.62	0.86	0.66	0.0032	0.85	1.73
	All	(527/232)	0.61	0.88	0.73	0.019	0.83	1.77
Fairfield + Oak Valley (2014)	Equator blush	(133/60)	0.82	0.63	0.37	-0.028	0.82	1.99
	Equator green	(132/60)	0.70	0.79	0.53	0.039	0.85	1.68
	Neck	(273/120)	0.72	0.79	0.53	-0.016	0.97	1.68
	All	(552/240)	0.70	0.82	0.64	-0.047	0.95	1.69
La Plaisante + Oak valley + Fairfield	Equator blush	(203/90)	0.78	0.68	0.52	0.00026	0.76	2.12
	Equator green	(205/90)	0.69	0.82	0.63	0.015	0.87	1.86
	Neck	(407/180)	0.72	0.77	0.65	-0.0091	0.94	1.75
	All	(826/360)	0.68	0.83	0.73	-0.011	0.91	1.76
La Plaisante + Koelfontein + Oak valley + Fairfield	Equator blush	(268/118)	0.75	0.78	0.55	-0.0048	0.77	2.17
	Equator green	(267/118)	0.69	0.87	0.60	0.02	0.87	1.89
	Neck	(541/236)	0.72	0.82	0.71	0.0019	0.88	1.87
	All	(1087/472)	0.66	0.91	0.78	-0.061	0.90	1.83

Table 4. Model performance for Fourier transformed near infrared (FT-NIR) total soluble solids (TSS) validation models using spectra of 'Forelle' pear fruit from independent farms and harvest seasons (La Plaisante, Koelfontein (2013), Fairfield and Oak Valley (2014), in the Western Cape Province, South Africa) for various fruit sides (equator blush, equator green, neck pooled for two sides and all sides). Models made using 2013 spectra were used to predict 2014 spectra and vice versa. Test set validation was used as a validation technique.

| Farm	Fruit side		LVs	R^2	RMSECV	RMSEP	Bias	RPD
La Plaisante + Koelfontein (2013)	Equator blush	(Cross Val)		0.52	0.84		-0.0014	1.44
		(T/Set val-Oak Valley)	9	0.60		0.96	0.026	1.59
	Equator green	(Cross Val)		0.71	0.778		-0.036	1.85
		(T/Set val-Oak Valley)	6	0.58		0.99	-0.29	1.62
	Neck	(Cross Val)		0.70	0.79		-0.0017	1.76
		(T/Set val-Oak Valley)	9	0.28		1.3	-0.66	1.37
	All	(Cross Val)		0.67	0.81		-0.011	1.75
		(T/Set val-Oak Valley)	8	0.05		1.49	0.89	1.28
Fairfield + Oak Valley (2014)	Equator blush	(Cross val)		0.84	0.61		-0.0061	2.48
		(val-La Plaisante	1	0.11		1.42	0.05	1.07
	Equator green	(Cross val)		0.76	0.73		-0.03	2.07
		(val-La Plaisante	1	0.14		1.4	0.056	1.08
	Neck	(Cross val)		0.78	0.71		0.00088	2.15
		(val-La Plaisante	1	0.34		1.23	-0.84	1.7
	All	(Cross val)		0.75	0.77		-0.023	1.99
		(val-La Plaisante	1	0.11		1.43	0.16	1.07
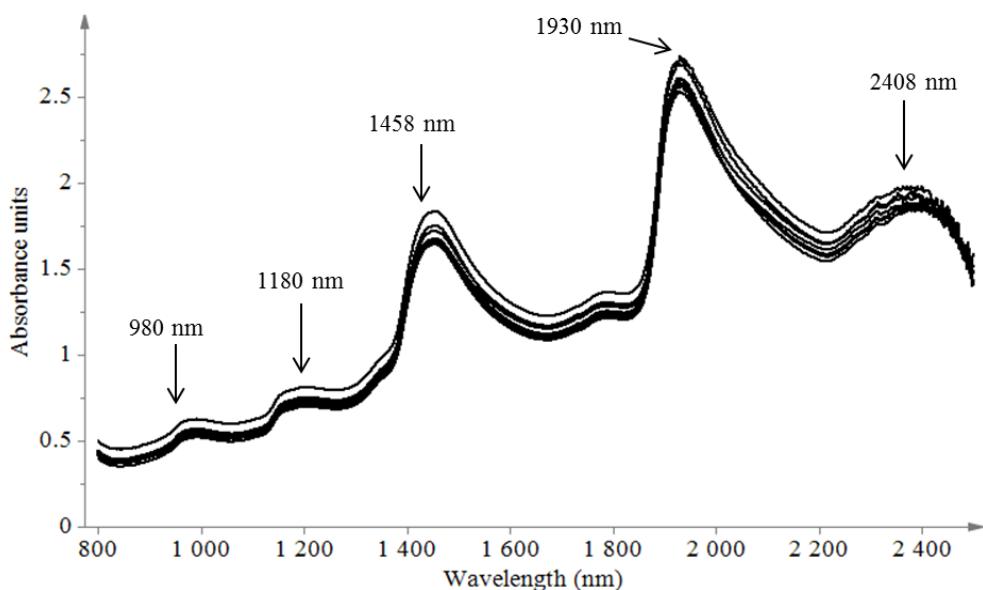
Fig. 1. Typical Fourier transformed near infrared (FT-NIR) absorbance spectra of 'Forelle' pear showing the whole spectral band between 800 and 2500 nm.
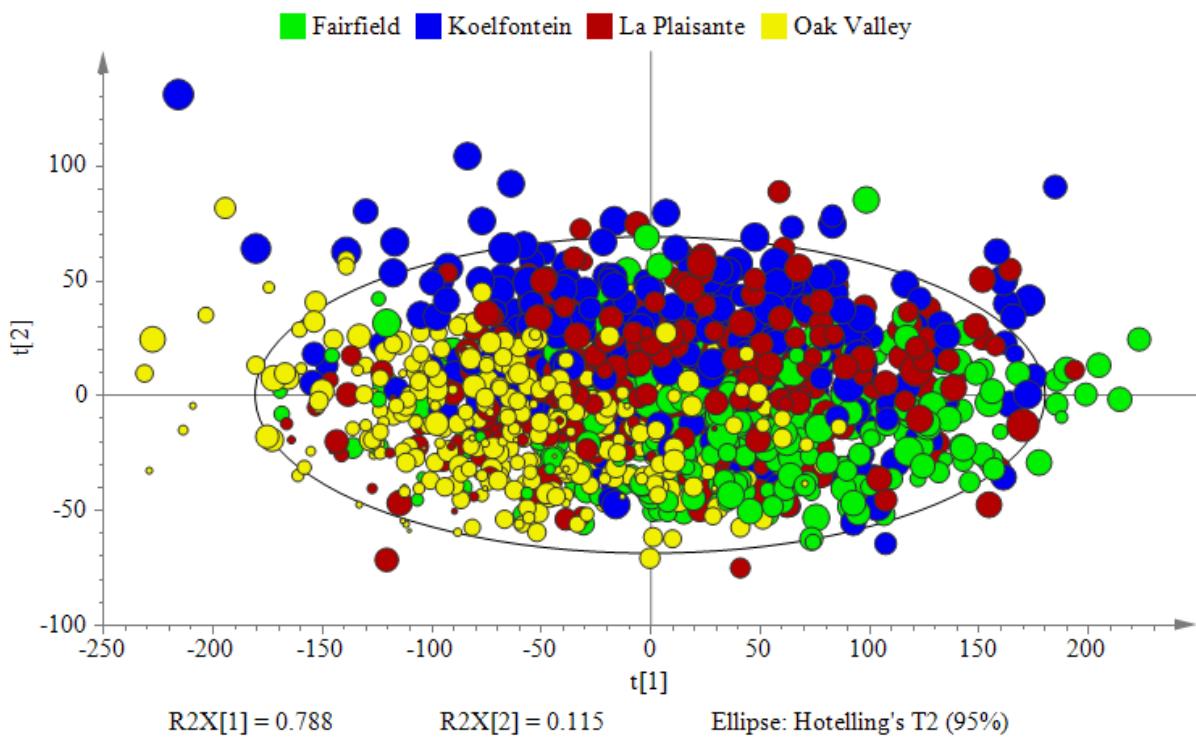
Fig. 2. Principal component analysis (PCA) scores plots for all the Fourier transformed near infrared (FT-NIR) absorbance spectra measured on 'Forelle' pears harvested from Koelfontein and La Plaisante in 2013 and from Fairfield and Oak Valley in 2014. Spectra were depicted with circles of sizes varying according to the actual measured TSS values.

169
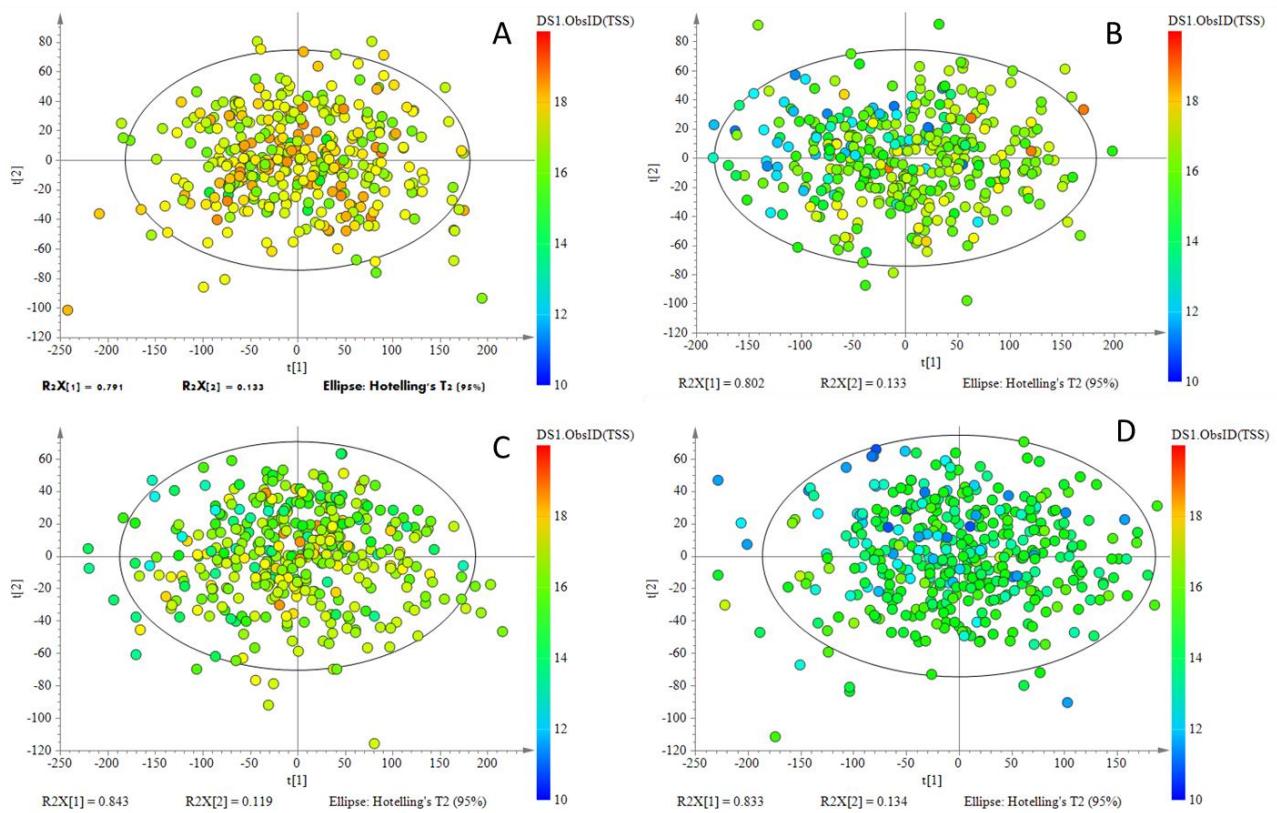
Fig. 3. Principal component analysis (PCA) score plots for all the Fourier transformed near infrared (FT-NIR) spectra measured on 'Forelle' pears from Koelfontein (A), La Plaisante (B), Fairfield (C), and Oak Valley (D). The spectra in the scores plots are depicted with circles with a colour scale varying according to actual TSS values. High TSS values (18+%) are depicted as red-orange and low TSS values (<12%) are depicted as blue, respectively.

170
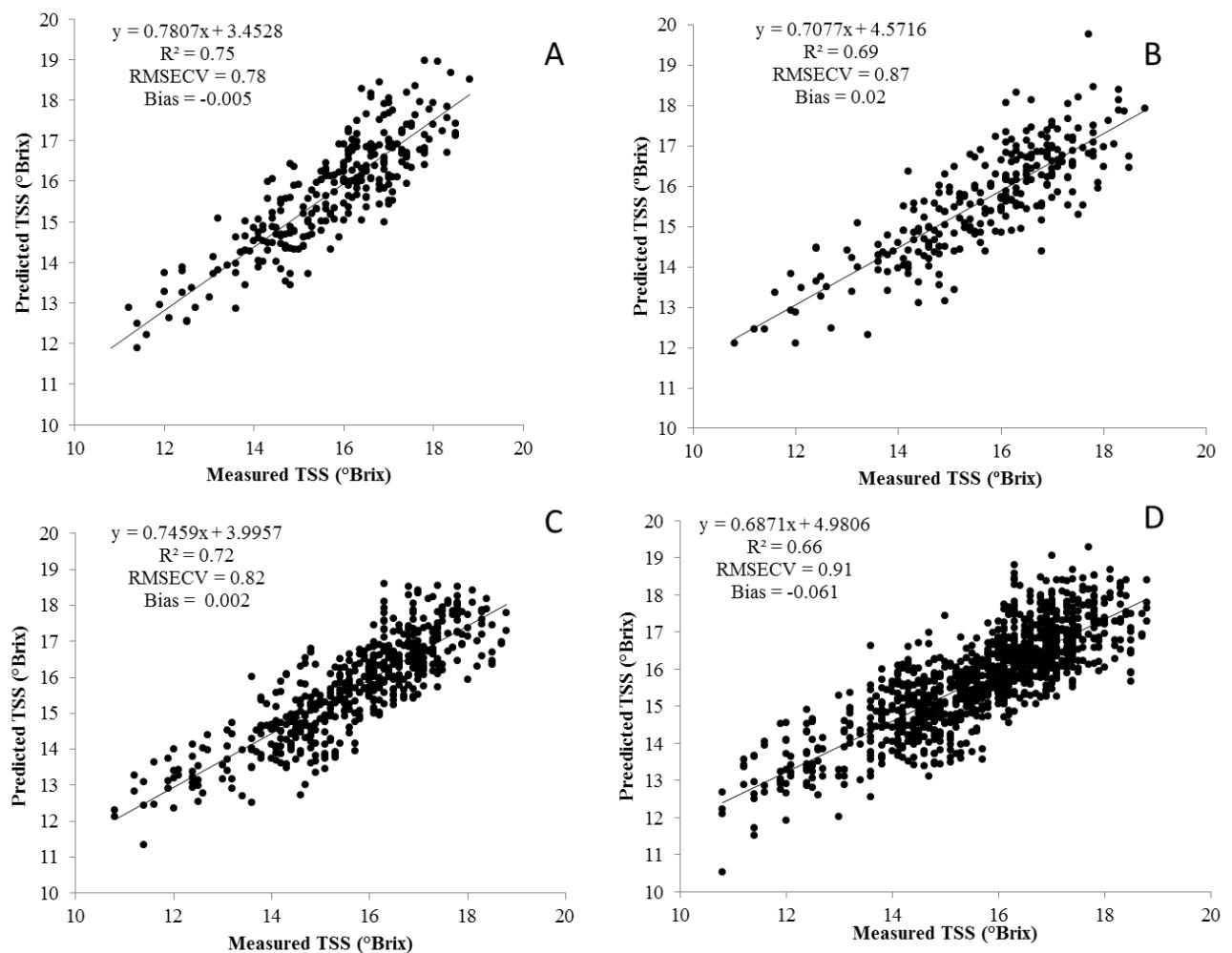
Fig. 4. Fourier transformed near infrared (FT-NIR) spectral predicted vs. actual total soluble solids (TSS) of 'Forelle' pear fruit from data combined for all sides (Koelfontein and La Plaisante (2013); Fairfield and Oak Valley (2014)). A) Using fruit spectra from the equator blush side, B) Using spectra from the equator green side C) Using spectra from the neck (both sides) D) Using all spectra.

171
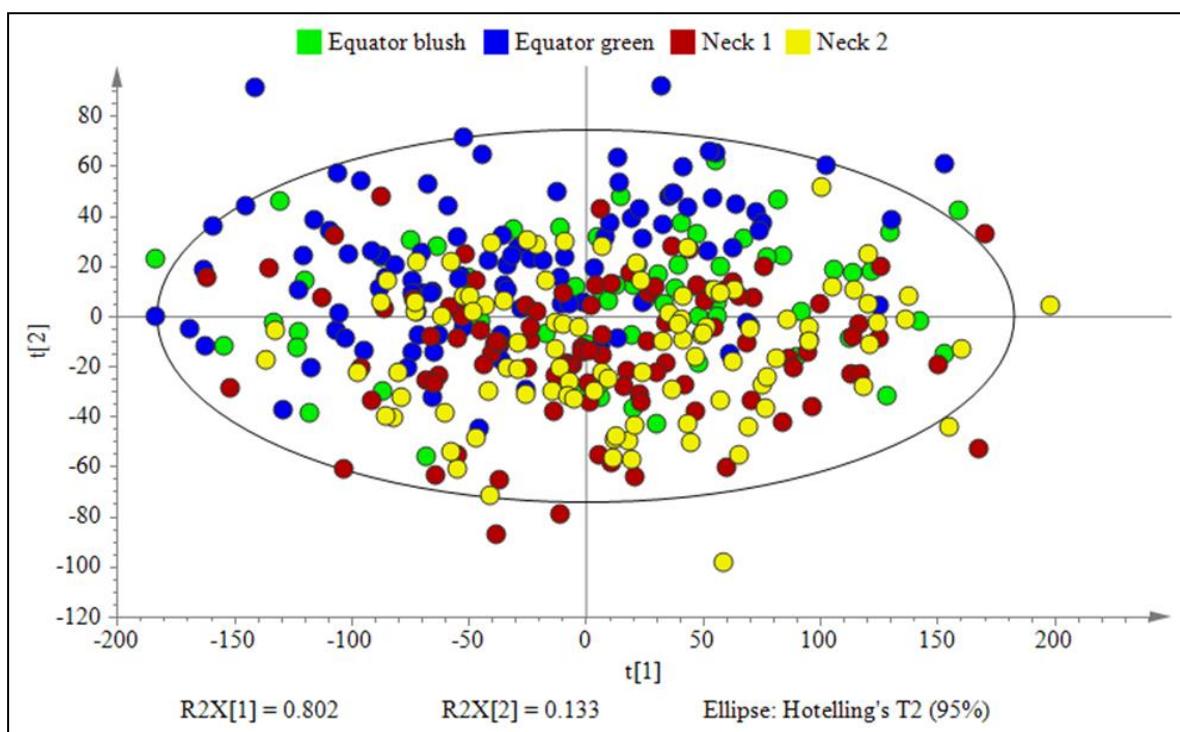
Fig. 5. PCA scores plot showing Fourier transformed near infrared (FT-NIR) spectra measured on 'Forelle' pears from La Plaisante showing separation due to side of the fruit where spectra were taken.

Supplementary Tables:

Table 1. Performance of Fourier transformed near infrared (FT-NIR) total soluble solids (TSS) calibration models developed using spectra from La Plaisante, Koelfontein (2013), Fairfield and Oak Valley (2014) farms for various fruit sides (equator blush, equator green, neck pooled for two sides and all sides). Models were done using cross validation. No test set validation was done due to limited number of spectra. (MSC pre-processed spectra)

Farm	Fruit side	# of samples	Number of factors	R^2	RMSECV	RPD_c	Bias	RMSEE	RPD
La Plaisante (2013)	Equator blush	92	8	0.81	0.64	2.28	0.0022	0.33	4.66
	Equator green	97	4	0.62	0.91	1.62	-0.011	0.86	1.75
	Neck	189	8	0.63	0.90	1.65	0.012	0.57	2.68
	All	387	9	0.68	0.85	1.76	0.04	0.67	2.26
Koelfontein (2013)	Equator blush	93	1	-0.07	0.71	0.97	-0.008	0.69	1.00
	Equator green	92	1	-0.03	0.71	0.98	-1.43E-005	0.70	1.02
	Neck	184	2	-0.02	0.71	0.99	-0.0028	0.70	1.01
	All	362	3	0.01	0.69	1.01	-0.004	0.67	1.04
Fairfield (2014)	Equator blush	97	7	0.56	0.74	1.5	0.0089	0.38	3.09
	Equator green	67	4	0.34	0.90	1.23	-0.019	0.77	1.48
	Neck	190	7	0.53	0.74	1.46	-0.02	0.49	2.27
	All	379	9	0.55	0.73	1.49	-0.054	0.56	1.97
Oak Valley (2014)	Equator blush	100	8	0.70	0.67	1.84	0.024	0.324	3.98
	Equator green	98	3	0.50	0.83	1.42	-0.037	0.74	1.6
	Neck	191	9	0.64	0.72	1.64	0.013	0.45	2.75
	All	391	9	0.60	0.78	1.68	-0.0014	0.60	2.07

RPD_c – relative prediction deviation (calibration)

Table 2. Performance of Fourier transformed near infrared (FT-NIR) total soluble solids (TSS) calibration models developed using combined data from the four farms harvested over two seasons (La Plaisante, Koelfontein (2013), Fairfield and Oak Valley (2014)) for various fruit sides (equator blush, equator green, neck pooled for two sides and all sides). Segmented cross validation (SCV) was used with 70% of spectra as calibration set and 30% as validation set. (MSC pre-processed spectra).

Farm	Fruit side	# of samples (cal / val)	Calibration				Validation	
			R^2	RMSECV	RMSEE	Bias	RMSEP	RPD
La Plaisante + Koelfontein (2013)	Equator blush	(133/57)	0.56	0.90	0.61	-0.066	0.87	1.73
	Equator green	(133/57)	0.50	0.95	0.56	0.06	0.95	1.42
	Neck	(262/116)	0.64	0.83	0.61	-0.018	0.90	1.64
	All	(527/232)	0.59	0.90	0.73	-0.029	0.9	1.63
Fairfield + Oak Valley (2014)	Equator blush	(133/60)	0.86	0.57	0.32	-0.0075	0.83	2.01
	Equator green	(132/60)	0.69	0.80	0.477	0.00121	0.85	1.68
	Neck	(273/120)	0.71	0.80	0.54	-0.0225	0.95	1.69
	All	(552/240)	0.67	0.85	0.67	-0.046	0.91	1.74
La Plaisante + Oak valley + Fairfield	Equator blush	(203/90)	0.84	0.59	0.393	-0.0171	0.77	2.09
	Equator green	(205/90)	0.74	0.75	0.52	-0.0064	0.91	1.79
	Neck	(407/180)	0.71	0.77	0.64	-0.0075	0.89	1.83
	All	(826/360)	0.66	0.87	0.76	-0.025	0.91	1.76
La Plaisante + Koelfontein + Oak valley + Fairfield	Equator blush	(268/118)	0.77	0.75	0.55	0.0222	0.78	2.12
	Equator green	(267/118)	0.68	0.87	0.60	-0.0163	0.89	1.85
	Neck	(541/236)	0.73	0.81	0.70	-0.0023	0.86	1.92
	All	(1087/472)	0.66	0.91	0.78	0.00865	0.88	1.86

GENERAL DISCUSSION AND CONCLUSIONS

Rising incomes and health education in most developed and developing countries have led to an increase in the number of consumers who are conscious of the foods they consume, particularly foods claimed to have health benefits such as fruits (Shewfelt, 2006; Huang, 2010). Internal quality and health benefits now play an increasing role in consumer choices of fruit and vegetables. Forelle (*Pyrus communis* L.) is the second most exported pear cultivar from South Africa (HORTGRO, 2014). The planted area of 'Forelle' has grown significantly in the last decade and volumes of exports have risen too (HORTGRO, 2014; Steenkamp, 2014). 'Forelle' owes its prominence to its exceptional blush, pear flavour and buttery texture when ripened (Crouch, 2011; Manning, 2009). However, 'Forelle' pears grown in South Africa are prone to mealiness, a textural disorder which causes fruit to lose juiciness during ripening at room temperature following cold storage (Crouch, 2011; Hurndall, 2011). The disorder is frequently associated with fruit harvested post-optimally and / or to fruit that has not been stored for a minimum period of 12 weeks at -0.5 °C (Carmichael, 2011; Crouch et al., 2005; Martin, 2002). Research on factors causing mealiness in 'Forelle', as well as practices to reduce mealiness, have been done in more than two decades, yet factors causing it are still poorly understood. Factors that are limiting to research on mealiness of 'Forelle' include that the condition cannot be detected from outside the fruit, but only after destructive assessment. Secondly, no biochemical cue has been directly linked to mealiness.

Previous research and reviews contend that mealiness in fruit may be in part a result of failure of cells to rupture at the cell wall leading to cell separation at the middle lamella (Barreiro et al., 1998; Harker and Hallet, 1992; McAtee et al., 2009). What is not clear is the mechanism responsible for the failure by cells to yield juice upon mastication. Research into factors leading to mealiness is complicated by the fact that mealiness does not show in the early stages of the value chain but rather manifests itself in the hands of the consumer. Unlike in apples where mealiness can be induced by applying room temperature (20 °C) and high relative humidity (95%) (Barreiro et al. 1998), in 'Forelle' pears no condition has yet been discovered that can induce mealiness. There is also no biomarker that can be used to guide whether or not mealiness will develop or environmental model linking any factors to mealiness. The current research was therefore carried out to shed more light on mealiness. The research was divided into two broad sections. The first section was aimed at enhancing our understanding of the biochemistry of mealiness while the second section sought to find

175

non-destructive methods of mealiness determination. The first section investigated the role of cell wall bound Ca^{2+}, cell sizes and cell numbers on the development of mealiness in 'Forelle' pear. In the second section, two non-destructive methods of mealiness determination, near infrared (NIR) spectroscopy and X-ray computed tomography (X-ray CT) were examined for the detection of mealiness in 'Forelle' pears. In summary, our research aimed to answer the following questions: i) Does the level of cell wall bound Ca^{2+} affect mealiness development in 'Forelle' pears? ii) Is mealiness a consequence of cell number and cell size variation among fruit? iii) Can NIR and X-ray CT be used to detect mealiness in 'Forelle' non-destructively?

A literature review was undertaken in order to gain understanding of mealiness. The literature review examined the history of mealiness in 'Forelle', drawing parallels from mealiness studies done in other fruit. The review showed that previous research has concentrated mainly on understanding the biochemical and physiological basis of mealiness. Studies have been done on the role of cell wall enzymes, particularly polygalacturonase (PG) and pectin methyl esterase (PME) (Obenland and Carroll, 2000; Villalobos-Acuna and Mitcham, 2008), the role of ethylene (Zhou et al., 2001) and pectin (Brummell et al., 2004; Crouch, 2011; Hobbs et al., 1991; Murayama et al., 2006; Villalobos-Acuna and Mitcham, 2008). Environmental factors such as maximum temperatures 6 weeks prior to harvest (Mellenthin and Wang, 1976), harvest maturity (Murayama et al. 1998), and storage duration and condition (Chen et al., 1983; Murayama et al., 2002) played a role in mealiness development in other pears. Research on 'Forelle' mealiness has focussed on temperatures 6 weeks prior to harvest (Crouch et al., 2005) which did not seem play a role, harvest maturity (Carmichael, 2011), fruit position in the canopy (Cronje et al., 2015), storage temperature, ethylene treatments (Martin, 2002), ripening (Crouch, 2011; Martin et al., 2003) and cell wall compositional differences between mealy and non mealy fruit (Crouch, 2011) which did play a role in mealiness incidence. The review also revealed that while a significant amount of research has been done on apples, peaches and nectarines, pear mealiness research has received relatively little attention. As a result, there is very little information relating to mealiness in general and 'Forelle' mealiness in particular.

The review was followed by experimental studies based on the objectives outlined above. The first experiment was aimed at establishing the role played by Ca^{2+} in the development of mealiness in 'Forelle' pears (Paper 1). Calcium plays a vital role in plant cells and in maintaining structural integrity in plant tissues and organs (Hepler and Winship 2010).

176

Calcium is involved in cell to cell communication as well as communication between cytoplasm and the extracellular environment (Decrock et al., 2011). Ca^{2+} also binds with phospholipids and proteins at the membrane surface, thereby reinforcing the cell membrane structure (De Freitas et al., 2010). A link between Ca^{2+}, the cell wall and mealiness has been reported in previous studies on peach and apple (Mignani et al., 1995; Saftner et al. 1998). Three fractions of Ca^{2+} were extracted sequentially. The extracted Ca^{2+} fractions were the alcohol soluble Ca^{2+} fraction, water soluble Ca^{2+} fraction which together with the alcohol soluble fraction was called the available free Ca^{2+}. The alcohol soluble fraction represents the Ca^{2+} which is readily released from the cell wall, including that contained in cell compartments. This fraction represents the labile Ca^{2+} fraction which plays a role in maintenance of membrane integrity and cell function (De Freitas and Mitcham, 2012). The last fraction extracted was the Ca^{2+} bound in the cell wall, measured on the cell wall residue by acid digestion. There was an interaction of orchard location and mealiness on the three Ca^{2+} fractions. Free Ca^{2+} constituted about 49-73% of the total cell Ca^{2+}, representing a significant fraction of cell Ca^{2+}. Therefore, any change in the concentration of this Ca^{2+} fraction may inevitably affect the cell integrity, movement of Ca^{2+} to and from the cell wall, as well as enzymatic reactions (De Freitas et al., 2010; Caffall and Mohnen, 2009; Hepler and Winship, 2010). In mealy fruit, the average concentration of free Ca^{2+} was 61% while non-mealy fruit had 66% free Ca^{2+}. In some orchards, mealy fruit had lower total Ca^{2+} compared to non-mealy fruit, although the averages for all locations were not significantly different. It can be argued that low total Ca^{2+} at these locations could have led to low available Ca^{2+} hence mealiness. Interventions that would improve total Ca^{2+} and thus also free Ca^{2+} may aid in reducing mealiness and therefore future studies should focus on Ca^{2+} sprays during the cell division phase (early stages of fruit growth), when uptake of Ca^{2+} into fruit is at its maximum (Wilkinson and Perving, 1964). Radiolabeled Ca^{2+} may also aid in tracking the movement of Ca^{2+} within a cell during ripening (Saftner et al., 1997). This method, when used at various storage and ripening intervals could aid in explaining the movement of Ca^{2+} as a result of ripening or further storage. Non-destructive methods in determining fruit Ca^{2+} of intact fruit would be particularly useful in such a study, however no non-destructive Ca^{2+} determination techniques are currently known. The link between free Ca^{2+} and mealiness in 'Forelle' as well as its role in cell-to-cell debonding in this pear is therefore still unclear and should be further explored.

The second experiment (Paper 2), investigated the influence of cell size and cell numbers on mealiness. Plant growth regulator treatments; 2,4-dichlorophenoxy acetic acid (2,4-D) amine salt formulation (Amine), gibberellin$_{4+7}$, prohexadione-Ca, forchlorfenuron and their combinations were applied in 2011 in four orchards (three from Ceres and one located in Wolseley, Western Cape), in order to incite variations in cell size and numbers. Plant growth regulators play various roles in fruit, in fruit and seed development, signalling and regulating processes such as cell proliferation, differentiation and cell expansion (Ozga and Reinecke, 2003; Theron, 2011; Dreyer, 2013). In this study, comparisons were made of the incidences of mealiness among the different plant growth regulator treatments and also the influence of treatment application on cell sizes and numbers. Results showed that cell diameter and cell volume were significantly affected by treatment application. Forchlorfenuron and the combination of gibberellins$_{4+7}$ + prohexadione-Ca caused an increase in cell size while 2,4-D Amine and the combination of prohexadione-Ca, gibberellins$_{4+7}$ and forchlorfenuron produced cells with sizes comparable to the control. However, the effects of the combined plant growth regulators were variable, probably due to the combined or independent effects of the different plant growth regulators in a specific orchard (Coenen and Lomax, 1997). Correlation studies showed a very weak but positive linear relationship between cell diameter and mealiness and a similar relationship between cell volume and mealiness. These data could statistically be further explored as taking out the outlier farm improved the correlation significantly (r=0.6, data not shown). Correlations with mealiness over various locations need to be carefully considered as mealiness may increase and decrease over the ripening time (Martin, 2002) and the ripening rate of fruit from each location may have varied. These studies should therefore perhaps be ideally done over ripening days to establish the point of maximum mealiness for each farm. Fruit from treatments that produced larger cells were found to be more susceptible to mealiness. Various explanations have been put forward to explain the high incidences of mealiness in larger celled fruit. Some argue that because of the large cell size, cell contact area between neighbouring cells is reduced making the cells prone to cell-to-cell debonding during ripening (Crouch, 2011; Harker and Hallett, 1992), resulting in tissue breakdown (Carpita and McCann, 2000). Other authors argue that as cells enlarge, vacuoles tend to occupy a greater proportion of the cell volume, compromising the load bearing capacity of the cell (Ozga et al., 2002), leading to tissue breakdown. In order to examine detailed microstructural differences between mealy and non mealy fruit, images of dried tissues taken at the end of shelf life from the hypanthium of mealy and non mealy fruit were examined through visualisation for features that could be peculiar to mealy fruit.

Mealy fruit had large intercellular air spaces, corroborating findings in apples (McAtee et al., 2009). This may support the hypothesis that lower cell-to-cell adhesion may cause tissue failure at ripening and therefore mealiness. Results from this study suggest that practices that aim at improving yield through cell enlargement such as thinning (Theron, 2011; Zhang et al., 2007) may lead to increase in mealiness. However, this still needs further investigation.

Paper 3 explores whether mealiness of 'Forelle' pear could be detected in intact pears, using X-ray CT. The research was the first attempt at non-destructive determination of 'Forelle' pears using X-ray CT as a technique which utilises electromagnetic radiation ranging from 0.01 to 10 nm (Kotwaliwale et al., 2014). The technique is growing in use owing to its ability to provide microstructural detail without destroying the structure of the material (Mendoza et al., 2007), with little or no sample preparation or chemical fixation required as in techniques such as scanning electron microscopy. Mealiness development in 'Forelle' was visualised over the shelf life period using macro X-ray CT. Microstructural differences between mealy and non-mealy fruit were observed using micro X-ray CT. Fruit that develop mealiness were seen to have a higher percentage of defects or localised regions of darker voxels that are less dense at the end of cold storage. Mealy fruit had a higher fraction of defects in the neck of the fruit compared to the neck of non mealy fruit. This observation was made in macro CT scans and confirmed using micro CT scans. Cells of mealy fruit were observed to be larger and ellipsoidal in shape while non-mealy cells were smaller and more rounded. Contrary to previous explanations that separation of cells and tissues in mealiness occurs during mastication or when a force is exerted (Harker and Hallet, 1992), our study showed that cell separation occurs well before fruit is cut, during shelf-life but also after storage. Lastly, our results have demonstrated the potential ability of X-ray computed tomography to determine mealiness non-destructively. To our knowledge, this was the first time that mealiness differences could be seen before ripening on 'Forelle' pears.

Papers 4 and 5 report investigations of Fourier transformed-near infrared spectroscopy (FT-NIR) as a non-destructive method of mealiness detection in 'Forelle' pears. FT-NIR is growing in use as a technology for non-destructive investigation of agricultural produce (Alander et al., 2013; Bobelyn et al., 2010; Nicolai et al., 2007, Magwaza et al., 2012; Wang et al., 2015). It has advantages in food evaluation as the NIR spectra are related to overtones and combination bands of chemical bonds such as C-H, O-H, and N-H, which have an influence in most foods (Alander et al., 2013, Louw and Theron, 2013). Studies were carried

out over two seasons (2013 and 2014). Near infrared spectral data were measured at four positions on each fruit at harvest and after shelf life. Measurement of the four positions was done to ensure validity if used in online grading where fruit may roll without maintaining one side and also to account for the variations in physicochemical attributes within the fruit.

Paper 4 describes the steps that were taken to determine if NIR spectra could distinguish between mealy and non-mealy fruit. Prior to analysing near infrared data, principal component analysis was done on maturity indices and other physicochemical attributes in order to determine parameters that relate to mealiness. Total soluble solids (TSS) content and TSS: titratable acidity (TA) ratio were found to be closely related to mealy 'Forelle' pears. TSS together with sensory analysis results were therefore used in supervised discriminant analysis to distinguish mealy from non-mealy fruit (Paper 4). Discriminant analysis was carried out using orthogonal partial least squares discriminant (OPLS-DA). Models produced had classification accuracies of 51%-95%. The best models were found when spectra from the equator blush were used. Examination of spectra showed that mealiness causes an increase in transmittance in specific regions of spectra. These shifts in spectra could be a result of scattering caused by changes in physicochemical characteristics in fruit related to mealiness (Piers et al., 2000). This study showed that FT-NIR spectra can be used to discriminate between mealy and non-mealy 'Forelle' pears, with room for further improvement with appropriate variable selection and pre-processing. To our knowledge, this study is the first discrimination study on pear mealiness which used FT-NIR spectra. The only other study was on apples by Huang and Lu (2010) which used hyperspectral imaging. Results of our study compare favourably with results from this study.

In the second NIR study (Paper 5), FT-NIR spectroscopy was evaluated for the quantification of TSS in 'Forelle' pears. Partial least squares (PLS) calibration models were built using spectra of fruit from each individual farm and then using spectra from combined farms. Independent calibration models were made for spectra from the equator blush, the equator green and two neck positions opposite each other, in order to cater for spatial variations within the fruit. Validated calibration models had root mean square error of cross validation (RMSECV) value ranges of 0.63-0.91, while validation models had root mean squared error of prediction (RMSEP) values of 0.76-0.94. Like in other FT-NIR studies with fruit from different geographical locations, model performance in this study varied. This was attributed to biological variability due to location, season and side of fruit where spectra were taken

(Bobelyn et al., 2010). PCA confirmed the variation in spectra due to location and position on fruit where spectra were acquired.

The study reveals a link between TSS and mealiness, which is further supported by the finding of Cronje et al. (2015) that outside canopy fruit with a higher TSS had a higher incidence of mealiness. It is unclear whether the relation of TSS to mealiness is due to riper pears at harvest or whether it is due to a carbon source sink relation with the outside canopy fruit having larger cell sizes resulting in a more porous structure compared to inside canopy fruit. This mechanism should therefore be further explored. Further studies could also determine whether other parameters shown by PCA to correlate with mealiness (juiciness and hue angle) could be used as biochemical markers and further non-destructively explored. Results from the two NIR studies showed the potential of using NIR for prediction of mealiness and TSS in 'Forelle' pears. Future studies are recommended on calibrations with 'Forelle' from wide geographical origins in South Africa over different seasons in order to increase variability in the samples and therefore robustness of these models.

In summary, the current study was an integrated study of mealiness, based on three major hypotheses, viz: (i) cell size and cell numbers have an influence on 'Forelle' mealiness development, (ii) the composition of cell wall free and cell wall bound calcium have an influence on mealiness development, and (iii) non-destructive techniques such as NIR spectroscopy and X-ray computed tomography have potential for identifying and predicting mealiness. Cell wall calcium determination, scanning microscopy, sensory mealiness determination, near infrared spectroscopy and X-ray computed tomography studies were carried out on 'Forelle' pear. The studies were preceded by a review of literature on mealiness in 'Forelle' and other related crops. The review showed that there is paucity of information on the mechanism of mealiness development, particularly on 'Forelle'. Cellular studies showed that mealiness was promoted by high cell wall free Ca^{2+} and was positively correlated with cell sizes. Possibly, rapid growth of mealy fruit cells may outpace calcium accumulation into bound forms in the cell wall, hence cells fail to form strong intercellular bridges, in turn resulting in mealiness. There were however, no differences in cell bound Ca^{2+}. Through scanning electron microscopy imagery and X-ray CT, mealy fruit were shown to have larger cells and large intercellular spaces. In addition, areas of large cell separation (lysigenous intercellular spaces), attributable to crumbling cells or even cell death were shown by X-ray CT. Previous studies have shown only schizogenous type of cell separation (Harker and Hallet, 1992). Lysigenous pore formation observed in this study was attributed to

cell death or cell shrinkage due to water loss. Our findings further show that cells are already separated in severe mealiness cases before any force is exerted as opposed to claims by other studies (Harker and Hallet, 1992). Interestingly, NIR spectroscopy was shown to be capable of discriminating between mealy and non-mealy fruit. NIR spectra are influenced by both absorption due to dissolved biocomponents such as TSS and also by scattering due to tissue macrostructure (Alander et al., 2013; Rinnan et al., 2009). Because mealy fruit have differences in both tissue structure as evidenced by micro CT scans and biochemistry as is seen in TSS differences, this is possibly the reason why it was readily possible to identify mealy fruit using NIR. Although our results were not conclusive in determining the link between mealiness and TSS, we managed to establish that indeed mealy fruit had higher TSS compared to non-mealy fruit and also that both mealiness and TSS can be predicted by NIR. Overall, the study enabled us a better understanding of mealiness and provided clues for future research.

A number of recommendations for further studies on promising technologies and applications were identified. The study also highlighted areas where limitations were observed and recommenations for future studies made. For example, our NIR study used a limited number of fruit and orchards, which influenced the model accuracy due to a limited range of TSS. Secondly, our sampling for TSS was done on equatorial slices, representing both the sunny and shaded sides of the fruit as is done commercially. This was, however, not specific to the point where FT-NIR spectra was acquired. Future studies are recommended to take TSS or the biochemical marker of interest on the position or closer to the position of NIR acquisition to cater for gradient within the fruit. There is need for further analysis of spectra at harvest to establish if TSS and mealiness could be predicted already at harvest. Models could perhaps be improved with relevant wavelength selection. Research is also recommended on the use of hand held spectrometers directed at specific sides of the fruit such as the equator blush. The accuracy of online commercial spectrometer models should be explored where spectral acquisition side may be less accurate but more locations can be used, which may improve the model accuracy. Other recommendations coming from this study include the need for further X-ray CT studies on pre-harvest fruit porosity to elucidate in order to determine if mealiness is present prior to harvest and whether it is related to environmental or field factors.

Literature Cited

Alander, J.T., Bochko, V., Martinkauppi, B., Saranwong, S., Mantere, T., 2013. A review of optical nondestructive visual and near infrared methods for food quality and safety. Int. J. Spectrosc. Hindawi Publishing Corporation. http://dx.doi.org/10.155/ 2013/341402.

Barreiro P., Ortiz, C., Ruiz-Altisent M., De Smedt V., Schotte S., Andani Z., Wakeling I., Beyts PK., 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples: a collaborative experiment. J. Texture Stud. 29, 509-525.

Barreiro, P., Ortiz, C., Ruiz_Altisent M., Ruiz_Cabello, J., Fernandez_Valle, M. E., Recasens, I., Asensio, M., 2000. Mealiness assessment in apples and peaches using MRI techniques. Magn. Reson. Imaging. 18, 1175-1181.

Bobelyn, E., Serban, A.S., Nicu, M., Lammertyn, J., Nicolai, B.N., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133-143.

Caffall, K.H., Mohnen, D., 2009. The structure, function, and biosynthesis of plant cell polysaccharides. Carbohydr. Res. 344, 1879-1900.

Carmichael, P.C., 2011. Effect of fruit maturation and ripening potential for optimum eating quality of 'Forelle' pears. MSc. Agric. Thesis, University of Stellenbosch, Department of Horticultural Sciences. Stellenbosch. South Africa.

Carpita N., McCann M., 2000. The cell wall. In: Biochemistry and Molecular Biology of Plants. Chapter 2, 52-108. Eds. Buchanan B.B., Gruissem, W. and Jones, R.L., Amer. Soc. Plant Physiologists, Rockville, Maryland.

Chen, P.M., Mellenthin, W.M., Borgic, D.M., 1983. Changes in ripening behavior of 'd'Anjou' pears (*Pyrus communis* L.) after cold storage. Scientia Hort. 21, 137-146.

Coenen, C., Lomax, T.L., 1997. Auxin-cytokinin interactions in higher plants: old problems and new tools. Trends Plant Sci. 2, 351-356.

Cronje, A., Crouch, E.M., Muller, M., Theron, K.I., van der Rijst, M., Steyn, W.J., 2015. Canopy position and cold storage duration affects mealiness incidence and consumer preference for the appearance and eating quality of 'Forelle' pears. Sci. Hortic. 194, 327-336.

Crouch, E.M., 2011. Cell wall compositional differences between mealy and non-mealy 'Forelle' pear (*Pyrus communis* L). PhD Thesis. Stellenbosch University.

Crouch, E.M., Holcroft D.M., Huysamer, M., 2005. Mealiness of 'Forelle' pears_qouvadis? Acta Hort. 671, 369-376.

De Freitas, S.T., Do Amarante, C.V.T., Labavitch, J.M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biol. Technol. 57, 6-13.

De Freitas, S.T., Mitcham, E.J., 2012. Factors involved in fruit calcium deficiency. Hortic. Rev. 40, 107-145.

Decrock, E., Vinken, M., Bol, M., D'Herde, K., Rogiers, V., Vandenabeele, P., Krysko, D.V., Bultynck. G., Leybaert, L., 2011. Calcium and connexin-based intercellular communication, a deadly catch? Cell Calcium 50, 310-321.

Dreyer, C., 2013. Fruit set and fruit size studies on 'Forelle' and 'Abate Fetel' pear (*Pyrus communis*. L). MSc (Agric) thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. Hort. Sci. 27, 1291-1294.

Hepler K.P., Winship, L.J., 2010. Calcium at the cell wall cytoplast interface. J. Integr. Plant Biol. 52, 147-160.

Hobbs, M.C., Easterbrook, K.M., Melton, L.D., 1991. Cell wall material composition of mealy fruit among ripening nectarines. J. Sci. Food Agr. 57, 141-145.

HORTGRO, 2014. Key Deciduous Fruit Statistics, Paarl, South Africa, pp 18-24.

Huang, M., Lu, R., 2010. Apple mealiness detection using hyperspectral scattering technique. Postharvest Biol. Technol. 58, 168-175.

Huang, S., 2010. Global trade of fruit and vegetables and the role of consumer demand. In: Hawkes, C., Blouin, C., Henson, S., Drager, N., Dubé, L., 2010. Trade, Food, Diet and Health: Perspectives and Policy Options. John Wiley and Sons, UK. Pp 60-67.

Hurndall, R.F., 2011. 'Forelle' dispensation procedures for 2011 season. http://www.decidous.co.za (accessed 10-09-2014).

Kotwaliwale, N., Singh, K., Kalne, A., Jha, S.N., Seth, N., Kar, A., 2014. X-ray imaging methods for internal quality evaluation of agricultural produce. J. Food. Sci. Technol. 51 (1), 1-15.

Louw, D.E and Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina*), using NIR spectroscopy. Postharvest Biol. Technol. 58, 176-184.

Magwaza, L.M., Opara, L.U., Nieuwoudt, H., Cronje, P.J., Saeys, W. Nicolai, B., 2012. NIR Spectroscopy applications for internal and external quality analysis of citrus fruit. Food Bioprocess Technol. 5, 425- 444.

Manning, N., 2009. Physical, sensory and consumer analysis of pear genotypes among South African consumers and preference of appearance among European consumers. MSc. Food Science. Thesis, University of Stellenbosch, Department of Food Science. Stellenbosch. South Africa.

Martin, E.M., 2002. Ripening responses of 'Forelle' pear. MSc (Agric) thesis. Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Martin, E.M., Crouch, I.J., and Holcroft, D.M., 2003. Ripening and mealiness of 'Forelle' pears. Acta Hort. 600, 449-452.

McAtee, P.A., Hallet, I.C., Johnston, J.W., Schaffer, R.J., 2009. A rapid method of fruit cell isolation for cell size and shape measurements. Plant Methods. 5 (5). 1-7.

Mellenthin, W.M., Wang, C.Y., 1976. Preharvest temperatures in relation to postharvest quality of 'd'Anjou' pears. J. Amer. Soc. Hort. Sci. 101, 302-305.

Mendoza, F., Verboven, P., Mebatsion, H.K., Kerckhofs, G., Wevers, M., Nicolai, B., 2007. Three-dimensional pore space quantification of apple tissue using X-ray computed microtomography. Planta. 226, 559-570.

Mignani, I., Greve, L.C., Ben-Arie, R., Stotz, H.U., Li, C., Shackel, K.A., Labavitch, J.M., 1995. The effect of GA_3 and divalent cations on aspects of pectin metabolism and tissue softening in ripening tomato pericarp. Physiol. Plant. 93, 108-115.

Murayama, H., Takahashi, T., Honda, R. and Fukushima, T., 1998. Cell wall changes in pear softening on and off the tree. Postharvest Biol. Technol. 14, 143-149.

Murayama, H., Katsumata, T., Horiuchi, O. and Fukushima, T., 2002. Relationship between fruit softening and cell wall polysaccharides in pears after different storage periods. Postharvest Biol. Technol. 26, 15-21.

Murayama, H., Katsumata, T., Endou, H., Fukushima, T. and Sakurai, N., 2006. Effect of storage period on the molecular mass distribution profile of pectic and hemicellulosic polysaccharides in pears. Postharvest Biol. Technol. 40, 141-148.

Obenland, D.M., Caroll, T.R. 2000. Mealiness and pectolytic activity in peaches and nectarines in response to heat treatment and cold storage. J. Amer. Soc. Hort. Sci. 125 (6), 723-728.

Ozga, J.A., Reinecke, D.M., 2003. Hormonal interactions in fruit development. J. Plant Growth Regul. 22, 73-81.

Ozga, J.A., van Huizen, R., Reineke, D.M., 2002. Hormone and seed-specific regulation of pear fruit growth. Plant Physiol. 128, 1379-1389.

Peirs, A., Lammertyn, J., Ooms, K., Nicolai, B.M., 2000. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. Postharvest. Biol. Technol. 21, 189-199.

Saftner, A.A., Conway, W.S., Sams, C.E., 1998. Effect of postharvest calcium chloride treatments on tissue water relations, cell wall calcium levels and postharvest life of 'Golden Delicious' Apples. J. Amer. Sci. Hort. Sci. 123 (5), 893-897.

Saftner, R.A., Buta, G., Conway, W.S., 1997. Effects of durfactants on pressure infiltration of calcium chloride solutions into 'Golden Delicious' apples. J. Amer. Soc. Hort. Sci. 122 (3): 386-391.

Shewfelt, R.L., 2006. Defining and meeting consumer requirements. Acta Hort. 712, 31-37.

Steenkamp, E., 2014. FEMA_ A blushing success. SA Fruit Journal. Aug/Sep 2014, 8-9.

Theron, K.I., 2011. Size matters: Factors influencing fruit size in pear. Acta Hort. 909, 545-556.

Villalobos-Acuna, M., Mitchan, E.J., 2008. Ripening of European pears: The chilling dilemma. Postharvest Biol.Technol. 49, 187-200.

Wang, H., Peng, J., Xie, C., Bao, Y., He, Y., 2015. Fruit quality evaluation using spectroscopy technology. A review. Sensors. 15, 11889-11927.

Wilkinson, B.G., Perving, M.A., 1964. Changes in the chemical composition of apples during development, nad near picking time. J. Sci. Fd. Agic. 15:146-152.

Zhang, C., Tanabe, K., Tan, H., Nakajama, H., Mori, M., Sakuro, E., 2007. Biologically active gibberellins and Abcissic acid in fruit of two late maturing Japanese pear cultivars with contrasting fruit size. J. Amer. Soc. Hort. Sci. 132 (4), 452-458.

Zhou, H., Dong, L., Ben‐Arie, R., Lurie, S., 2001. The role of ethylene in the prevention of chilling injury in nectarines. J. Plant Physiol. 158, 55-61.