DRY MATTER AND FRUIT QUALITY:
MANIPULATION IN THE FIELD AND EVALUATION WITH NIR SPECTROSCOPY

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Summary

Growers strive to improve the quality of fruit they produce, in terms of eating potential and its ability to store well after harvest. Assessment of quality is made via a set of recognised parameters, the two most important being firmness and soluble solids content (SSC). However fruit harvest dry matter (DM) is fast becoming a valuable indicator of quality, not only due to its relationship with postharvest SSC via starch hydrolysis, but also because it is a parameter based on fruit biology and is heavily affected by field conditions and cultivation practices.

Cultural techniques, such as root pruning, are increasingly employed to curtail shoot growth and to shift tree physiology in favour of fruit production. Root pruning modifies carbohydrate partitioning in trees and its translocation to developing fruit, so can influence harvest fruit quality. However it can also reduce fruit size, advance maturity and carries the risk of causing water and nutrient stress in developing fruit. Supplementary irrigation may help alleviate these side effects and improve harvest fruit quality. A move towards non-destructive methods of measuring fruit quality, such as near infrared (NIR) spectroscopy, is also fuelling interest in DM, as it is a technique ideally suited for analysis of samples with high carbohydrate content.

Results from the ‘Clara Frijs’ pear field experiment showed that root pruning advanced fruit maturity, increased DM and SSC at harvest while also reducing average fruit size and shoot length. The effects of root pruning on fruit DM were evident as early as 51 days after full bloom (DAFB). Root pruning also improved return bloom. In contrast, non-root pruned trees produced large fruit with low DM and SSC that did not improve in quality
during storage. Supplementary irrigation helped counter the effect of root pruning on fruit size, but it also reduced DM and SSC and had no clear effect on fruit maturity or shoot growth. Furthermore, the results from this experiment show a strong linear correlation between preharvest, harvest and postharvest fruit DM making it possible to reliably estimate fruit quality well in advance of harvest.

Results from the NIR spectroscopy experiments demonstrated that the complexity of absorbance spectra and the collinearity of DM and SSC are a problem for models, as they seem unable to easily distinguish between forms of carbohydrate. However variable selection proved a useful tool for highlighting subtle differences in prediction models for pear DM and SSC, particularly at longer wavelengths. The standard of prediction models for pear SSC were better than expected, especially considering starch was still present in the fruit. This raised questions concerning the influence of pear physiology, principally the presence of stone cells, on prediction model accuracy.

To conclude, the work presented here demonstrates that DM links many aspects of fruit husbandry. It remains a relatively stable parameter during preharvest fruit development, but it is also possible to manipulate its value in fruit by altering tree physiology. Together with the prospect of non-destructive prediction, these attributes combined have the potential to improve fruit production by providing an insight into the status of fruit as they develop on the tree and an idea of their potential quality at harvest and after storage.
**Sammendrag**

Producenter af æbler og pærer agerer i et stærkt konkurrencepræget marked, hvor en vigtig succesparameter er frugtens spisekvalitet og holdbarhed efter endt lagring. Frugtkvalitet bedømmes ud fra en række generelt accepterede målbare parametre, hvoraf fasthed og opløseligt tørstofinhold (OT) er de vigtigste. I de senere år har der været interesse for at undersøge tørstofindholdet (TS) som en kvalitetsparameter. Det skyldes, at TS er tæt knyttet til OT via hydrolyse af stivelse, men også at TS er en parameter der relaterer sig til frugters biologi og som kan påvirkes af dyrkningsforhold og dyrkningstekniske tiltag.


Resultater fra forsøgene med NIR spektroskopi viste at det er vanskeligt at udvikle modeller der kan skelne mellem forskellige former for kulhydrat fordi absorptionsspektrerne er meget komplekse og der er et stort sammenfald mellem målinger af TS og OT. Det kan konkluderes, at variabelselektion er et brugbart værktøj til at fremhæve de subtile forskelle der er i modellerne for både TS og OS, især ved lange bølgelængder. De opnåede modeller var meget akkurate og det er særligt bemærkelsesværdigt i de tilfælde, hvor der stadig var stivelse tilbage i frugten. Pærer adskiller sig anatomisk fra andre frugtarter ved at indeholde stenceller, og spørgsmålet er om disse kan influere på resultatet.

Det her præsenterede arbejde viser at TS er en interessant parameter at arbejde med i fruktproduktion. Frugtens TS kan manipuleres ved kulturtekniske tiltag og betydelige forskelle i TS kan opnås og relateres til træets fysiologi. Forskelle i TS kan påvises på et
tidligt tidspunkt i frugtens udvikling og forskellene opretholdes igennem hele frugtens udvikling. Når det sammenkædes med muligheden for at måle TS ikke-destruktivt, åbnes der mulighed for at kunne manipulere frugtkvaliteten mens frugten stadig er på træet, eller som minimum, at få et relevant rettidig forvarsel om det givne års frugtkvalitet, som kan bruges til at optimere beslutningsprocesserne omkring lagrings- og salgsstrategi.
Abbreviations and symbols

Fruit quality
DM – dry matter (%)
SPI – starch pattern index
SSC – soluble solids content

Root pruning and irrigation
ABA – Abscisic acid
DAFB – days after full bloom
DI – deficit irrigation
ED – evaporative demand
ET – evapotranspiration
FI – full irrigation
NI – no irrigation
NP – non-root pruned
PGRs – plant growth regulators
PRD – partial root zone drying
RH – relative humidity
RP – root pruned
WUE – water use efficiency

Spectroscopy
IR – infrared
Abbreviations and Symbols

MIR – mid-infrared
NIR – near infrared
Si – Silicon
InGaAs – Indium Gallium Arsenide

\[ E \quad \text{energy} \]

\( h \) – Planck’s Constant (that there is a proportionality between the energy \((E)\) of a photon and the frequency of its associated electromagnetic wave)

\( \lambda \) - wavelength, nm \((1 \text{ nm} = 10^{-9} \text{ m})/1 \text{ mm} = 1 \text{ mm})\) (the distance between two peaks)

\( \nu \) - frequency (the number of waves that pass through a fixed point per second)

\( \tilde{\nu} \) – wavenumber, \( \text{cm}^{-1} \) (the number of waves per cm)

\[ A = \text{absorbance (-Log}_{10}(P/P_0)) \]

\( P_0 \) - incident radiation

\( P \) - transmitted radiation

\( R \) - reflectance

\( T \) - transmittance \((P/P_0)\)

\( c \) - concentration

\( \varepsilon \) - molar absorptivity/molar extinction coefficient

\( l \) - path length of sample

Statistics and chemometrics

ANOVA – analysis of variance
CARS – competitive adaptive reweighted sampling
E – residual variance
iPLS – interval partial least squares (regression)
Log(1/R) – reflectance to absorbance
Log(1/T) - transmittance to absorbance
LV – latent variable
MSC – multiplicative scatter correction
PC – principle component
PCA – principle component analysis
PLS – partial least squares (regression)
RMSEC – root mean square error of calibration
RMSECV – root mean square error of cross-validation
RMSEP – root mean square error of prediction
RPD – residual predictive deviation
R² – coefficient of determination
S. Dev. – standard deviation
S-G – Savitsky-Golay
SNV – standard normal variate
X – Spectral data
x - a single spectrum
Y - reference data
y – a single reference data point
Publications

Paper 1.

Predicting apple (cv. Elshof) postharvest dry matter and soluble solids content with NIR spectroscopy

Paper 2.

The effect of root pruning and supplementary irrigation on ‘Clara Frijs’ (Pyrus communis L.) pear quality
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Paper 3.

Predicting pear (cv. Clara Frijs) preharvest dry matter and soluble solids content with NIR spectroscopy
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Paper 4.
Effects of root pruning and irrigation regimes on pear trees: growth, yield and yield components
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SUBMITTED to Horticultural Science

An additional paper submitted for peer review and included in this thesis, but not discussed:

Paper 5.
Prediction of postharvest dry matter, soluble solids content, firmness and acidity in apples (cv. Elshof) using NMR and NIR spectroscopy – a comparative study
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Chapter 1. Introduction

Apples and pears are important horticultural crops, and together with citrus fruit, they are the three most consumed fruit types in Europe (CBI, 2011). The apple and pear cultivars used in this project (‘Elshof’ and ‘Clara Frijs’, respectively) are both extensively grown in Denmark. ‘Elshof’ is a mutant of ‘Elstar’ and is of medium size with a bright blush and yellow background colour (www.prognosfruit.eu). Danish production of ‘Elshof’ is approximately 24,500 tonnes per annum. It maintains good postharvest quality and can be kept in cold storage for up to three months. ‘Elshof’ is a popular cultivar in northern Europe due to better colouration than ‘Elstar’, even in reduced light conditions (www.thefruitfirm.com/eng/varieties/apple/elshof). ‘Clara Frijs’ is the most popular pear cultivar in Denmark, with average annual production at approximately 6,000 tonnes (www.prognosfruit.eu). It is a medium sized, bright green pear when mature, rarely with a blush, turning yellow as it senesces. Danish consumers prefer to eat it soon after harvest when still crisp.

1.1 Background and context

Quality is an essential aspect of apple and pear production as growers aim to consistently produce high quality fruit for maximum return. For the purposes of this PhD, fruit quality is defined and discussed in terms of the standard industry parameters used to measure it. They reflect its quantitative and sensory properties and include: fruit size, soluble solids content (SSC), colour, firmness, acidity and starch. Apart from size and colour, the parameters listed determine fruit internal quality.
In addition to the aforementioned parameters, fruit dry matter (DM) has emerged in recent years as another indicator of internal quality in apples (McGlone et al., 2003; Palmer et al., 2010). However, to the author’s knowledge, there is no equivalent research regarding the relationship between DM and fruit quality in pears. The interest in DM is driven by a need for an objective measurement of quality based on fundamental fruit biology, but also one that reflects potential flavour and allows more precise preharvest, harvest and postharvest evaluation of fruit (Harker et al., 2009; Jordan et al., 2000; Palmer et al., 2010). DM is essentially a reflection of fruit carbohydrate content, where SSC and starch are the major constituents. The hydrolysis of starch into SSC during fruit ripening makes DM a valuable and accurate indicator of potential postharvest SSC, or of actual SSC once hydrolysis is complete (Jordan et al., 2000; McGlone and Kawano, 1998; McGlone et al., 2003).

The focus on DM emphasises the connection between fruit quality and fruit physiology. Fruit physiological development (and that of the tree) is the result of the interaction of numerous cultural practices (e.g. pruning, thinning), environmental inputs (e.g. water, nutrition, light, CO₂) and physiological processes (e.g. light interception, photosynthesis, respiration, transpiration) (Wünsche and Lakso, 2000a), as well as the inherent characteristics of the cultivar. These processes fundamentally affect preharvest fruit development and dictate how harvested fruit looks, tastes and behaves in storage (Kader, 2002).

The control of vegetative growth and the production of high quality fruit are inextricably linked and are two very important considerations in Danish orchard production. The
withdrawal of synthetic plant growth regulators (PGRs) from the market has prompted interest and research into alternative methods of curtailing vegetative growth without serious compromise to fruit quality. Root pruning is one such alternative method known to control vegetative growth in orchard trees, to alter carbohydrate partitioning (Geisler and Ferree, 1984; Saure, 2007), temporarily limit water uptake (Green and Clothier, 1999) and possibly control biennial bearing (Ferree, 1992). However it can also alter fruit quality and produce fruit that are below marketable size (Khan et al., 1998a; Schupp and Ferree, 1987b). A complementary strategy, such as supplementary irrigation, may help counteract some of the negative effects of root pruning on fruit quality. In addition restricting the water supply may have the same beneficial effect on fruit quality as supplementary irrigation.

The interest in DM as a measure of fruit quality runs parallel to a move towards faster, non-destructive methods of quality assessment, including near infrared (NIR) spectroscopy and multivariate data analysis (chemometrics) which creates models to ‘predict’ internal fruit quality. The value of spectroscopy and chemometrics in measuring the internal quality of a wide range of fruit has been thoroughly investigated over the last ten years as illustrated in the review paper by Nicolaï et al. (2007). The most commonly predicted parameters using NIR spectroscopy are fruit firmness and SSC (Bobelyn et al., 2010; Fan et al., 2009; Liu et al., 2008; Park et al., 2003). There is some published research concerning DM prediction in apples (McGlone et al., 2003; McGlone and Martinsen, 2004) but none for pears.
The relationship between DM and SSC is valuable in terms of fruit quality but their prediction with NIR spectroscopy can be problematic as models may not be able to distinguish between the two parameters. McGlone et al. (2003) suggest longer NIR wavelengths may be able to distinguish between them. Longer wavelength spectra have been used to predict DM of avocado (Schmilovitch et al., 1997; Wedding et al., 2011;), but not in the prediction of both DM and SSC in other fruit.

1.2. Project hypotheses, aims and objectives

This background knowledge has given rise to four general hypotheses:

1. Root pruning increases preharvest DM in ‘Clara Frijs’ pears but compromises overall harvest fruit quality.
2. Supplementary irrigation of root pruned pear trees has the potential to improve harvest fruit quality and size.
3. Preharvest and harvest DM of pears is an indicator of postharvest fruit quality.
4. Prediction of apple and pear DM and SSC is possible using NIR spectroscopy and chemometrics.

These hypotheses produced a thesis project composed of two distinct halves and thus with two overall aims:

1. To understand how root pruning affects preharvest DM and harvest fruit quality and size of field grown ‘Clara Frijs’ pears and how this may alter their postharvest quality after storage. In addition to assess the value of supplementary irrigation in mitigating the negative effects of root pruning on quality (Paper 2). An underlying intention here was to produce fruit with a wide variation in DM for use in Aim 2.
2. To predict apple and pear DM and SSC using NIR spectroscopy and chemometrics. Plus to determine the relationship between the two parameters and whether prediction models have the ability to discriminate between them. (Papers 1 and 3)

Specific objectives:

1. To measure DM in pears from root pruned and supplementary irrigated trees throughout their development – from preharvest to postharvest storage.
2. To perform quality analysis on these pears prior to harvest, at harvest and at intervals during postharvest storage to evaluate the effect of DM on fruit quality.
3. To obtain preharvest and harvest NIR spectra of these pears and construct prediction models for DM and SSC using chemometrics.
4. To predict DM and SSC of ‘Elshof’ apples during postharvest storage.
5. To compare prediction models for DM and SSC based on two different wavelength ranges (300-1100 nm; 1000-2500 nm) and to assess their ability to differentiate between the parameters.

1.3. Thesis outline

This thesis will provide an overview of the relevant concepts and literature and to discuss the results obtained in relation to existing knowledge. Its structure reflects this:

- Chapter 2 – Fruit Quality: the concept and its assessment, plus an outline of preharvest factors that may influence it.
- Chapter 3 – Dry Matter: its composition and accumulation in developing fruit and importance as a measure of fruit quality.
Chapter 4 – Manipulating Fruit Quality: the change in carbon allocation in trees caused by root pruning, using supplementary irrigation to maintain fruit quality and an outline of the effects on tree and fruit DM.

Chapter 5 – Near Infrared Spectroscopy: basic principles and the relevance of spectroscopy in predicting fruit quality, including a review of existing NIR literature.

Chapter 6 – Chemometrics: principles and some methods employed in this thesis.

Chapter 7 – NIR Spectroscopy and Fruit Quality: a review of the research to date and some issues arising concerning the prediction of fruit quality.

Chapter 8 – Discussion, Conclusions and Perspectives: examination of results from this PhD project in relation to current research, plus concluding remarks and future perspectives.
Chapter 2 – Fruit Quality

This chapter will provide an overview of the concept of fruit quality, its importance in fruit production and the parameters used during this PhD to measure it.

2.1. The concept

Quality is a highly mutable concept. Its definition is very much dependent on the commodity in question; it’s intended use and the interests of those intent on defining it, be they researchers, growers, retailers or consumers (Kader, 1999). The term broadly implies a degree of excellence in a commodity and suitability for its intended use (Abbott, 1999). Quality can refer to a set of legal standards established for fresh produce to facilitate global trade (Barreiro et al., 2004; Harker et al., 2008). It can also be highly subjective, founded on personal, cultural and sensory preferences (Shewfelt, 1999), or be based on a series of measurable attributes (Abbott, 1999).

Indeed, concepts of quality vary according to context. They change and merge as a commodity moves along the supply chain, from farm to cold store, to retailer and finally to consumer (Crisosto and Costa, 2008). For instance, a consumer may evaluate a commodity on quantifiable criteria, such as size, firmness and colour, while other less estimable concepts like texture, visual appeal and mouth feel are also very relevant in shaping and satisfying expectations of quality (Kader, 1999).

For the purposes of this thesis, quality will be defined according to the definition provided by the OCED (2012): ‘the degree, measured with objective criteria, to which a commodity has reached a sufficient stage of development such as to enable its quality, after harvesting
and postharvest handling (including ripening, where required) to be at least the minimum acceptable to the final consumer’. The ‘objective criteria’ employed here to measure fruit quality are standard industry parameters, namely soluble solids content (SSC), firmness, fruit size, colour, acidity, starch and the Streif Index. A more recent parameter is dry matter (DM) and is discussed in Chapter 3. These parameters ensure fruit satisfy legal requirements (or Grade Standards) and are good indicators of conditions in the field and of fruit maturity, as well as providing an idea of eating experience. More detailed information regarding these parameters and their measurement is provided at the end of this chapter in sections 2.4. and 2.5., respectively.

2.2. Fruit maturity

The assessment of preharvest fruit quality is essential for monitoring the progress of a crop. Perhaps the most important reason for doing so is to estimate optimum fruit physiological maturity for better postharvest quality and to minimise storage losses.

Fruit maturity at harvest is vital in dictating postharvest storage life and future eating quality (Kader, 2002). However the stage of maturity where fruit is able to ripen successfully after harvest or is at its best for storage does not invariably coincide with the period when fruit has the most appeal to consumers (Kingston, 1992). Therefore maturation can be considered as a developmental process leading to two related, but distinct, classifications of maturity: physiological maturity and commercial maturity.

Physiological (or technical) maturity can be defined as the developmental stage where fruit can continue to ripen independently from the tree after harvest and where storage
potential is optimal (Kader, 2002). Ripening encompasses a set of physiological changes that occur between the latter stages of growth and development through to the early stages of senescence, which result in the characteristic eating quality of fruit (Watada et al., 1984). Some climacteric fruit, including apples and pears, require a period of cold storage after harvest to stimulate the production of enzymes involved in the biosynthesis of ethylene, before proper ripening at room temperature (Villalobos-Acüna et al., 2011). Other fruit such as ‘Clara Frijs’ pears are an exception to these general rules. These pears still need to be physiologically mature when harvested, but their organoleptic quality is best when they are still firm and green not long after picking. Therefore they do not require much time in cold storage or further ripening to improve their postharvest quality. However, fruit harvested when physiologically mature is not always commercially mature (Figure 2.1.) in order to tolerate storage and transportation (Kader, 2002) and have the best chance of ripening.

Figure 2.1. The relationship between maturity and fruit organoleptic quality (López-Carmelo, 2004).
Commercial (or horticultural) maturity is the stage where fruit has the preferred characteristics for a particular use by consumers (Watada et al., 1984), and for most fruit this follows physiological maturity. With reference to Figure 2.1., this means fruit destined for storage requires a different level of ripeness (somewhere after physiological maturity but before commercial maturity) compared to fruit for immediate consumption.

Defining maturity implies it is measurable and that techniques exist to quantify it. Maturity indices are based on characteristics that are known to change as fruit mature. Indeed most of these indices double-up as indicators of quality (as listed in section 2.1.) and are a compromise between the need to harvest fruit when it is physiologically but not commercially mature, whilst also ensuring the best potential eating quality for the consumer (Kader, 2002).

2.3. Preharvest determination of fruit quality

By its very definition, determining physiological maturity requires a certain level of preharvest quality assessment to pre-empt an ideal harvest date. Preharvest can be defined as the period in fruit development between fruit set until just before harvest. Fruit quality is inextricably linked to its physiology, therefore the process of fruit development, and the external factors affecting it, can be seen as determinants of harvest and storage quality (Kader, 2002). This also implies an alignment between certain preharvest quality attributes and particular fruit internal developmental processes (Figure 2.2.).
Understanding the relationship between preharvest development and harvest and postharvest quality may improve orchard management and overall fruit quality. Furthermore early estimations of quality may be useful in terms of scheduling harvest and adjusting inputs such as water and fertiliser. Field practices are crucial determinants of quality as postharvest treatments and storage can only maintain quality characteristics defined in the field (Manganaris et al., 2008). Determination of preharvest fruit quality predominantly focuses on measurement of soluble solids content, firmness and starch content, three indices that are also tested at harvest and during storage.

**2.3.1. Preharvest factors influencing harvest and postharvest fruit quality**

Numerous factors influence preharvest fruit development and have an impact on quality after harvest and in storage. They include climatic factors such as light intensity, rainfall and temperature; cultural factors such as nutrition, pruning and training techniques, crop
load and the use of plant growth regulators (PGRs); and genetic influences arising from cultivar choice and rootstock (Asín et al., 2007; Behboudian et al., 2011; Johnson et al., 2002; Kays, 1999; Sams, 1999). Knowledge about the effects of external factors on tree growth and fruit quality allows manipulation of tree and fruit development for the benefit of the grower and consumer. Two factors that have formed a major part of the experimental impetus behind this thesis are root pruning in combination with supplementary irrigation. Root pruning is a technique adopted to replace the use of chemical PGRs for the control of vegetative growth in orchard fruit production. A restricted water supply can also limit vegetative growth (Ebel et al., 1995) and both can reduce fruit size and alter quality. Root pruning and water stress and their effects on fruit quality, particularly DM, will be discussed in greater detail in Chapter 4.

2.4. Fruit quality parameters
The ‘measured criteria’ of fruit quality form a crucial part of this thesis and are the means by which apple and pear samples were evaluated experimentally. Measurement of these indices is time consuming and based on a selected number of samples but this is standard industry practice for estimating quality. Although measured independently these parameters make greater sense when viewed holistically. Indeed many are interlinked, either physiologically, for example through the relationship between dry matter (DM) and soluble solids content (SSC) via the hydrolysis of starch (Palmer et al., 2010) or through consumer preference for sweeter apples, only if they are firm too (Harker et al., 2008). Below is a brief overview of some of the quality parameters used in this thesis. Further elaboration of their assessment is given in section 2.5.
2.4.1. Fruit size

Size is an important parameter when marketing fruit as it can significantly affect its appeal to consumers (Kays, 1999). Fruit size is also related to total yield per tree and so has an impact on the commercial value of the crop. Fruit development is characterised by an initial period of cell division and expansion, followed by continued cell expansion until harvest (Bain and Robertson, 1951; Denne 1963; Mallardi and Hirst, 2010). Cell number (division) and volume (expansion) at harvest, and by implication fruit size and weight, are influenced by the rate and duration of these phases (Coombe, 1976; Pratt, 1988). Field conditions, crop load and seed development are other factors influencing fruit size. Fruit size has a knock on effect on other quality attributes including firmness, dry matter and soluble solids content.

2.4.2. Colour

Colour is hugely important in terms of consumer preference and quality assessment. A fall in skin chlorophyll content is correlated with advancing maturity (Abbott, 1999) and depending on the fruit, it is often the most obvious sign of maturity (Wills et al., 2007). ‘Clara Frijs’ pears have a uniform surface colour, which makes colour measurements a good indicator of fruit quality. Other fruit, including apple cultivars such as ‘Elstar’, have an unevenly distributed skin blush, making colour measurements based on single points on the fruit surface an inaccurate indicator of maturity.

2.4.3. Firmness

Firmness is a good measure of maturity and for many fruit, including apples and pears, it is also used as a measure of eating quality (Kingston, 1992). However firmness in the
laboratory does not invariably translate into good mouth-feel or texture. It is a complex phenomenon based on the physical characteristics of cells Harker et al. (1997) but also related to other parameters such as SSC (Harker et al., 2008). The cell walls of apple and pear flesh are composed of cellulose that provides strength and pectic substances that make contact with adjoining cells and confer flexibility (Harker et al., 1997). This flexibility is essential during fruit development as cells expand and fruit grow larger. These pectic substances are solubilised as fruit ripen causing a loss of cohesion between cells and so affecting general fruit firmness and juiciness.

2.4.4. Dry matter (DM)
DM is the main parameter of interest in this thesis due to its relationship to SSC through the solubilisation of starch in ripening fruit. DM, its composition and importance as a quality parameter are discussed in detail in Chapter 3.

2.4.5. Soluble solids content (SSC)
As apples and pears mature, starch is converted into sugars thereby increasing SSC and making fruit taste sweeter. The extractable juice in apples and pears contains soluble compounds including fructose, glucose, sucrose, sorbitol, organic acids and inorganic salts (Kingston, 1992; Wills et al., 2007). The ratio of sugars varies depending on the fruit and the cultivar (Wu et al., 2007) and influences taste. Fructose is sweeter that sucrose, which is sweeter than glucose (Kader, 2002). Chapter 3 has more information on SSC and its relationship to DM.
2.4.6. Acidity

The principle acid in apples and pears is malic acid (Ackermann et al., 1992; Colaric et al., 2007). Acidity is an important component of fruit flavour and in combination with SSC, contributes to overall organoleptic quality. Total organic acid content declines in fruit as they mature, ripen and store, with apples having a reasonably high acid content compared to pears. However fruit acidity should be considered in conjunction with other quality parameters, especially firmness and SSC as consumer studies show a strong relationship between the three (Harker et al., 2008).

2.4.7. Starch

The starch pattern index (SPI) has long been used as an indicator of maturity. Apples and pears as they ripen show a characteristic starch pattern after iodine staining. The value of the starch-iodine test as a method of measuring fruit maturity relies on the relationship between starch hydrolysis and physiological maturity (Reid et al., 1982). Hydrolysis in apples and pears begins in the core area and moves through the cortical tissue in a pattern determined by the variety (Reid et al., 1982).

2.4.8. Streif Index

The Streif Index was developed to provide a more stable and less subjective way of assessing fruit maturity using indices whose individual value can vary from year to year. It combines three standard indices: firmness/(SSC*SPI), with the index decreasing as fruit mature until they reach a predetermined harvest threshold value (Peirs et al., 2000).
Table 2.1 lists the industry recommended harvest quality standards for ‘Clara Frijs’ pears and ‘Elshof’ apples. Apart from size, firmness and SSC are the two most important parameters. As yet there are no recommended minimum values for DM for either fruit.


<table>
<thead>
<tr>
<th>Parameter</th>
<th>‘Clara Frijs’ pear</th>
<th>‘Elshof’ apple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight/diameter</td>
<td>&gt; 55 cm</td>
<td>&gt; 55 cm</td>
</tr>
<tr>
<td>Colour</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Firmness</td>
<td>5 kg cm⁻²</td>
<td>6-7 kg cm⁻²</td>
</tr>
<tr>
<td>SSC</td>
<td>&gt; 11 %</td>
<td>&gt; 12 %</td>
</tr>
<tr>
<td>DM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acidity</td>
<td>-</td>
<td>&gt; 1 %</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Streif Index</td>
<td>-</td>
<td>0.32</td>
</tr>
</tbody>
</table>

2.5. Assessment of fruit quality in this project

Experimental evaluation of fruit quality during the course of this PhD was based on standard protocols. Measurements were taken from two points located at the equator of each fruit. For apples the first was taken on the blush side (Point 1) and directly opposite (Point 2). Point 1 for pears was located on the ‘shoulder’ side with Point 2 again directly opposite. Colour, DM and SSC measurements were obtained from these locations with firmness data taken in between Points 1 and 2.
Figure 2.3. Directional orientation of fruit spatial variability (Peiris et al., 1999). From left to right: proximal-distal, circumferential and radial.

These protocols are standard (McGlone et al., 2002a and 2003; Slaughter et al., 2003), and are an attempt to balance out within-fruit spatial variation in quality. Peiris et al. (1999) investigated differences in DM and SSC of numerous fruit, including apples, running along three directional orientations (Figure 2.3.). Their results show apple SSC increased linearly along the proximal-distal axis and radially from core to surface but circumferential measurements had the least variation. Preliminary work on pears conducted during the early stages of this PhD, found a within fruit range of 12.5-13.8 % DM and 12.1-13 °Brix in ‘Clara Frijs’ pears (unpublished data).

2.5.1. Fruit size
For the purpose of this thesis, individual fruit size was based on weight (FX300i scales, A&D Co., Ltd., 0.01 g accuracy) and diameter recorded with a digital calipers (Whitworth Ltd.).

2.5.2. Colour
Apple and pear colour was measured using a hand held colorimeter (Chroma Meter, CR-400, Konica Minolta Sensing Inc., Japan) incorporating the CIE (Commission Internationale de l'Eclairage/ International Commission on Illumination) $L^*a^*b^*$ and
$L^*C^*H^*$ colour spaces (Figure 2.4). $L^*a^*b^*$ is a 3-D co-ordinate system containing three axes, $L^*$, $a^*$ and $b^*$, where the vertical axis, $L^*$ is lightness (black=0 and white=100) and on the horizontal axes, $a^*$ is the trend from red to green and $b^*$ is the trend from blue to yellow.

The $L^*C^*H^*$ colour space shares some features of $L^*a^*b^*$, but it is a more straightforward measurement and considered a more appropriate measure of colour (McGuire, 1992). This space is visualised as a sphere, again with three axes. Just as in $L^*a^*b^*$, $L^*$ is lightness, whilst on the horizontal axes, $C^*$ represents Chroma or saturation (the brighter the colour, the higher the value), and $H^*$ is hue angle ($0^\circ =$ pure red, $90^\circ =$ pure yellow, $180^\circ =$ pure green and $270^\circ =$ pure blue). Both systems function on the principle that the combined coordinates of the three values define a colour. In fact the two colour spaces are interconnected as $a^*$ and $b^*$ are the ground values for calculating hue angle and Chroma. Hue angle is the most commonly used parameter when measuring fruit colour (McGuire, 1992).
Commercial graders, such as the AWETA Rollerstar used in Paper 4, grade fruit according to percentage blush area so provide a more general measure of skin colour.

### 2.5.3. Firmness

Firmness measurements, using a bench-mounted penetrometer (GS20 Fruit Texture Analyser, Güss Ltd., South Africa), were based on the pressure necessary (kg cm\(^{-2}\)) to push a metal probe of a specific size into fruit flesh to a certain depth (Figure 2.5.). The tip of the probe is convex and its diameter varies according to the type of fruit being tested, with softer fruit, such as apples, requiring a larger probe (11 mm) and pears an 8 mm probe.
2.5.4. Dry matter

Apple and pear DM were determined using two 10 mm diameter plugs of tissue taken perpendicular to the fruit surface. After the core was removed each plug was weighed on a pre-weighed foil tray. The samples were then oven dried at 70 °C for 20 h until constant weight and re-weighed. DM was expressed as percentage dry weight of the initial fresh sample.

2.5.5. Soluble solids content

SSC of juice was assessed using a temperature compensated digital refractometer (RFM712, Bellingham Stanley Ltd., UK).
2.5.6. Acidity

Fruit acidity measurements are based on titratable acidity and are usually calculated on a per replicate basis. 200 g of diced flesh, including skin, was blended (Wareing Laboratory blenders, UK) with 100 g of deionised water for 2 minutes. Acidity was determined by titrating the puree with 0.1 M NaOH to pH 8.1 using an autotitrator (7195-Titrino, Metrohm, Switzerland). Results were expressed as mg g⁻¹ of malic acid equivalent (MAE).

2.5.7. Starch

The SPI of apple and pear samples was measured by dipping a cross-section of flesh (~3 cm from the calyx) in a standard iodine solution for 30 s and after 2 mins visually ranking the slices on a visual scale from 1 (black, starch present) to 9-10 (white, no starch present). The index is specific to fruit type and cultivar as the arrangement of cells and pattern of starch degradation is different for each. Figure 2.6. shows a sample SPI chart for ‘Red Delicious’ apples.

![Figure 2.6. SPI chart for apple cv. Red Delicious (www.omfra.go.on.ca).](image-url)
2.6. Non-destructive quality assessment

All the aforementioned methods of quality analysis are standard practice within the fruit industry. However many of them share one major disadvantage – they are all destructive measurements. In recent years there has been a shift towards developing alternative non-destructive methods of fruit quality analysis with near infrared (NIR) spectroscopy receiving particular attention (McGlone et al., 2003; Nicolaï et al., 2007; Park et al., 2003). Aside from its value as a quality parameter, dry matter is of interest as NIR spectroscopy is especially suited to the prediction of the constituents present in carbohydrate. An essential part of this PhD project has been to investigate the possibility of predicting apple and pear DM using NIR spectroscopy. The principles of NIR spectroscopy are explained in Chapter 5. and a short review of its application in fruit quality assessment is given in Chapter 7.
Chapter 3 - Dry Matter

The focus on DM as a quality parameter necessitates investigation into its composition and mode of synthesis in developing fruit. This chapter also elaborates on some reasons for its prominence as a parameter. This naturally leads to an examination of ways of increasing fruit DM accumulation in the field. This is provided in Chapter 4.

3.1. Definition

The internal quality of apples and pears is based on constituents of their flesh and their concentration. The dominant constituent of fruit is water, with carbohydrate, minerals, lipids, proteins, organic acids, phenolic compounds, vitamins making up the remainder (Ackermann et al., 1992; Colaric et al., 2007; Kader, 2002). Once water is excluded these constituents can collectively be referred to as ‘dry matter’. DM can be defined as the ratio of fruit dry weight (DW) to fresh weight (FW) and is expressed as a percentage (DW/FW*100). This is the definition used throughout this thesis. The term DM is often used interchangeably with other definitions such as dry matter concentration (D, dry mass per volume of plant organ) and dry matter content (DMC, dry mass per fresh mass) (Shipley and Vu, 2002).

3.2. Composition

Approximately 90 % of fruit DM is composed of carbohydrates – in soluble and insoluble forms (Suni et al., 2000). The main soluble carbohydrates determining soluble solids content (SSC) are fructose, comprising between 3.9-5.7 % on a fresh weight basis of a ripe apple; sucrose, between 3.5-4.6 %, and glucose between 0.8-1.0 %, with sorbitol playing a lesser role (Ackermann et al., 1992).
Insoluble forms include starch and various structural carbohydrates, such as cellulose, hemicellulose and pectin (Gibson, 2012; Tao et al., 2009). Pear flesh differs slightly to that of apples as its cell walls contain small amounts of lignin in the form of brachysclereids or ‘stone cells’ (Tao et al., 2009). These originate from sclerenchyma cells and are randomly distributed throughout pear flesh providing support to non-meristematic parts of the fruit.

Pectin is a complex polysaccharide located in the cell walls of fruit. It is present in primary cell walls as well as in the middle lamellae where it helps bind cells together. The solubilisation of pectin (and hemicellulose) during ripening and the softening of the middle lamellae cause cells to separate from each other and for fruit, such as pears, to lose firmness (Ahmed and Labavitch, 1980; Ben-Arie, 1979). However, species within the genus *Pyrus* exhibit different softening characteristics during ripening. For example, the European pear ‘La France’ softens dramatically during ripening. Alternatively, the Chinese pear (*P. bretschneideri* Rehd.) ‘Yali’ produces ethylene but does not soften to the same extent and remains crisp, whilst firmness in the non-climacteric Japanese pear (*P. pyrifolia* Nakai.), does not change markedly (Hiwasa et al., 2004). The authors argue the mechanism and extent of pectin and hemicellulose solubilisation during ripening differs for each species. In contrast, lower levels of pectin solubilisation in apples means they stay relatively crisp upon ripening (Percy et al., 1996).

Limited contribution to fruit DM is made by organic acids, minerals, lipids, proteins, phenolic compounds and vitamins (Coombe, 1976). Malic acid accounts for approximately 90 % of the organic acid content of apples with a concentration of between 0.4-1.0 % in mature fruit (Ackermann et al., 1992; Suni et al., 2000). It is a major substrate for
metabolism (Berüter, 2004) and is synthesised in the fruit mainly via glycolysis. Additional acids include citric and succinic plus a few minor other ones.

3.3. Dry matter in preharvest fruit development

Developing fruit are a strong photosynthate sink and accumulate carbohydrate in soluble and/or insoluble forms. Fruit containing carbohydrate in soluble forms, such as strawberries, raspberries, peaches, grapes or citrus, must remain on the plant until they are almost ripe to ensure they can be stored postharvest and maintain marketable quality. The major increase in SSC in these fruit does not occur until late in development, signalling the onset of ripening. Therefore harvesting fruit too early compromises their final sugar content. In contrast, fruit such as avocados, kiwifruit, apples and pears also store insoluble carbohydrate, primarily as starch, which is hydrolysed into soluble sugars as fruit mature and ripen after harvest. This permits greater efficiency in the accumulation of carbohydrate, as starch is a more compact and osmotically inactive metabolite (Kavakli et al., 2000).

Cumulative fruit growth in apples and pears, measured as FW, usually follows a sigmoid curve (Figure 3.1) (Bain and Robertson, 1951; Denne, 1960; Jackson, 2011; Schechter et al., 1993b). Fruit growth and development can be divided into two distinct, but overlapping, phases: cell division and cell expansion. The cell division phase (I) coincides with a period of significant translocation of carbohydrate into fruit (Nelmes and Preston, 1967; Percy et al., 1996). Nevertheless growth rate remains low during this phase, suggesting that initial carbohydrate imports are used primarily for cell biosynthesis (Schechter et al., 1993a).
Figure 3.1. Fruit growth (size, FW) over time plotted as a sigmoid curve (Jackson, 2011).

The main cell division phase in apples occurs in the early weeks after full bloom (Figure 3.2), with small increases in cell number occurring later in the season. It seems to vary greatly. Schechter et al. (1993a) reported cell division in ‘Idared’ apples lasted approximately 40 DAFB, its rate peaking after 15. Denne (1960) reported it lasting 6-7 weeks in ‘Cox’s Orange Pippin’ and up to 12 weeks in ‘Miller’s Seedling’ apples in the UK. Sterling (1954) reported cell division in ‘Bartlett’ pear lasting for between 6-8 weeks after full bloom and for approximately 6 weeks in kiwifruit (Walton and De Jong, 1990).
Figure 3.2. Stages of fruit development in ‘Royal Gala’ apples (Janssen et al., 2008). DAA = Days after full bloom (DAFB). A, fruit set, 0 DAFB; B, 14 DAFB; C, 35 DAFB; D, 60 DAFB, E, 87 DAFB; F, 132 DAFB; G, 146 DAFB. H illustrates a general timeline of fruit development, including major physiological events. Ripening is shown as a solid and dashed red line – solid from the time of the climacteric and dashed for the period just before.

Although cell expansion (II) is a separate process, it begins soon after fruit set and continues alongside the main cell division phase, lasting until harvest (Denne, 1960). The end of the main period of cell division and the shift to cell expansion marks a change in fruit metabolism to carbohydrate accumulation (Ackermann et al., 1992; Berüter 1985). It is also associated with terminal shoot formation (Forshey et al., 1987) and coincides with
‘June drop’ (Schechter et al., 1993a). Figure 3.2 illustrates the different stages of apple development, from fruit set to maturity and ripening. It also shows the overlap between cell expansion and starch (carbohydrate) accumulation and later starch hydrolysis as fruit near maturity.

Figure 3.3. Fruit dry weight (DW) during development in three apple cultivars (Schechter et al., 1993b). Inside the insert box: Phase I, cell division; phase II, cell expansion. ‘Delicious’ harvested 139, ‘Empire’ 126 and ‘McIntosh’ 112 DAFB.

Fruit DW increases in a mostly linear manner after 14-40 DAFB (Figure 3.3.) suggesting sink demand for assimilates is established during the cell division phase and remains relatively constant after it, despite changeable tree source-sink relationships and environmental fluctuations (Schechter et al., 1993a). In contrast, fruit DM follows a U-shaped curve (Figure 3.4.), falling to a minimum towards the end of the cell division phase. This may be related to high fruitlet respiration rates during this period (Schechter et al., 1993a). DM rises for a time after this, possibly due to a decline in fruit respiration (Jones et
al., 1981), but levels off as cell expansion continues and more water and carbohydrate are translocated into fruit (Schechter et al., 1993a).

Carbohydrates translocated from leaves into the vacuoles of developing apples consist of approximately 60-80 % sorbitol and 20-40 % sucrose (Berüter, 2004; Hansen, 1970; Klages et al., 2001; Li et al., 2012) and both are primarily used for sugar and starch synthesis and accumulation (Figure 3.5.). Water is essential to this process, both in aiding cell expansion and by acting as a medium for solute translocation (Coombe, 1979).

Figure 3.4. Fruit dry matter (DM) during development in three apple cultivars (Schechter et al., 1993b). Inside the insert box: Phase I, cell division; phase II, cell expansion. ‘Delicious’ harvested 139, ‘Empire’ 126 and ‘McIntosh’ 112 DAFB.
Figure 3.5. Schematic illustration of the translocation of soluble sugars into developing fruit (modified from Berüter et al. (1997), Hansen (1970) and Li et al. (2012)).

Sorbitol primarily serves as a precursor of fructose, whilst sucrose is the source for both glucose and fructose (Berüter, 1989 and 2004; Hansen, 1970). Sucrose is simultaneously translocated into fruit, broken down into glucose and fructose and re-synthesised in a cyclical manner (Berüter et al., 1997). Fructose accounts for approximately 80% of the total carbon flux in fruit. Almost all the sorbitol and half the sucrose are converted to it, meaning it accumulates to a much greater extent than glucose (Li et al., 2012).

Glucose concentration in apples is at its highest during early development where it is used for both general growth and maintenance (Figure 3.6.). It is the preferred precursor of starch so with the end of cell division and the onset of starch synthesis, its concentration
declines only to increase again during starch degradation (Berüter, 1989; Berüter et al., 1997).

Figure 3.6. Changes in carbohydrate concentration in developing apples (Berüter et al., 1997).

Starch concentration in apple reaches a maximum approximately 100 DAFB, depending on cultivar (Berüter, 1989). Apple DM at harvest is composed of approximately 15-20 % starch and 10-12 % soluble sugars (Pavel and De Jong, 1995). In contrast, the equivalent values for kiwifruit are 50 % and 7 %, respectively (Walton and De Jong, 1990). The relatively similar SSC for both fruit at consumption illustrates that postharvest starch hydrolysis contributes more to kiwifruit SSC than to apple.

Starch synthesis is a major carbon consuming process in developing fruit and Berüter et al. (1997) suggest that optimum synthesis occurs in apples when an abundant carbon supply is available to the fruit. The temporary formation of starch during fruit development could
be viewed as a mechanism for regulating osmotic potential (osmoregulation) in cells by lowering solute concentration to maintain cell turgor pressure (Hsiao, 1973) and to guarantee an osmotic gradient for continued sucrose unloading and translocation. Maintaining turgor is essential for cell growth as enlargement of storage cells can only take place when there is positive turgor pressure.

### 3.4. Importance as a parameter

DM is essentially a reflection of total fruit carbohydrate, and its two most relevant components, in terms of fruit quality, are starch and SSC. This relationship to fruit SSC, once the hydrolysis of starch into sugars is complete, makes DM interesting (Palmer et al., 2010). Furthermore its relative stability at harvest, apart from small losses due to respiration, means DM has the potential to be a reliable indicator of postharvest SSC (Crisosto et al., 2012; McGlone and Kawano, 1998).

SSC is a recognised attribute when evaluating quality and flavour for many types of fruit including apples (Palmer, 2007); kiwifruit (Crisosto and Crisosto, 2001); mangoes (Subedi et al. 2007). Recent work has focused on DM as a quality parameter in its own right, with research concentrating on kiwifruit (Harker et al., 2009; Jordan et al., 2000; McGlone et al., 2002b); avocados (Clark et al., 2003; Wedding et al., 2010; Woolf et al., 2003) and apples (McGlone et al., 2003; Palmer et al., 2010). To the author’s knowledge, no published research exists concerning pear DM.

Early research on avocado DM intended to find an alternative measure of horticultural maturity as external appearance was not an accurate indicator. Maturity was previously
based on percentage oil content but this method was slow and expensive, so fell out of favour (Ranney et al., 1992). Research by Morris and O’Brien (1980) and Lee et al. (1983) focused on the close relationship between avocado oil and DM during fruit maturation. This eventually prompted the global avocado industry to adopt DM as an official indicator of harvest fruit maturity (Woolf et al., 2000). In the US (California), minimum DM for avocado is 19-25 %, depending on the cultivar (Kader and Arpaia, 2000). In Australia it is 21 % for all cultivars (McCarthy, 2001) although consumer studies suggest a preference for 25 % (Harker et al, 2009).

Research on the relationship of apple SSC to DM has taken inspiration from that on kiwifruit (Palmer, 2007; McGlone and Kawano, 1998; McGlone et al., 2002a and 2002b). Consumer studies have shown kiwifruit with high harvest DM are perceived as having more flavour when ripe and are more likely to be purchased again (Burdon et al., 2004; Harker et al., 2009; Jaeger et al., 2011). A minimum kiwifruit DM of 15.1 % (and 5.5-6.5 SSC) at harvest ensures SSC rises above the industry threshold of 12.5 % after ripening (Crisosto and Crisosto, 2001). However the bulk of research to date concerning consumer preference for apples is related to SSC and so only indirectly related to DM. However Palmer et al. (2010) demonstrated significant consumer liking and acceptability for apples with higher DM compared to those with lower percentages. No minimum DM % threshold exists for apples within the industry at present.

However consumer acceptability is based on the eating experience of the fruit as a whole and on the interaction between attributes. Harker et al. (2008) found consumer acceptability for ‘Red Delicious’ apples with a SSC above 13 °Brix only improved when
firmness was greater than 5.4 kg\(^{-1}\) (53 N). On the other hand, for a sweet and high acid cultivar such as ‘Braeburn’, acceptance of firmer (>5 kg\(^{-1}\) (49 N)) sweeter (>13 °Brix) fruit declined due to a shift in the sugar:acid ratio. The ratio of sugar:acid is also relevant to kiwi and based on consumer tests, Crisosto et al. (2012) found a preference for ripe fruit with high TA (≥1.2 %) only if DM was greater than 16.1 %.

Average DM varies between fruit species and cultivars. Many factors can influence this variability including crop load, canopy cover, period of time spent on the tree and whether the fruit is from a late or earlier harvest (maturity level). For example, early cropping cultivars tend to have lower DM. Schechter et al. (1993b) compared three apple cultivars and found the earliest, ‘McIntosh’, had the lowest DM (13.6 %) relative to the latest, ‘Delicious’ (15.3). Furthermore this difference can be detected as early as 4 weeks after full bloom at the beginning of the main period of cell expansion. Gamble et al. (2010) found an 18 % difference in DM between early harvested avocados (20 %) and late harvested fruit (38 % DM). Indeed there is variability between orchards, within an orchard, within a tree and within fruit. Palmer et al. (2010) found a 1.3-2 % (13-20 g/kg\(^{-1}\)) difference in DM between orchard samples and a 7 % (70 g/kg\(^{-1}\)) difference between individual fruit. Tables 7.2. and 7.4. (Chapter 7.) give an insight into the variability of fruit DM, even within the same cultivar, particularly for apple, avocado and kiwifruit. This biological variability poses a problem regarding the potential of NIR spectroscopy to predict fruit DM and SSC and is discussed in Chapter 7.
Chapter 4 – Manipulating Fruit Quality

The removal of chemical PGRs in commercial fruit production in Denmark, coupled with the interest in DM as an indicator of fruit quality, has led to a re-evaluation of cultural practices that control vegetative growth, improve flowering and alter carbohydrate partitioning in trees. Root pruning is one such practice, and it was once widely used in European gardens to reduce fruit tree size and vigour (Rivers, 1865).

This chapter will outline the principles behind the practice of root pruning. Special emphasis will be made on how it affects carbohydrate production, partitioning and storage in trees and fruit. Supplementary irrigation will be discussed in terms of its potential for counteracting the negative effects root pruning has on some aspects of fruit quality.

4.1. Root Pruning

The control of vegetative growth in favour of fruiting is a primary consideration in orchard production, especially in high-density plantings. A canopy management strategy, such as root pruning, prompts a uniform response in the tree and is a highly effective and economical method of reducing vigour to optimise leaf light interception and enhance photoassimilate production and partitioning to fruit. The overall aim of root pruning is to balance the desired reduction in vegetative growth and improvement in fruit quality, against the possible impact on preharvest fruit abscission and on harvest fruit size. Root pruning has been successful in controlling vegetative growth in apple (Ferree, 1992; Ferree and Rhodus, 1993; Khan et al., 1998a) and in pear (Vercammen et al., 2004; Asín et al, 2007).
4.1.1. Principles

Root pruning is not a new technique, but its use in commercial fruit production has been sporadic due to a reliance on chemical plant growth regulators (PGRs), conflicting evidence concerning its effect on trees (Saure, 2007) and a lack of affordable tractor mounted machinery. However over the last ten years, root pruning has become more widespread mainly due to the prohibition of certain PGRs in European apple and pear production.

Figure 4.1. Root pruning with a tractor mounted subsoiler blade.

Root pruning severs part of the root system of a tree by dragging a subsoiler blade, mounted on a toolbar and offset to the side of a tractor (Schupp and Ferree, 1987a), vertically through the soil along a row of trees (Figure 4.1.). Tree vigour and water
availability determine the distance the cut is made from the trunk and whether both sides of the row are cut in the same season (Schupp and Ferree, 1988; Vercammen et al., 2004).

4.2. Impact of root pruning on tree physiology

Shoot growth determines canopy size and also the ability of a tree to intercept sunlight for photosynthesis. On the other hand, root growth determines the extent to which a plant is able to explore the surrounding soil for water and nutrients (Hsiao and Xu, 2000). Growth of the two organs functions in competition, for assimilates and water, but also in coordination by adapting and redistributing growth in relation to changes in the environment (Hsiao and Xu, 2000; Saure, 2007). Root pruning has a fundamental effect on this physiological balance - on the tree canopy by reducing shoot vigour and on the root system itself and ultimately on fruit quality. Some of the major outcomes of root pruning are described in this section.

4.2.1. Restriction of nutrient and water access and uptake

Root pruning restricts water and nutrient uptake in two specific ways. It removes access to stored reserves in severed root tissue whilst also reducing the overall root volume available for nutrient and water uptake from the soil. Carbohydrate reserves stored in different parts of the tree, particularly in the roots (Hansen, 1967b), are crucial to apple and pear seasonal growth and determine their potential to fruit and produce vegetative growth (Khan et al, 1998b; Samid et al., 1999). Reserves can be defined as materials produced in excess of current assimilate and respiratory demands, which may be later removed from storage to support metabolism and growth (Samid et al., 1999).
The seasonal nature of root growth and activity means carbohydrate reserves do not really begin to decrease until spring (Hansen and Grauslund, 1973; Keller and Loescher, 1989). During bud break, but before the export of assimilates begins, between a $\frac{1}{2}$-$\frac{2}{3}$ of the energy required for early growth of extension shoots and leaf spurs and for flower initiation relies on stored carbohydrate (Hansen, 1971; Lakso and Goffinet, 2013). Hansen and Grauslund (1973) found root DM decreased considerably during sampling (late winter to mid-summer), even when accounting for possible root death during winter. Consequently reducing root volume through pruning not only has a profound effect on the amount of carbohydrate a tree can mobilise at the start of the growing season, but may also dictate how much can be stored by the end of it (Khan et al. 1998b).

Root pruning temporarily reduces root volume in the upper soil layer, where a tree extracts most of its water (Green and Clothier, 1999; Woodall and Ward, 2002). The ability of a tree to take up water is affected by the size and distribution of its root system (Grossnickle, 2005). There is also a fundamental relationship between water availability and assimilate production, as photosynthesis can be limited by a lack of water due to insufficient root growth, just as root growth can be limited by a lack of access to assimilates and water (Grossnickle, 2005). Greenhouse studies on young apple trees have demonstrated root pruning can significantly reduce net photosynthesis, transpiration and water potential (Geisler and Ferree, 1984; Schupp and Ferree, 1990). Woodall and Ward (2002) found root-pruned pine trees compensate for this loss of water uptake ability by increasing uptake through the remaining roots.
4.2.2. Functional equilibrium – vegetative inhibition and root (re)growth

The equilibrium between roots and shoots is an important morphological attribute and demonstrates the capacity of a tree to adapt and redistribute its growth by translocating resources to restore it (Saure, 2007).

The imbalance root pruning creates between the above and below ground parts of a tree prompts a redirection of assimilates in favour of the root system to support regrowth, whilst also reducing leaf growth and lateral extension (Geisler and Ferree, 1984; Maggs, 1964). Root pruning also temporarily reduces cytokinin concentration in roots and encourages the transport of auxin (and assimilates) to them to promote regrowth. Research suggests these new roots are formed until a threshold auxin concentration is reached (positive feedback) and any further growth is inhibited (negative feedback) (Saure, 2007; Wilson, 1988).

Root pruning increases total root surface area, as root regrowth from the pruning cut is finer and more branched (Geisler and Ferree, 1984; Ferree, 1994). Figure 4.2. clearly illustrates this phenomenon. Indeed root regeneration can occur to such an extent, that given time, the root:shoot ratio returns to its pre-root pruned value (Richards and Rowe, 1977). In addition, the reduction in top growth appears to be proportional to the severity of root pruning, with some less severe reductions in root volume having no effect (Geisler and Ferree, 1984; Khan et al., 1998a). This suggests that a threshold level of root removal must be reached to sufficiently alter the equilibrium between roots and shoots for the technique to be effective. The high concentration of roots in the upper soil layer is the main reason
why root pruning is so successful in controlling vegetative growth in apple and pear trees (Ferree, 1992; Schupp and Ferree, 1987a, 1988).

Figure 4.2. Root regrowth after root pruning (Photo: Karen Dam).

4.2.3. Modification of source-sink relationships and crop load

Carbon allocation within a plant depends on the relationship between photosynthetic source organs (leaves) and sink organs (stem, branch and root sapwood, and fruit). The source-sink relationship is a set of processes governing the flow of assimilates from source organs via a transport path to sink organs (Génard et al., 2008; Marcelis, 1996). A source organ can be defined as a net exporter of assimilates and its strength can be defined as: source size x source activity (Marcelis, 1996). On the other hand, a sink organ has the competitive power to attract assimilates and its strength can be defined as: sink size x sink activity. Size refers to the total biomass of the sink or source tissue, with sink/source
activity being the rate of uptake/production of photosynthates per unit biomass of sink/source tissue (Marcelis, 1996). Some organs function as a sink during one stage of development and a source during another. For example, young expanding leaves will import assimilates initially but will export them later in the season. Root pruning modifies this source-sink relationship and causes a tree to shift from vegetative to more reproductive growth (Khan et al., 1998a).

This source-sink relationship is central to the understanding of DM partitioning in fruit trees. Assimilates, produced in source organs by photosynthesis, can be stored at source or transported via the phloem to different sink organs. Fruit act as a strong sink, and their presence accelerates the translocation of assimilates from proximate leaves compared to leaves on fruitless spurs (Hansen, 1967a). The power of fruit as assimilate sinks can have a major impact on tree photosynthetic activity leading to an overall increase in net carbohydrate assimilation (Maggs, 1963; Wünsche and Ferguson, 2005).

In addition to its severity, the efficacy of root pruning in promoting DM partitioning and curtailing vegetative growth appears to be related to crop load. Palmer (1992) found DM production per unit leaf area in heavy cropping ‘Crispin’ apple trees was double that of deblossomed ones. Furthermore a heavy crop load can inhibit shoot and root growth (Avery, 1970; Geisler and Ferree, 1984; Schupp, 1990). Alternatively light cropping trees, or those with no fruit, maintain sink strength early in the season by producing shoot (Palmer, 1992) or root growth (Marsal et al. 2002). However these trees have limited ability to sustain this strength later in the season when shoot growth ceases due to a lack of suitable alternative sinks. Despite diverting photosynthates into shoot and trunk
thickening they are insufficient sinks, so to compensate for the lack of sink availability, their photosynthetic potential and stomatal conductance falls (Palmer, 1992; Wünsche et al., 2005).

The nature of the sink organ and its size has a role in determining the type of assimilate produced. According to Naschitz et al. (2010), a heavy crop load encourages the synthesis of soluble sugars, mainly sucrose and sorbitol in apple leaves, for translocation to fruit (the sink organ) via the phloem (see section 3.3. of Chapter 3). Alternatively a lower crop load, and so a lower sink strength, promotes the accumulation of starch, initially in leaves then in roots and other woody tissue.

4.2.4. Flower initiation

The process of flower initiation is a complex one and there are conflicting views as to the relationship between flower bud initiation, nutrient reserves and root regeneration in trees after root pruning. Root pruning is thought to promote flower bud initiation and reduce the tendency for biennial bearing (Ferree, 1992; Geisler and Ferree, 1984; Schupp and Ferree, 1990), but the results are by no means conclusive. Some report a shift from vegetative to reproductive growth after root pruning, characterised by an increase in return bloom (Asín et al., 2007; Khan et al., 1998a) and a reduction in biennial bearing (Ferree, 1992). Others report no improvement in return bloom (Schupp and Ferree, 1987a) or change in the tendency for biennial bearing (McArtney and Belton, 1992).

Flower bud initiation is the physical start of flower bud development and is a distinct process to floral induction, which occurs before initiation and commits a bud to become
floral. Flower bud initiation begins the previous season, pausing during winter dormancy, and is only completed after bud break the following year. The attainment of a critical number of nodes (leaf primordia) is the prerequisite for buds to undergo the transition from a vegetative to floral stage (Bertelsen et al., 2002; Hirst and Ferree, 1995). The rate of node development during the growing season must be fast enough to ensure the critical number is reached before the end of the season. The first visible sign of transition is the flattening of the apical dome and occurs approximately 50 DAFB (Foster et al., 2003). The process can continue until winter dormancy.

A decline in shoot carbohydrate reserves may be responsible for a drop in flower bud initiation after root pruning (Hansen and Grauslund, 1973). These reserves would ordinarily be used by developing shoots and for flower initiation, but root pruning prompts their redirection to roots for regrowth. Alternatively, improved return bloom may be related root regeneration after pruning due to increased nutrient uptake as well as greater cytokinin production and translocation to shoots (Geisler and Ferree, 1984). However improved flowering has also been associated with a reduction in root growth (Zaerr and Bonnet-Masimbert, 1987), presumably as resources are retained for use in the canopy.

Increased flower bud initiation is related to high light levels and reduced competition from shoot growth as this improves assimilate partitioning (Palmer and Jackson, 1977; Palmer, 1992). Of relevance is the negative relationship between crop load and flower bud initiation, but this is also linked to gibberellin concentration in seeds of fruit (Wünsche and Ferguson, 2005). The effect of timing of root pruning on flower bud initiation is possibly more directly related to the amount of shoot and root growth rather than timing alone.
Apart from root pruning and differences in genetic predispositions between cultivars, alternate bearing is also influenced by rootstock vigour, tree age and is related to spur bearing habit (Geisler and Ferree, 1984). An improvement in flower bud formation may be linked to a better and more stable availability of resources due to less competition from vegetative growth together with improved light interception for flower bud initiation. However other authors have found no relationship between root pruning and a reduction in alternate bearing (McArtney and Belton, 1992; Schupp and Ferree, 1987a).

4.3. Timing

Timing appears to be a crucial factor in the success of root pruning in curtailing vegetative growth and in minimising any adverse effects on fruit yield. McArtney and Belton (1992) found root pruning during the later stages of dormancy more effective at reducing shoot length than root pruning at petal fall. This is in general agreement with Schupp and Ferree (1987b) who recommended root pruning during dormancy or at full bloom. They also found root pruning later in the growing season (at June drop or preharvest) had no effect on shoot growth but increased preharvest fruit abscission. Bearing all this in mind, Vercammen et al. (2004) suggest root pruning no later than 2-3 weeks before flowering to minimise stress to the tree and discourage early fruit drop. However Ferree (1992) found the timing of root pruning made little difference to the reduction in shoot length and had no effect on preharvest fruit abscission. Although the recommendation is that it be done earlier in the growing season so as not to compromise cumulative yield.
4.4. Impact of root pruning on fruit quality

Root pruning can have a profound effect on fruit quality. Although the extent varies and is related to other factors, including crop load and environmental conditions.

4.4.1. Fruit size and yield

The main effect of root pruning on fruit quality is on harvest fruit size. Studies have shown a reduction in fruit size in many cases (Baugher et al., 1995; Ferree, 1992; Khan et al., 1998a; Miller, 1995; Schupp, 1990; Schupp and Ferree, 1987b; Schupp and Ferree, 1989) but not in others (Asín et al., 2007; Elfving et al., 1991). An important factor to consider is crop load, which is negatively correlated to fruit size and positively to yield (McArtney and Belton, 1992; Robinson and Lakso, 1989). However, even when taking crop load into account, a drop in relative fruit size after root pruning suggests the consequent fall in the number of leaves and bourse shoots, a reduced root volume and the translocation of assimilates for root regrowth often make it hard for a tree to supply sufficient water and assimilates to developing fruit (Khan et al., 1998a).

Cell number is an important contributor to fruit size, but cell expansion and volume are the main determinants (Coombe, 1976; Pritchard, 1994). Cell expansion relies on water availability and on solute uptake (Hsiao and Xu, 2000) and is facilitated by turgor pressure, which is generated by differences in osmotic potential. Solute accumulation creates differences in osmotic potential between the inside and outside of cells, thus increasing cell sap osmotic pressure (Pritchard, 1994). This draws water into cells by osmosis, which increases turgor and helps expand cell walls as they grow. Therefore
anything that upsets this water and solute uptake has the potential to alter cell expansion and fruit size.

4.4.2. Dry matter and soluble solids content

According to Wünsche and Lakso (2000a), DM partitioning is an important determinant of crop performance and yield. However to the author’s knowledge, there is no previous research specifically concerning changes in fruit DM of apples or pears as a direct result of root pruning. However existing literature regarding the general movement and partitioning of carbohydrate in root-pruned trees is useful for providing insight.

Fruit are a strong assimilate sink and there is a positive correlation between crop load and total carbohydrate partitioning to fruit (Khan et al., 1998a). Indeed DM production in heavy cropping trees can be double that of non-fruiting ones (Palmer, 1992). Whilst also considering the effect of crop load, the physiological changes brought about by root pruning could have a positive effect on fruit DM.

There is a fundamental relationship between crop DM production and seasonal accumulated light interception (Monteith, 1977; Robinson and Lakso, 1991; Wünsche and Lakso, 2000a). Therefore improved light interception, due to a reduction in canopy size after root pruning, has the potential to increase fruit DM. However DM production is also a function of the efficiency of light use and its distribution within the canopy (Jackson, 1980; Robinson and Lakso, 1989). These two factors also affect flowering, fruit set and general fruit quality (Wagenmakers and Callesen, 1989; Wünsche and Lakso, 2000a). Therefore sufficient, localised light exposure is needed at fruiting sites within the canopy.
for greater efficiency in converting light energy into fruit (Robinson and Lakso, 1991; Wünsche and Lakso, 2000b). Fruit development, 3-5 weeks after full bloom, is essentially supported by carbohydrate supplied from proximal spur leaves (Hansen 1971; Wünsche and Lakso, 2000b). Therefore a reduction in light interception at this time can alter carbohydrate distribution in favour of vegetative sinks and limit its supply to fruitlets (Garriz et al., 1998). The linear nature of the increase in fruit DW between approximately 14-40 DAFB and the suggestion that sink strength is established during this time (Figure 3.3. in Chapter 3) (Schechter et al., 1993a) makes optimisation of carbohydrate supply during cell division and early cell expansion all the more relevant.

The close relationship between DM and SSC, outlined in Chapter 3, suggests that any increase in DM is likely to prompt a rise in SSC. Therefore the increase observed in SSC in fruit from root-pruned trees (Elving et al., 1991; Ferree, 1992; Schupp and Ferree, 1987a, 1988), was probably accompanied by a similar increase in DM (although this was not measured in their research). It is also important to note the role of fruit water content when discussing DM and SSC, as both parameters are measured in relation to it. Water can skew DM and SSC values for individual fruit and give higher results.

4.4.3. Maturity, colour and firmness

Root pruning is considered by some authors to have minimal impact on apple maturity (Elving et al., 1991; Ferree and Knee, 1997), whilst others report that it can advance maturity by several weeks (McArtney and Belton, 1992). Differences in colour and firmness of fruit from root-pruned trees appear to be relatively independent of maturity. Improvements in apple colour (Baugher et al., 1995; Ferree, 1992; Schupp and Ferree,
1988, 1989) are possibly due to better light interception and better spur quality. The increase in fruit firmness (Elfving et al., 1991; Ferree, 1992) brought about by root pruning may be related to a reduction in fruit size and to a higher tissue density as a greater percentage of their volume is composed of cell wall material (Sams, 1999). Increased tissue density is also relevant when considering DM of fruit from root pruned trees.

4.5. Postharvest quality of fruit from root pruned trees

Given the changes root pruning can produce in fruit quality at harvest, there is not much specific research concerning postharvest behaviour of fruit from these trees. Nevertheless harvest fruit quality determines storage potential and postharvest quality, with differences apparent at harvest usually maintained.

Work by Elfving et al. (1991) found apples from root-pruned trees were firmer at harvest and remained so even after 19 weeks of storage. They also contained more starch at harvest and hydrolysed it at a slower rate. Fruit with a lower water content at harvest can lose less in cold storage and when left at room temperature (Kilili et al., 1996; Lopez et al., 2011). This could be due to modifications in skin structure or epicuticular waxes as a result of water stress (Behboudian et al., 2011). Another possible explanation could be linked to the reduced initial water content of these fruit, as fruit weight loss is a function of original water content (Nguyen et al., 2006).

4.6. Root pruning and water stress

Root pruning is an invasive technique and, as the previous sections have highlighted, its effect on tree vegetative and reproductive growth can be significant. Although relatively
uncommon in temperate climates, the impact of root pruning on tree physiology can be exaggerated by water stress, especially in drier years or when the crop load is high. The following sections will discuss the potential effects of water stress on fruit trees as a result of root pruning and the possibility of using supplementary irrigation to mitigate its effects without eliminating its benefits.

A sufficient water supply throughout the growing season is essential for maintaining tree evapotranspiration (ET) at its maximum potential, as there is a positive correlation between ET and carbon assimilation (Steduto et al., 2007). Therefore if the water supply is sufficiently restricted for the rate of ET to fall below the level dictated by evaporative demand there will be a reduction in biomass production (Chaves et al., 2002; Fereres and Soriano, 2007). Water availability is also essential for other plant processes, including, hormonal regulation, assimilate translocation and respiration. However water stress affects them differently as photosynthesis and the translocation of assimilates are not inhibited at levels of stress that inhibit cell expansion, which is especially sensitive (Hsiao et al., 1976).

As mentioned, in relation to fruit size in root-pruned trees (Section 4.4.1.), cell expansion is aided by turgor pressure. So to sustain growth, there must be water available for transportation into the cell. Any environmental change that inhibits this movement or availability of water can impact on growth, be it in leaves, roots or fruit (Behboudian and Mills, 1997). Furthermore plant water status is not only affected by soil water status, atmospheric conditions (radiation, wind speed, air temperature and humidity) also play a part.
The literature on deficit irrigation provides the best insight into the effects of water stress on tree physiology and fruit quality as the technique essentially uses a level of water stress to force a change in tree growth and development. Indeed water stress has a similar impact on trees as root pruning.

4.6.1. Impact of water stress on tree physiology

In common with root pruning, water stress can change the growth pattern of a tree by forcing a redistribution of its resources in an attempt to restore/maintain the status quo (Chaves et al., 2002). Leaf growth is more inhibited than root growth under conditions of low soil water availability (Munns, 2002; Sharp and Davies 1989) with roots limiting the supply of water to shoots until their own requirement for water is satisfied (Hsiao and Xu, 2000). Newly emerging leaves and shoots are the most sensitive to water stress, as they are furthest away from the roots and are connected to the rest of the canopy via immature stems and petioles that are highly resistant to water movement. Therefore any reduction in water supply may slow their growth leading to a smaller canopy and a reduced water requirement (Hsiao and Xu, 2000). The greater efficiency of pre-full bloom root pruning in curtailing shoot growth is possibly related to this sensitivity.

Reduced water availability can modify root development as the tree tries to ameliorate water stress by mining for new sources of soil water. Roots of well-irrigated plants tend to proliferate in the upper soil layers, whilst root growth of plants under conditions of low soil water availability tends to be more explorative as they grow deeper and longer into new soil in search of more moisture (Hsiao and Xu, 2000).
Return bloom can be improved the following season under conditions of reduced water availability (Asín et al., 2007; Marsal et al., 2002; Mitchell et al., 1986) or inhibit it (Kilili et al., 1996; Behboudian et al., 1998). However in some instances there is no effect (Mills et al., 1994). It can also limit the tendency for biennial bearing (Behboudian and Mills, 1997).

**4.6.2. Impact of water stress on fruit quality**

Water stress can reduce harvest fruit size in apple (Ebel et al., 1993; Mpelasoka et al., 2001) and in pear (Marsal et al., 2000). However the extent of its effect on fruit size seems to be dependent on its duration and severity, the stage at which it is applied (Behboudian and Lawes, 1994; Behboudian and Mills, 1997) and the crop load of the tree (Ebel et al., 1995; Marsal et al., 2002).

Water stress increases SSC in apple (Ebel et al., 1993 and 1995; Lopez et al., 2011; Mills et al., 1994; Mpelasoka et al., 2001) and in pear (Behboudian and Lawes, 1994; Raese et al., 1982). This may be due to advanced maturity and the faster conversion of starch into sugars (Mpelasoka et al. (2001), fruit dehydration (Lopez et al., 2011) or osmotic adjustment (Behboudian and Lawes, 1994), whereby drought stressed plants and fruit actively accumulate solutes (principally sorbitol) and decrease plant water potential in order to maintain cell turgor (Berüter, 1989; Davies and Lakso, 1979). However it is often hard to tell if the increase in SSC is due to dehydration or osmotic adjustment unless fruit DW is also compared (Behboudian and Lawes, 1994). According to research on deficit-irrigated apples, fruit with higher SSC and starch levels at harvest can also exhibit slower starch hydrolysis (Ebel et al., 1993; Elfving et al., 1991). This may be related to higher SSC as it may be sufficient to satisfy the increase in energy requirements for respiration during
climacteric ripening, thus slowing hydrolysis (Ebel et al., 1993). Lopez et al. (2011) argue that any changes in quality of deficit-irrigated fruit are more related to adaptations to water stress rather than actual changes in maturity.

There is not much information concerning water stress and DM of fruit, except that they tend to have a higher percentage (Kilili et al., 1996; Lopez et al., 2011; Mpelasoka et al., 2001). It may be due to improved carbohydrate partitioning to fruit instead of trunk thickening (Ebel et al., 1995) or the increase could be for similar reasons as in SSC: due to less cellular expansion and hydration (Mpelasoka et al., 2001) and to osmotic adjustment. The accumulation of carbohydrate in water stressed apples may play a role in colour development, as sucrose is important for anthocyanin synthesis (Mills et al., 1994; Westwood, 2009). The reduction in vegetative growth associated with water stress may also be a factor in improved fruit colour due to better light interception.

**4.7. Supplementary irrigation of root pruned trees**

Temporary water deficits during the growing season are possible in fruit trees, especially if the crop load is high, and it coincides with warm, dry weather (Janssens et al., 2012; Behboudian et al., 2011). Pears can be prone to water stress and even in a temperate climate, where evaporative demand is generally low, irrigation of root pruned, field-grown trees could be beneficial to ensure consistent fruit size and yield (Janssens et al., 2011).

Regulation of the water supply to root pruned trees seems essential in order for the combination not to be counterproductive by encouraging vegetative growth, especially in situations where soil fertility is high or where there is a possibility of heavy rainfall.
(Behboudian and Mills, 1997; Ferree, 1992). A technique such as deficit irrigation supplies water at a rate below the evaporative demand of the tree throughout the growing season or at specific periods during crop development. It is a management system that is intended to limit vegetative growth (Forshey and Elfving, 1989; Richards and Rowe, 1977) and benefit fruit quality (Behboudian and Mills, 1997; Fereres and Evans, 2006), as well as conserving water.

Any attempt to develop a strategy combining root pruning and supplementary irrigation must consider how to co-ordinate both around the annual cycle of shoot, root and fruit development. Early season root pruning seems best (before full bloom), for maximum impact on vegetative growth reduction and minimal fruit abscission (Behboudian et al., 2011; Vercammen et al., 2004). However an early season water deficit during full bloom can inhibit fertilisation and fruit set and promote fruitlet abscission (Behboudian and Mills, 1997). Alternatively supplementary irrigation may also be of benefit if given later in the season, during the main fruit expansion period, after shoot growth has peaked.

Irrigation seems to be most beneficial to fruit quality if supplied later in the season, during fruit expansion. Shoot growth is also past its peak then and the fruit load is sufficient to act as a strong sink. Furthermore sensitivity to water stress affects plant processes differently: photosynthesis and translocation of assimilates are not inhibited at levels of water stress/water potentials that inhibit cell expansion, which is especially sensitive (Hsiao et al., 1976).
In summary, root pruning has a profound effect on all aspects of tree physiology and fruit quality, not all of them beneficial. The possibility of using supplementary irrigation to counter some of the negative effects on fruit quality was the impetus behind the research documented in Papers 2. and 4.
Chapter 5 – Near Infrared Spectroscopy

The term ‘spectroscopy’ (spec-tros-co-py [/pekˈtraːskəpə/]) originates from the Latin ‘spectrum’ (appearance, image) and the Greek ‘skopia’ (‘to view’). It is the science of the interactions between electromagnetic radiation and the atoms and molecules of a sample, with the intention of identifying and/or quantifying the constituents of that sample. It has a variety of applications in the pharmaceutical, agricultural and horticultural industries. Between 1930-1980 the total number of papers published on near infrared spectroscopy (NIR) specifically was approximately 255. This quadrupled in the 1980s as a result of increased research (Wang and Paliwal, 2007). Over the last 20 years, developments in probes and fibre-optics, detectors and advances in chemometric analysis have firmly established its value as a technique. This chapter outlines the basic principles of NIR spectroscopy and instrumentation and its application in predicting fruit quality.

5.1. The electromagnetic spectrum

The electromagnetic spectrum ranges from long wave radiation, used for radio communication, to short wave gamma radiation and includes the visible spectrum between approximately 380-780 nm (Figure 5.1). Beyond the visible spectrum is the infrared region. It is invisible to the naked eye, but as it is close to the red region of the visible spectrum it is commonly referred to as the infrared region. It is further split into:

- Near Infrared (NIR/Near IR) – between 780-2500 nm.
- Mid Infrared (MIR/Mid IR) – between 2500-5000 nm.
- Far Infrared (FIR/Far IR) – between 5000-10000 nm (5-10 µm).
There are no precisely defined boundaries between bands of the electromagnetic spectrum, as radiation located on the cusp of a region will possess properties of the two spectral regions that border it.

Figure 5.1. The electromagnetic spectrum (www.math.montana.edu), emphasising visible radiation. \( \tilde{\nu} = \text{wavenumber}, \lambda = \text{wavelength}. \)

The energy \( (E) \) of electromagnetic radiation is directly related to its wavelength and frequency. Wavelength \( (\lambda) \) is the distance over which a wave’s shape repeats and is measured in nanometers \( (1 \text{ nm} = 10^{-9} \text{ m}) \). Shorter wavelengths have higher frequency \( (\nu) \) (the number of waves passing a fixed point per second) and so higher wavenumber \( (\tilde{\nu}) \) and energy. Wavenumber can be defined as the number of wave cycles in one centimetre. It is reciprocal to wavelength and is measured in inverse centimetres \( (\text{cm}^{-1}) \). Spectroscopists often prefer to use \text{cm}^{-1} \) rather than nm as a unit of measurement.
5.2. Principles of spectroscopy

Molecules can be considered as having two groups of properties: static and dynamic. Static properties include atomic composition, isomeric structure and morphology whilst dynamic properties refer to molecular energy states (translational, electronic, rotational and vibrational) (Miller, 2001). The dynamic properties of a molecule are directly responsible for spectra and are related to its static properties. This relationship is the foundation of spectroscopic analysis.

5.3. Vibrational spectroscopy

NIR spectroscopy is commonly referred to as vibrational spectroscopy and also includes Raman and mid-infrared (MIR) spectroscopy techniques. They all exploit the same principle: that there is a relationship between the energy of molecular bond vibrations and that of light radiation. Vibration energy refers to the energy of the bond-induced periodic oscillations of atoms in a molecule – similar to the movement of two balls connected by a spring (Miller, 2001). These oscillations, or vibrations, have frequencies and modes determined by the molecule’s atomic properties and the spatial arrangement of the atoms. For instance water (H$_2$O) has three atoms in a non-linear arrangement and three vibrational modes (Figure 5.2).
However molecules do not vibrate in harmony. In fact they are intrinsically anharmonic, because atomic nuclei if pressed sufficiently close together will repel each other and if forced far enough apart will disassociate (Osborne, 2000) (Figure 5.3). The greater the anharmonicity, the more intense the vibrations will be. The most anharmonic bonds are created when molecules are composed of atoms with different atomic masses, such as when a lighter H is bonded to a heavier carbon, oxygen or nitrogen molecule. Anharmonicity makes NIR spectroscopy possible as the energy of these vibrations matches that of radiation in the NIR region. Furthermore, absorbance of electromagnetic radiation can only occur if the periodic vibrations of the molecule are sufficient for an electric dipole moment (a temporary change in the molecular polarity of a molecule) to occur (Wilson, 1994). The extent of anharmonicity and the change in dipole moment dictate the intensity of a vibrational band and allow transitions to higher energy levels, thus creating NIR spectra.
Figure 5.3. Schematic illustration of the anharmonic oscillator model (Morse Curve). \( v \), potential energy; \( m_1 \) and \( m_2 \), mass of atoms (after Miller, 2001).

The molecular bond vibrations of a sample are stimulated by applying energy in the form of electromagnetic radiation. These bonds are typically at their lowest-energy state (ground state) and the stimulus causes them to become excited and transition to a higher-energy level (Miller, 2001).

According to quantum theory, a molecular vibration is elicited when a molecule absorbs a quantum of energy, \( E \), corresponding to its vibration frequency \( (v) \) (as per the equation \( E = h\nu \), where \( h \) is Planck’s constant). A fundamental vibration results when the molecule at its lowest energy or ground state absorbs one quantum of energy \( (v = 1) \). The first overtone \( (v = 2) \) is excited when two quanta are absorbed and so on to higher overtones. Therefore for each fundamental vibration there exists a corresponding series of overtones and
combinations. Each successive overtone is approximately an order of magnitude less intense that the preceding one (Osborne, 2000). Quantum theory also only permits certain vibrational transitions in the NIR range – namely overtone and combination bands (simultaneous excitation of two or more fundamental vibrations) (Wetzel, 1998).

5.4. Spectral band assignment

NIR spectra are characterised by broad undulating peaks spanning several wavelengths composed of overlapping overtone and combination bands. In contrast to IR peaks, which are narrow and diagnostic, NIR peaks can span between 100-150 nm (Walsh et al., 2000). For example Figure 5.4. shows typical absorbance spectra for ‘Elstar’ apples. The peak between 1400-1500nm is composed of O-H and N-H 1st overtones whilst the smaller peak at ~1150 nm contains additional C-H 2nd overtones. Furthermore the chemical structure of carbohydrate is complex and the presence of other atoms modifies the vibrational frequencies of all the bonds contained in that molecule (Greensill, 2000). This makes specific spectral band assignment difficult.

Figure 5.4. Typical apple (cv. Elstar) absorbance (Log(1/R)) spectra between 1000-1800 nm, minus noise.
Figure 5.5. shows a band assignment chart, including the main functional groups present in NIR spectra. However as interpretation of NIR spectra is not straightforward and as most band assignments are based on empirical rather than molecular evidence (Xiaobo et al., 2010) it is best to see it as a general reference.

Figure 5.5. Overtone and combination band assignments of NIR spectra (www.brukeroptics.com)

5.5. Sample and radiation interactions

The Beer-Lambert Law is fundamental to spectroscopy and is based on the principle of linear absorbance of light in a sample. However the interaction of light with a sample is more complex and is heavily dependent on the nature of the sample in question.
5.5.1. Absorbance and the Beer-Lambert Law: the theory

The Beer-Lambert Law states that the amount of light absorbed ($A$) by a sample depends on the concentration ($c$) of a compound in a sample; the distance the light must travel through ($l$, path length); and the specific molecular properties of the sample as these influence the wavelengths absorbed ($\varepsilon$, molar absorptivity or extinction coefficient). The Beer-Lambert Law is written:

$$A = -\log_{10}(P/P_0) = \varepsilon lc$$

However it is difficult to measure the actual absorbance of light. It is far simpler to consider light in terms of what is transmitted or reflected through or by a sample, as both modes are directly related to absorbance. The definition of transmittance ($T$) according to the Beer-Lambert Law states that $T = P/P_0$, where $P$ is transmitted radiation and $P_0$ is incident radiation. $P_0$ changes as light passes through a sample as some radiation is absorbed or scattered, therefore the relationship between transmittance and concentration or pathlength is logarithmic rather than linear. Conversely the relationship between absorbance and concentration or path length is linear, making the relationship between transmittance and absorbance, logarithmic ($A = -\log_{10}(P/P_0)$).

5.5.2. Absorbance and the Beer-Lambert Law: the practice

The Beer-Lambert Law relies on the definition of path length in order to determine sample concentration. However in practice the movement of light through a biological sample, especially if it is solid, is rarely linear and unidirectional. Scattering alters path length making it hard to define and so invalidates the Law (Osborne, 2000). For example in some liquid samples light is still transmitted, but there can be scattering due to particles present
(fat globules in milk). This is referred to as diffuse transmittance (Figure 5.6.). Diffuse transmittance spectroscopy is possible using wavelengths between ~800-1100 nm on solid samples between 1-2 cm thick as the radiation is intense enough to allow a certain amount to pass through the sample (Osborne, 2000).

![Figure 5.6. Schema of some of the interactions of light radiation with an object (Abbott, 1999).](image)

Longer NIR spectra (~1100-2500 nm) do not penetrate as far in a solid sample therefore the amount of radiation scatter means transmittance beyond 10 mm is negligible. If light does not penetrate the sample and no absorption takes place, specular reflectance occurs. Diffuse reflectance is a common method used in research relating to fruit quality prediction (Bureau et al., 2009; Lu et al., 2000; Park et al., 2003). This occurs where most of the incident radiation is reflected whilst some is absorbed or transmitted (depending on sample size and thickness). Chemometric transformation can mathematically relate diffusely reflected radiation (R) to concentration (c) and so satisfy the requirements of the Beer-Lambert Law. Other adaptations of diffuse reflectance include transflectance, where radiation is transmitted through the sample, reflected on a ceramic tile and then
transmitted back through the sample before reaching the detector. Alternatively, interactance involves two fibre-optic probes (one lighting and the other detecting) located at different points on the surface of the sample. It is a compromise between reflection and transmission modes and is commonly used when predicting fruit quality (Clark et al., 2003; Delwiche et al., 2008; McGlone et al., 2003). These spectral acquisition modes can also be converted to absorbance using chemometrics as they represent equivalent information. That is, transmittance or reflectance spectra will have maximum intensities at wavelengths where absorption is weakest. Likewise, as in Figure 5.4., absorbance spectra will have maximum intensities at wavelengths where absorption is the strongest.

5.6. Instrumentation

In simple terms, a spectrometer is composed of a radiation source; a means of selecting specific wavelengths (monochromator); a mechanism of exposing a sample to radiation (fibre optic probe); a detector to collect the return signal and some method of recording the data. According to Nicolaï et al. (2007) instruments are classified according to monochromator type and include:

1. Filter – a wheel carrying a number of filters.
2. Scanning – a grating or prism is used to separate individual wavelengths.
3. Fourier Transform (FT) – repeatedly passes a beam of light containing many frequencies through a sample, modifying it each time to contain another combination of frequencies.
4. Photodiode Array (PDA) – a fixed grating focuses radiation onto an array of SI (silicon, 350-1100 nm) or InGaAs (indium gallium arsenide, 1100-2500 nm) photodiode detectors.
5. Laser – a laser light source or tunable laser instead of a monochromator.

6. Acoustic Optic Tunable Filter (AOTF) – shift the frequency of light using sound waves.

7. Liquid Crystal Tunable Filter (LCTF) – use liquid crystal elements to select radiation and exclude others.

Two instruments were used during the course of this PhD project. A LabSpec 5000 Vis/NIR spectrometer was used during the first year of the project. It is a semi-portable machine with a spectral range of 350-1050 nm (Si) and 1000-2500 nm (InGaAs) when both detectors are fitted. Paz et al. (2009) used a similar instrument in their paper concerning the prediction of pear quality. During the second year an AgriQuant is an FT-NIR analyser fitted with interchangeable InGaAs and Si detectors was used. Both instruments were fitted with hand held fibre optic probes.

Figure 5.7. The LabSpec 5000 (left) (www.asdi.com) and the AgriQuant FT-NIR (right) (Photo: Connie Damgaard).
Chapter 6 – Chemometrics

The complex chemical composition of fruit, as discussed in Chapter 3, together with the high absorbance of water and excitation of overtone and combination bands in the NIR region means that spectra are highly convoluted and collinear (Nicolaï et al., 2007). Collinearity is common in spectroscopy due to the large number of variables contained in a spectrum of often more than a hundred wavelengths when the sample set is usually much smaller. However even if the sample set were larger, the limited source of variation within it simply prevents its elimination in spectral data.

Radiation scattering and sample or instrument variation add further complication, making it hard to assign spectra to particular functional groups or chemical components. In addition, the data generated often consists of hundreds or thousands of variables (Bobelyn et al., 2010). Chemometric methods are specifically designed for dealing with this otherwise problematic data.

6.1. Principles

Chemometrics aims to explore and model the underlying relationships in chemical systems via multivariate statistics, applied mathematics and computer science (Geladi and Dåbak, 1995). Multivariate methods, as used in chemometrics, are essentially tools that consider multiple variables simultaneously and so view a chemical problem holistically. By taking all variables into account chemometric models can reveal unexpected patterns in the data (Wold, 1995). Chemometrics uses *a posteriori* information to generate new hypotheses from novel representations of the data so does not assume all hypotheses *a priori* (Wold,
It is also highly graphical in its approach thus enabling visual identification of the inner structure of large datasets.

### 6.1.1. The importance of models

Chemometric modelling relates experimentally determined variables to each other. There are two major types of model: those for exploratory analysis and those for prediction. In the case of spectroscopic data, exploratory models show how individual spectra relate to each other, which spectra are similar or different, trends in spectral behaviour, as well as providing information about outliers or extreme spectra. Principal component analysis is the most popular method for exploratory data analysis in chemometrics.

Prediction of fruit quality requires the construction of a regression model. This model serves to ‘train’ the instrument to allow prediction of future samples. Therefore a regression model is a quantitative way of linking spectral information to sample components. Clearly a model’s success depends on the quality and breadth of information used to build it. Therefore preliminary data exploration and preprocessing are vital in optimising model success by eliminating outliers and reducing noise and scatter.

### 6.1.2. Noise

Variability or noise is generally present in a dataset or measurement. The deviation between a measured value and a model can be attributed to three major things. Firstly, lack of complete control over experimental conditions (temperature, sample concentration etc.) makes it impossible to maintain exactly the same conditions next time the experiment is undertaken. With reference to fruit, a persistent source of noise is the biological
variability of samples, from the same tree, cultivar or indeed within the same fruit (Bobelyn et al., 2010). Furthermore fruit are dynamic entities; they change with time either during development on the tree or in postharvest storage. Secondly, the spectrometer is not strictly stable as it is influenced by factors such as temperature and humidity. Finally, modelling errors due to the inherent simplification and approximation required to build the model. Wold (1995) concludes that all chemical data are contaminated with noise, be it random (environmental) or systematic (due to restrictions of the model).

6.2. Data preprocessing

As mentioned, the success of a model depends on the quality of the data used to build it. Before modelling takes place any preprocessing required must be completed to ensure optimal extraction of relevant information. Depending on the method of acquisition, spectra may need to be transformed from reflectance or transmittance to absorbance. Radiation scatter due to particle size, especially in solid samples such as fruit, is a major cause of variation in spectra. Certain transformations are able to reduce the influence of particle size and scatter removing spectral baseline shifts and changes in slope. Other techniques help distinguish overlapping spectral features.

6.2.1. Spectral transformation

Transmission spectra can be quantified using the Beer-Lambert Law (where concentration is approximated as log(1/T) and reflectance measurements generally converted to log(1/R). However sample interactions are not straightforward and spectra are complicated by variation in sample morphology and temperature. Kubelka-Munck transformation does
account for scattering but log(1/R) transformation is considered preferable for agricultural products. However Nicolaï et al. (2006) argue there does not seem to be any great advantage in transforming spectra.

### 6.2.2. Mean centring

This is a very important step in data preprocessing and requires subtracting the mean value from each variable. It highlights similarities and differences between samples. It centres the data cloud in a variable space and therefore highlights similarities and differences between samples. Centring is an essential step for most chemometric methods.

### 6.2.3. Auto-scaling or weighting

This divides each variable by its standard deviation and is necessary when data are measured in different units (mg, g, %, °Brix etc.). Variables become more comparable as no single variable can dominate or skew the model. Principle Component Analysis (PCA) and PLS regression (both explained later in this chapter) look for systematic variation, whilst scaling allows subtle variations to play the same role as larger ones. However this is not required or recommended with spectra as the units are the same and by scaling, important information can be lost or noise over emphasised (Wold et al., 1987).

### 6.2.4. Spectral smoothing – Savitsky-Golay

Spectral smoothing functions to increase the signal to noise ratio to maximise signal intensity. Savitzky-Golay transformation is often employed when using derivative spectra to remove scatter and noise without distorting the true signal responsible for the recorded intensities. The transformation performs noise reduction while also preserving the
character of the spectra (Ruffin and King, 1999). Although some argue against the use of smoothing as it removes information before it is clear whether it is useful (Nicolaï et al., 2007).

### 6.2.5. Minimising scatter - standard normal variate (SNV) and multiplicative scatter correction (MSC)

In instances of high scatter, there can be additive and multiplicative effects where both baseline shift and slope are evident in the spectra. Neither of these effects is present in reference spectra. Both phenomena are dealt with effectively these two transformations.

Standard normal variate (SNV) removes interference caused by scatter while and multiplicative scatter correction (MSC) corrects baseline shifts and differences in pathlength that affect absorbance. SNV centres each spectrum and scales it by its own standard deviation (Candolfi et al., 1999). By treating each spectrum individually, the slope of the spectra is not altered but their baseline is shifted (offset correction). MSC is based on the presumption that all samples have the same scatter coefficient at all wavelengths. It corrects the scatter level to that of an ‘ideal’ sample's spectrum, which is usually the average spectrum (Geladi et al., 1985). Both methods preserve original spectral orientation.

### 6.3. Data exploration – principle component analysis (PCA)

Principle component analysis (PCA) lies at the core of chemometric analysis. It is a method of condensing large amounts of data into a few representative components that describe variation in the dataset/data matrix without omitting any useful information. PCA is an
unsupervised step in multivariate analysis and enables visualisation of the latent structure and pattern of dataset and allows early identification of outliers (Wold et al., 1987). It can be expressed as:

$$X = TP' + E = \text{explained variance} + \text{residual variance}$$

PCA transforms a data matrix (X) of potentially correlated variables into a new set of orthogonal variables known as principle components (PCs) (or latent variables, LVs) (Esbensen, 2009; Wold et al., 1987). The number of PCs is less than or equal to the number of original variables. PCs are connected to the original data matrix via a scores matrix (T), containing quantitative information about samples, and a loadings matrix (P), containing quantitative information about variables. PCA is performed by repeated subtraction of the largest variation in the data until all that remains is unsystematic or unexplained (Wold et al., 1987). Therefore the 1st PC accounts for the largest variation in the data with each succeeding component including the highest remaining variance possible on the condition that it is unrelated to the preceding one.

Unexplained variance is referred to as a residual (E) and is not part of the model per se. Residual values reveal samples badly described by the model and are used to identify and confirm sample and variable outliers. If residuals are large then there is a large amount of unexplained variance and any model created does not sufficiently ‘fit’ or represent the data (Esbensen, 2009).

During exploratory analysis of data for Paper 3., PCA was used to find patterns in preharvest and harvest quality data for pears grown under different irrigation strategies in 2011. According to the scores plot (Figure 6.1.), harvest quality of NP (non-root pruned)
fruit was very different to RP fruit and to fruit from other treatments. The loadings plot shows NP pears had the lowest DM and SSC and were the greenest and firmest. This was confirmed by the reference data ANOVA and LDuncan test results used in Paper 2.

Figure 6.1. Scores plot (top) and Loadings plot (bottom) for pear harvest quality analysis (2011). NI, non-irrigation; DI, deficit irrigation; FI; full irrigation; RP, root-pruned; NP, non-root pruned.

When using PCA it is assumed that the phenomena we are looking for will be represented in the dataset. Furthermore as PCs are ordered according to decreasing explained variance,
it implies that the first PCs correlate with the information we are looking for, whilst the latter ones are noise and so irrelevant in clarifying the data structure.

### 6.4. Partial least squares (PLS) regression

PLS regression is a supervised method commonly used in spectroscopy for correlating spectral data (X) with chemical reference data (Y). PLS regression is similar to PCA in that it uses latent variables. However in PLS regression the decomposition of X is guided by variation in Y (i.e. the co-variance between X and Y is maximised) so variation in X directly correlated to Y is extracted. An important benefit of PLS regression is that it can analyse data with strongly correlated, noisy and numerous X-variables (in comparison to Y), whilst simultaneously modelling several response variables (Wold et al., 2001). Its purpose is to build a linear model to predict y (a chemical component) from X (a spectrum). In order to obtain good prediction, the model needs to be calibrated on samples that span the range of future Y samples as the model cannot extrapolate (Wold et al., 2001). Residuals are again applied to aspects of the data not explained by the model.

PLS regression has two functions: predicting Y values of future objects and identification of information in X that is important for Y in order to understand the relationship between the two. It is a standard tool in chemometrics and has been used in a variety of research articles concerning the prediction of quality in fruit (Table 7.2., Chapter 7.).

### 6.5 Model validation

Employing NIR spectroscopy to assess fruit quality requires assessment (validation) of a regression model's robustness and predictive ability. Nicolaï et al. (2007) defined a robust
model as one whose predictive accuracy is relatively insensitive to unknown changes in external factors. Such factors include within orchard (tree location, shading, access to water), within tree (fruit position, light exposure etc.) and within fruit biological variability.

Validation determines the optimal number of PCs to include to avoid overfitting, detects outliers and estimates prediction error. Cross-validation is used when there are only a limited number of samples, so all are required for calibration and validation. It divides the dataset into a (uneven or even) number of segments (containing one or more samples) and builds successive regression models, each time leaving out a different segment. On each run, the omitted segment is predicted using the model. The cumulative prediction error of these models is estimated as the root mean square error of cross-validation (RMSECV). In order to get more robust estimation of prediction errors the cross-validation procedure can be repeated several times (repeated cross-validation).

Esbensen and Geladi (2010) consider all variants of cross-validation as structurally sub-optimal to the true objective of validation, which is to assess prediction performance and estimate the most realistic prediction error. They argue that cross-validation merely functions as an internal sub-setting variance simulation of test set validation. It misses the critical sampling variance (a manifestation of two independent samplings), which can only be incorporated into the validation by basing it on a proper independent test set. Therefore it is essential the researcher make provision for obtaining a proper test set based on the same protocol used to select the calibration set (but based on different samples e.g. from a different day or harvest). The best alternative according to Esbensen and Geladi
(2010) is splitting the dataset into two subsets: a calibration set and a (dependent) test set. However this is only possible when the dataset is large enough to be divided. There are two ways of selecting test set samples: by random selection (which can be repeated to assess the subset sampling variance) or by y-sorted systematic selection. The latter is the one closest resembling as proper test set and is the method used in the chemometric analysis for Paper 1. Although it does not fill the ‘gap’, illustrated in the predicted versus measured plots (see Figure 3., Paper 1.), sorting the y-values before splitting ensures similar coverage of the experimental domain by the two subsets.

6.6. Model evaluation

Model robustness and accuracy are based on the evaluation of certain attributes including root mean squared error of calibration (RMSEC); of cross-validation (RMSECV) and of prediction (RMSEP); the number of PCs; determination coefficient (R² value); regression coefficients and residual predictive deviation (RPD). RMSE values are measures of the average difference between predicted and measured response values for calibration, cross-validation or prediction models (in cases where errors are distributed normally). For model validation, the optimal numbers of PCs chosen is a balance between keeping the RMSECV value at a minimum whilst also avoiding overfitting. The suggested number of PCs also informs the number recommended for the prediction model. R², the coefficient of determination, is used in regression analysis to measure how well a model fits by evaluating the strength of the relationship between observed and expected values. RPD is defined as the ratio between the standard deviation of the reference values (SD) and the standard error SECV or SEP) of the model. It is useful for evaluating how well a model can
predict data (Nicolaï et al., 2007; Cozzolino et al., 2008). The greater the value, the more robust the model and the higher the probability it can predict chemical data (Table 6.1).

<table>
<thead>
<tr>
<th>RPD</th>
<th>Classification</th>
<th>Application</th>
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<tbody>
<tr>
<td>0.0-2.3</td>
<td>V. poor</td>
<td>Not recommended</td>
</tr>
<tr>
<td>2.4-3.0</td>
<td>Poor</td>
<td>V. rough screening</td>
</tr>
<tr>
<td>3.1-4.9</td>
<td>Fair</td>
<td>Screening</td>
</tr>
<tr>
<td>5.0-6.4</td>
<td>Good</td>
<td>Quality control</td>
</tr>
<tr>
<td>6.5-7.9</td>
<td>V. good</td>
<td>Process control</td>
</tr>
<tr>
<td>8.0-</td>
<td>Excellent</td>
<td>Any application</td>
</tr>
</tbody>
</table>

For accurate prediction model evaluation these indices should be viewed in relation to one another. For instance, the range of the dataset influences the $R^2$ value as the broader it is the more likelihood of linearity. Furthermore including the RMSEP value and number of PCs used to build the model gives a more rounded view of model accuracy. The RPD is a valuable tool, but a narrow data range and a small sample set can bias the result. Regression coefficients are a numerical value expressing the link between variation in the predictors and variation in the response. A small regression coefficient may be a sign of an unimportant or noisy variable but, unless data are auto-scaled, it may also be due to large measurement values or the interaction between several variables (Esbensen, 2009).
6.7. Variable selection

Variable selection optimises model predictive ability by excluding noisy variables and those that do not explain variation in Y. Variable selection can be performed before or during regression modelling. However outliers should be removed and all preprocessing should be complete beforehand. There are numerous methods, but only the two used during this PhD will be described: interval partial least-squares regression (iPLS) and continual adaptive reweighted sampling (CARS).

6.7.1. Interval partial least squares (iPLS) regression

iPLS (Nørgaard et al., 2000; Xiaobo et al., 2010) develops local PLS models based on equidistant sub-intervals of the full spectrum thereby focusing on important regions and excluding less relevant ones. One of its main advantages is that it is a graphical approach so gives a clear overview of spectral data and important variables. The method compares RMSECVs of these local models to a model based on all variables. The local model yielding the lowest RMSECV is recommended. $R^2$ and number of PCs are also evaluated to ensure a rounded model overview. This method was employed in Paper 1. in an attempt to improve DM and SSC prediction models.

Models based on iPLS usually require a different number of components compared to full spectrum ones to capture the relevant variation in $y$ (Xiaobo et al., 2010). However the dimensions on which the local models are based must all be the same to allow a fair comparison.
6.7.2. Competitive adaptive reweighted sampling (CARS)

This technique was employed in Paper 3., and it functions by selecting an optimal combination of wavelengths from the full spectrum and, together with PLS regression, uses the principle of ‘survival of the fittest’ to build a regression model (Li et al., 2009). CARS has four successive steps. Firstly, a fixed ratio of samples (80-90 % of the calibration set) is selected using Monte Carlo (MC) sampling and a PLS regression model built. The absolute value of the regression coefficient is used to evaluate wavelength importance. Enforced wavelength selection is then employed using the exponentially decreasing function (EDF) and adaptive reweighted sampling (ARS) randomly selects important variables. Finally, the subset with the lowest RMSECV is chosen. Consideration only of the regression coefficient value for each variable is a major drawback of CARS. It does not always reflect the real importance of a variable as it may change when different samples are collected in a calibration set (Zheng et al., 2012).
Chapter 7 - NIR Spectroscopy and Fruit Quality

Investigation of fruit internal quality with NIR spectroscopy dates back as far as 1956 (Davies and Grant, 1987), and since then it has been a huge focus of research. Of all the non-destructive quality assessment techniques, NIR spectroscopy is arguably the most advanced as regards instrumentation, applications, accessories and availability of chemometric software (Magwaza et al., 2012).

The primary interest has been in the prediction of quality attributes such as SSC, firmness, acidity and pH to ensure proper estimation of harvest time and grading for postharvest storage. The relevance of NIR in harvest and postharvest assessment of fruit is evidenced by the amount of research devoted to it and attempts to incorporate the technology into on-line grading machines (Abbott, 1999; Nicolaï et al., 2007).

7.1. Prediction of fruit quality

As previously mentioned, lighter hydrogen atoms attached to heavier carbon, nitrogen and oxygen atoms are responsible for the presence of strong overtones and combinations in the NIR region. This restricts the number of compounds visible in the NIR region to mainly organic ones (Xiaobo et al., 2010), making NIR spectroscopy ideal for analysis of samples, such as fruit, with high water and carbohydrate contents. NIR spectra of complex molecules, such as those found in fruit, typically have broad peaks containing multiple absorption bands. The absorption of water dominates spectra, with overtone bands of O-H bonds located at 760, 970 and 1450 nm and a combination band at 1940 nm (Polesello and Giangiacomo, 1983), by broadening and shifting peaks, even of molecules not associated with O-H groups (Lin and Ying, 2009). Nevertheless absorption in this region is extremely
weak, and only permits identification of macro-constituents at concentrations above 1 %, making fruit an ideal subject (McGlone and Kawano, 1998). Most intact fruit is measured in reflectance mode as it gives a better predictive outcome and contact with the fruit is not required in order to obtain spectra. Fruit size is a factor in transmission mode due to low light penetration (Sanchez, 2013).

NIR spectroscopy has been used extensively to predict maturity and storage quality of a number of fruit ranging from kiwifruit to prunes. Nicolaï et al. (2007) in their review paper provide a comprehensive assessment of previous research. As this thesis is concerned with the prediction of apple and pear fruit quality, only papers relating to these two fruit types will be discussed. Much more research has been carried out on apples, approximately 280 papers to date (Sanchez, 2013), than on any other fruit. Table 7.1. presents a selection of recent research on apple quality prediction using NIR spectroscopy, outlining the wavelength ranges used and parameters measured.
Table 7.1. Selection of NIR spectroscopy papers, including wavelengths used and parameters measured

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mode</th>
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<th>Postharvest</th>
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<td></td>
<td>SSC</td>
<td>light penetration, skin transmittance</td>
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<td>Liu and Ying (2005)</td>
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<td></td>
<td></td>
<td>SSC, TA</td>
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<td></td>
<td></td>
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<td>prediction of maturity and storage life</td>
</tr>
<tr>
<td>Pear</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Jiang and Zhu (2013)</td>
<td>reflectance</td>
<td></td>
<td></td>
<td></td>
<td>Firmness, SSC</td>
<td></td>
</tr>
<tr>
<td>Li et al. (2013)</td>
<td>reflectance</td>
<td></td>
<td></td>
<td></td>
<td>Firmness, pH, SSC</td>
<td></td>
</tr>
<tr>
<td>Liu et al. (2008)</td>
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<td>Nicolai et al. (2008)</td>
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<td></td>
<td></td>
<td></td>
<td>Firmness, SSC</td>
<td>storage for 6 months, then SL for 7 days</td>
</tr>
<tr>
<td>Paz et al. (2009)</td>
<td>reflectance</td>
<td></td>
<td></td>
<td></td>
<td>Firmness, SSC</td>
<td></td>
</tr>
<tr>
<td>Xia et al. (2012)</td>
<td>reflectance</td>
<td></td>
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</tr>
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</table>
Harvest and postharvest quality prediction of apple has received the most attention (Table 7.2.). Given their relationship to fruit quality and maturity, it is not surprising the main attributes of interest have been SSC and firmness. The success of SSC prediction varies, with validation $R^2$ values for ‘Golden Delicious’ apples, for example, ranging between 0.57-0.95 and RMSEPs of 0.49-1.05 °Brix (Bobelyn et al., 2010; Lu et al., 2000; Paz et al., 2008; Ventura et al., 1998). Harvest and postharvest assessment of pear (Table 7.3.) has achieved $R^2$ values of between 0.48-0.82 and RMSEPs of 0.2-1.08 °Brix (Jiang and Zhu, 2012; Li et al., 2013; Liu et al., 2008; Nicolaï et al., 2008; Paz et al., 2009; Xu et al., 2012). According to McGlone and Kawano (1998) an RPD of 3 (SD/SECV or SEP), which in theory corresponds to a $R^2$ of 0.89, is generally considered the minimum value for a model to be of any use for grading or sorting purposes. Many of the models in Tables 7.2. and 7.3. have RPD values well below this. When calculated, Liu and Ying (2005), Lu et al. (2000) and Park et al., (2003) in Table 7.2. have RPDs >3 for prediction of SSC. However it is worth noting that their prediction models were made using 10-15 LVs. Variation in the standard of prediction models for SSC can partly be attributed to its instability as a parameter if starch is still present in fruit at harvest (Palmer et al., 2010).
Table 7.2. Selection of NIR spectroscopy papers on apple, including wavelengths used, best models and statistics. LV, latent variables; R^2, coefficient of determination; RMSECV and RMSEP, root mean square error of cross-validation and prediction; RPD, residual prediction deviation; S.D, standard deviation. * denotes firmness measured in N. 1st and 2nd der., derivative spectra; Abs, absorbance; Cal+val., calibration and validation models; Cross-val., cross-validation; Ext. val., external validation; iPLS, interval PLS regression; Log(1/R), spectra transformed to absorbance; MPLS, modified PLS regression; MSC, multiplicative scatter correction; PLS, partial least squares regression; SG, Savitzky-Golay; SNV, standard normal variate.

<table>
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<tr>
<th>Authors</th>
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<th>Modeling</th>
<th>Testing</th>
<th>Parameter</th>
<th>R^2</th>
<th>RMSECV/RMSEP</th>
<th>LVs</th>
<th>RPD</th>
<th>Best models sample size</th>
<th>Ref.</th>
<th>Range cal/val</th>
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</thead>
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<tr>
<td>Bobelyn et al. (2010)</td>
<td>Braeburn</td>
<td>800-1690</td>
<td>Log (1/R), SNV</td>
<td>PLS</td>
<td>Ext. val</td>
<td>SSC</td>
<td>0.66/0.77</td>
<td>0.87/0.74</td>
<td>7</td>
<td>1.71/2.13</td>
<td>1086</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. Delicious</td>
<td>800-1690</td>
<td>Log (1/R), SNV</td>
<td>*</td>
<td>*</td>
<td>SSC</td>
<td>0.88/0.85</td>
<td>0.6/0.71</td>
<td>7</td>
<td>2.89/2.22</td>
<td>1295</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fuji</td>
<td>800-1690</td>
<td>Log (1/R), SNV</td>
<td>*</td>
<td>*</td>
<td>SSC</td>
<td>0.74/0.77</td>
<td>0.76/0.7</td>
<td>6</td>
<td>1.99/1.93</td>
<td>335</td>
<td>-</td>
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<tr>
<td>Bobelyn et al. (2010)</td>
<td>Braeburn</td>
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<td>PLS</td>
<td>Ext. val</td>
<td>Firmness</td>
<td>0.67/0.4</td>
<td>9.8/17.66</td>
<td>8</td>
<td>1.7/1.6</td>
<td>1086</td>
<td>-</td>
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<tr>
<td>G. Delicious</td>
<td>800-1690</td>
<td>Log (1/R)</td>
<td>*</td>
<td>*</td>
<td>Firmness</td>
<td>0.41/0.2</td>
<td>6.38/8.37</td>
<td>8</td>
<td>1.3/1.34</td>
<td>1295</td>
<td>-</td>
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<tr>
<td>Fuji</td>
<td>800-1690</td>
<td>Log (1/R)</td>
<td>*</td>
<td>*</td>
<td>Firmness</td>
<td>0.55/0.03</td>
<td>7.8/23.4</td>
<td>8</td>
<td>1.5/1.3</td>
<td>335</td>
<td>-</td>
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<tr>
<td>Fan et al. (2009)</td>
<td>Red Fuji</td>
<td>850-920 nm</td>
<td>Log (1/R), 2nd der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.98/0.05</td>
<td>0.29/0.38</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Fuji</td>
<td>812-2357</td>
<td>Log (1/R), MSC, 1st + 2nd der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.91/0.95</td>
<td>0.005/0.004</td>
<td>10</td>
<td>-</td>
<td>333 (235/98)</td>
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<tr>
<td>Liu and Ying (2005)</td>
<td>Fuji</td>
<td>812-2357</td>
<td>Log (1/R), MSC, 1st + 2nd der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.91/0.95</td>
<td>0.005/0.004</td>
<td>10</td>
<td>-</td>
<td>333 (235/98)</td>
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<tr>
<td>Lu et al. (2000)</td>
<td>G. Delicious</td>
<td>800-1700 nm</td>
<td>Log (1/R), 20-SG, MSC</td>
<td>PCR</td>
<td>Cross-val</td>
<td>SSC</td>
<td>0.92/0.88</td>
<td>0.48/0.49</td>
<td>15</td>
<td>-</td>
<td>(13.9 ± 1.5)</td>
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<tr>
<td>McGlone et al. (2002a)</td>
<td>Royal Gala</td>
<td>500-1000 nm</td>
<td>Log (1/T), smoothing</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC (harv)</td>
<td>0.83</td>
<td>0.7/0.72</td>
<td>10</td>
<td>1.7</td>
<td>915</td>
<td>(11.3 ± 1.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>Firmness</td>
<td>0.78</td>
<td>0.06/0.05</td>
<td>4</td>
<td>1/1.8</td>
<td>915</td>
<td>(2.9 ± 1.9)</td>
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</tr>
<tr>
<td>McGlone et al. (2002a)</td>
<td>Royal Gala</td>
<td>500-1000 nm</td>
<td>Log (1/T), smoothing</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>SSC (postharv)</td>
<td>0.67</td>
<td>0.32/0.5</td>
<td>10</td>
<td>1.8</td>
<td>897</td>
<td>(12 ± 0.8)</td>
</tr>
<tr>
<td></td>
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<td>*</td>
<td>*</td>
<td>Firmness (harv)</td>
<td>0.83</td>
<td>7.7/7.7</td>
<td>10</td>
<td>1.8</td>
<td>915</td>
<td>(80.7 ± 11.4)</td>
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<td>McGlone et al. (2002a)</td>
<td>Royal Gala</td>
<td>800-1000nm</td>
<td>15-SG, 2nd der, MSC</td>
<td>PLS</td>
<td>Cal + val</td>
<td>DM</td>
<td>0.97</td>
<td>0.44</td>
<td>8</td>
<td>(105/84)</td>
<td>(41.4 ± 1.5)</td>
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<td></td>
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<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>SSC</td>
<td>0.94</td>
<td>0.3</td>
<td>9</td>
<td>-</td>
<td>(12.03 ± 2.2)</td>
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<td></td>
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<tr>
<td>McGlone et al. (2002a)</td>
<td>Royal Gala</td>
<td>500-1000 nm</td>
<td>Log (1/T), smoothing</td>
<td>PLS</td>
<td>Cal + val</td>
<td>DM</td>
<td>0.97</td>
<td>0.44/0.34</td>
<td>9</td>
<td>-</td>
<td>81 (66/53)</td>
<td>(13.5 ± 1.2)</td>
<td></td>
</tr>
</tbody>
</table>
Table 7.2. Continued. Selection of NIR spectroscopy papers on apple, including wavelengths used, best models and statistics. LV, latent variables; $R^2$, coefficient of determination; RMSECV and RMSEP, root mean square error of cross-validation and prediction; RPD, residual prediction deviation; S.D, standard deviation. * denotes firmness measured in N. 1st and 2nd der., derivative spectra; Abs, absorbance; Cal+val., calibration and validation models; Cross-val., cross-validation; Ext. val., external validation; iPLS, interval PLS regression; Log(1/R), spectra transformed to absorbance; MPLS, modified PLS regression; MSC, multiplicative scatter correction; PLS, partial least squares regression; SG, Savitzky-Golay; SNV, standard normal variate.

<table>
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<tr>
<th>Authors</th>
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<th>Preprocessing</th>
<th>Modeling</th>
<th>Testing</th>
<th>Parameter</th>
<th>$R^2$ cal/val</th>
<th>RMSECV/RMSEP</th>
<th>LVs</th>
<th>RPD</th>
<th>sample size</th>
<th>Ref.</th>
<th>Range cal/val</th>
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<td>Park et al. (2003)</td>
<td>Red Delicious</td>
<td>800-1100 nm</td>
<td>Log (1/R)</td>
<td>PCR</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.96/0.28</td>
<td>0.34/0.28</td>
<td>10/10</td>
<td>9.6-18.8 (12.1 ± 1.5)</td>
<td>950/470 (470/470)</td>
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<tr>
<td>Paz et al. (2008)</td>
<td>Fuji</td>
<td>900-1700 nm</td>
<td>SNV, derivs</td>
<td>MPLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.94/0.87</td>
<td>0.95/0.77</td>
<td>10/10</td>
<td>10.5-19.3 (14.2 ± 1.4)</td>
<td>9.6-14.1 (11.7 ± 0.8)</td>
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</tr>
<tr>
<td>Peirs et al. (2000)</td>
<td>Various</td>
<td>580-2000 nm</td>
<td>MA, 2nd der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.92/0.59</td>
<td>0.49/0.38</td>
<td>7/7</td>
<td>244/244</td>
<td>22/22</td>
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<tr>
<td>Peirs et al. (2003b)</td>
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<td>380-2000 nm</td>
<td>none</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>-</td>
<td>0.80</td>
<td>9</td>
<td>446 (448)</td>
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<tr>
<td>Peirs et al. (2005)</td>
<td>Cox’s O. Pippin</td>
<td>380-2000 nm</td>
<td>Log (1/R)</td>
<td>PLS</td>
<td>Cal + val</td>
<td>Streif</td>
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<td>0.14</td>
<td>6</td>
<td>326</td>
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<td>G. Delicious</td>
<td>819-987 nm</td>
<td>Log (1/R), 1st der</td>
<td>MLR</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.59/0.105</td>
<td>0.94/1.06</td>
<td>5/5</td>
<td>150 (50/50)</td>
<td>10.2-15.2 (13.1 ± 1.4)</td>
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<td>Walsh et al. (2004)</td>
<td>-</td>
<td>734-933 nm</td>
<td>SG 2nd der</td>
<td>PLS</td>
<td>Cross-val</td>
<td>SSC</td>
<td>0.96/0.22</td>
<td>1.04/1.15</td>
<td>3/3</td>
<td>85 (85/85)</td>
<td>9.4-16.6 (12.9 ± 1.5)</td>
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<td>Xiaohe et al. (2007)</td>
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<td>MSC, mean centring</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.66/0.18</td>
<td>1.04/1.15</td>
<td>3</td>
<td>85 (85/85)</td>
<td>9.4-16.6 (12.9 ± 1.5)</td>
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<td>Zude et al. (2006)</td>
<td>G. Delicious</td>
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<td>Abs, Autoscaling</td>
<td>PLS</td>
<td>Cross-val</td>
<td>SSC (preharv)</td>
<td>0.2/0.07</td>
<td>1.2/1.26 (RMSEC/CV)</td>
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Best models
Table 7.3. Selection of NIR spectroscopy papers on pear, including wavelengths used, best models and statistics. LV, latent variables; R², coefficient of determination; RMSECV and RMSEP, root mean square error of cross-validation and prediction; RPD, residual prediction deviation; S.D, standard deviation. * denotes firmness measured in N. 1st der., derivative spectra; Cal+val., calibration and validation models; Independent, independent test set; iPLS, interval PLS regression; Log(1/R), spectra transformed to absorbance; MA, moving average; MPLS, modified PLS regression; PLS, partial least squares regression; SNV, standard normal variate.

<table>
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<tr>
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<th>Authors</th>
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<th>Modeling</th>
<th>Testing</th>
<th>Parameter</th>
<th>Best models</th>
<th>R² cal/val</th>
<th>RMSECV/RMSEP</th>
<th>LVs</th>
<th>RPD</th>
<th>sample size</th>
<th>Ref.</th>
<th>Range cal/val</th>
</tr>
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<tr>
<td></td>
<td>Jiang and Zhu (2012)</td>
<td>Huangguan</td>
<td>1000-2500 nm</td>
<td>SNV</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.88/0.82</td>
<td>0.34/0.4</td>
<td>0.78/0.72</td>
<td>9</td>
<td>7.4</td>
<td>99 (66/33)</td>
<td>9.7-14.3 (11.8 ± 0.96)/9.8-14 (11.7 ± 0.96)</td>
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<tr>
<td></td>
<td>Li et al. (2013)</td>
<td>Various</td>
<td>400-1800 nm</td>
<td>Log (1/R), 7-MA, SNV, 1st der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.9/0.82</td>
<td>0.33/0.42</td>
<td>0.81/0.76</td>
<td>8</td>
<td>9.2-12.7</td>
<td>9.2-12.7 (10.8 ± 0.96)</td>
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<td>Liu et al. (2008)</td>
<td>Fengshui</td>
<td>350-1800 nm</td>
<td>Log (1/R), SG 1st der</td>
<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.74/0.72</td>
<td>1.15/1.33</td>
<td>0.82/0.82</td>
<td>8</td>
<td>11 ± 1.6</td>
<td>8.5-13.3 (11 ± 1.6)</td>
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<td>Nicolaï et al. (2008)</td>
<td>Conference</td>
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<td>PLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.6</td>
<td>/0.44</td>
<td>/0.35</td>
<td>14</td>
<td>-</td>
<td>840 (420/420)</td>
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<td></td>
<td>Paz et al. (2009)</td>
<td>Blanquilla</td>
<td>900-1700 nm</td>
<td>SNV</td>
<td>MPLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.52/0.78</td>
<td>0.8/0.59</td>
<td>-</td>
<td>168 (146/22)</td>
<td>10.7-17.7 (13.9 ± 1.2)/11.9-15.4 (13.9 ± 1)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conference</td>
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<td>*</td>
<td>Independent</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<td>*</td>
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<tr>
<td></td>
<td>Blanq + Conf</td>
<td>*</td>
<td>*</td>
<td>Independent</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<td>*</td>
<td>*</td>
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</tr>
<tr>
<td></td>
<td>Xu et al. (2012)</td>
<td>Chinese Royal</td>
<td>533-929 nm</td>
<td>none</td>
<td>iPLS</td>
<td>Cal + val</td>
<td>SSC</td>
<td>0.87/0.82</td>
<td>0.43/0.54</td>
<td>-</td>
<td>2.47 (165/82)</td>
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</table>
Prediction of DM with NIR spectroscopy has so far centred on avocado and kiwifruit (Table 7.4.), two crops that are the foundation of DM research in general and with industry recommended harvest DM thresholds. Although not so extensively researched, some literature does exist concerning harvest and postharvest DM prediction in apple, with $R^2$ values between 0.87-0.97 and RMSEPs between 0.24-0.43 % DM (McGlone et al., 2003; McGlone and Martinsen, 2004). DM RPD for McGlone et al. (2003) is excellent, at approximately 6.3 whilst for McGlone and Martinsen (2004), who had a smaller number of reference samples with a narrower range, their RPD works out below 2.8.

Preharvest prediction of quality has not figured greatly in research to date, with the exception of work by Peirs et al. (2000 and 2005) and Zude et al. (2006) on apple SSC and physiological maturity. Peirs et al. (2000) predicted SSC with PLS regression, achieving $R^2$ values of 0.89 and RMSEPs of between 0.58-0.59 °Brix. Zude et al. (2006) best predictions, using a narrower wavelength range, had $R^2$ calibration values of 0.20-0.41 and RMSECs of between 0.81-1.2 °Brix.

To the author’s knowledge, there is no previous research regarding the preharvest prediction of DM. However its rising importance as a quality parameter and its link to postharvest quality (Harker et al., 2009; Palmer et al., 2010), plus the establishment of minimum harvest percentages for avocado and kiwifruit, makes preharvest estimation of DM of other fruit, including apples and pears, an interesting possibility.
Table 7.4. Selected avocado and kiwifruit research, including wavelengths and model statistics. LV, latent variables; R^2, coefficient of determination; RMSEC, RMSECV and RMSEP, root mean square error of calibration, cross-validation and prediction; RPD, residual prediction deviation; S.D, standard deviation. 1st and 2nd der., derivative spectra; 2nd poly, 2nd polynomial; Cal+val., calibration and validation models; Cross-val., cross-validation; PLS, partial least squares regression; SG, Savitzky-Golay.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cultivar</th>
<th>Selected spectra</th>
<th>Preprocessing</th>
<th>Modeling</th>
<th>Testing</th>
<th>Parameter</th>
<th>Best model</th>
<th>RMSECV or CV/RMSEP</th>
<th>LVs</th>
<th>RPD (cal/val)</th>
<th>Sample size</th>
<th>Ref.</th>
<th>Range cal/val (mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Avocado</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Clark et al. (2003)</td>
<td>Hass</td>
<td>750-1051 nm</td>
<td>15-SG 2nd der</td>
<td>PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.88</td>
<td>1.3/1.3</td>
<td>12</td>
<td>-</td>
<td>240 (180/60)</td>
<td>27.7-36.8 ± 2</td>
<td></td>
</tr>
<tr>
<td>Walsh et al. (2004)</td>
<td>Hass</td>
<td>734-931 nm</td>
<td>SG 2nd der</td>
<td>PLS</td>
<td>Cross-val</td>
<td>DM</td>
<td>0.79/</td>
<td>1.14/</td>
<td>-</td>
<td>2.2/</td>
<td>100 (20.5 ± 2.3)</td>
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<td></td>
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<tr>
<td>Wedding et al. (2010)</td>
<td>Hass</td>
<td>843-2444 nm</td>
<td>25-SG 2nd der, 2nd poly</td>
<td>PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.81/0.76</td>
<td>1.47/1.53</td>
<td>5</td>
<td>-</td>
<td>629 (199/430)</td>
<td>18.2-35 ± 3.4/19.4-34.2 ± 3.1</td>
<td></td>
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<tr>
<td>Wedding et al. (2013)</td>
<td>Hass</td>
<td>843-2444 nm</td>
<td>25-SG 2nd der, 2nd poly</td>
<td>PLS</td>
<td>Cross-val</td>
<td>DM</td>
<td>0.93/0.92</td>
<td>1.39/1.49</td>
<td>7</td>
<td>3.8/3.5</td>
<td>(209/399)</td>
<td>16.1-32.6 (25.6 ± 5.2)/16.5-36.1 ± 5.4</td>
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<tr>
<td><strong>Kiwifruit</strong></td>
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<tr>
<td>Clark et al. (2004)</td>
<td>Hort16A</td>
<td>800-1000 nm</td>
<td>25-SG, 1st der, 2nd poly</td>
<td>PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.92</td>
<td>0.47/0.47</td>
<td>10 max</td>
<td>-</td>
<td>(2642/898)</td>
<td>(16.6 ± 1.6)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.92/0.93 ± 0.27</td>
<td>10 max</td>
<td>-</td>
<td>(2663/882)</td>
<td>(11.7 ± 3.4)</td>
<td></td>
</tr>
<tr>
<td>Lü et al. (2010)</td>
<td>Zhonghua</td>
<td>various</td>
<td>Log (1/R), MSC</td>
<td>(si)PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.88-0.9/0.88-0.39-0.44/0.41-0.11 or 12</td>
<td>127 (74/38)</td>
<td>13.5-18.7 (16.2 ± 1.08)</td>
<td></td>
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<tr>
<td>McGlone &amp; Kawano (1998)</td>
<td>Hayward</td>
<td>800-1100 nm</td>
<td>10-SG 2nd der</td>
<td>PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.90/</td>
<td>0.42/</td>
<td>-</td>
<td>3.1/</td>
<td>(16.5 ± 1.4)/16.3 ± 1.3)</td>
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<td></td>
<td></td>
<td></td>
<td>0.90/0.39 ± 0.27</td>
<td>-</td>
<td>3.1/</td>
<td>(12.9 ± 1.3)/12.7 ± 1.2)</td>
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<tr>
<td>McGlone et al. (2002b)</td>
<td>Hayward</td>
<td>800-1000 nm</td>
<td>5-SG 2nd poly</td>
<td>PLS</td>
<td>Cal+val</td>
<td>DM</td>
<td>0.94/</td>
<td>0.29/</td>
<td>9</td>
<td>-</td>
<td>179 (120/59)</td>
<td>(17.7 ± 1.3)</td>
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<td></td>
<td></td>
<td></td>
<td>0.94/0.39 ± 0.27</td>
<td>8</td>
<td>-</td>
<td>(14.5 ± 1.3)</td>
<td></td>
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</tr>
<tr>
<td>Walsh et al. (2004)</td>
<td>Hayward</td>
<td>734-931 nm</td>
<td>SG 2nd der</td>
<td>PLS</td>
<td>Cross-val</td>
<td>DM</td>
<td>0.9/</td>
<td>0.38/</td>
<td>-</td>
<td>3.2/</td>
<td>144 (5.3 ± 1.2)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.86/0.24 ± 0.07</td>
<td>5</td>
<td>3.1/</td>
<td>(13.7 ± 1.21)</td>
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</tbody>
</table>
According to the literature (Table 7.1.) most research on the prediction of apple DM and SSC has centred on shorter wavelength NIR spectra (<1100 nm), sometimes incorporating some longer wavelengths of the visible spectrum (<780 nm). One reason is that this range allows the use of cheaper silicon detectors. Furthermore spectra between 700-1200 nm contain a number of overlapping carbohydrate and water absorption bands (Williams and Norris, 1987), which are relevant when predicting of both DM and SSC. Prediction of pear SSC has predominantly used longer wavelength spectra between 350-1800 nm and there is some research on apple using spectra up to 1700 nm or even 2000 nm. Most authors are reluctant to use longer NIR radiation due to reduced penetration depth. However longer wavelengths, between 1100-1300 nm, 1600-1700 nm and 2100-2400 nm contain information relevant to the prediction of carbohydrate and can produce reasonable prediction models (e.g. Lammertyn et al., 2000; Paz et al., 2009; Peirs et al., 2005).

The possibility of using DM to predict postharvest SSC of apples is well documented (McGlone and Kawano, 1998; McGlone et al., 2002b; McGlone et al., 2003). However McGlone et al. (2003) raise an issue concerning the lack of success when predicting apple harvest SSC (R² = 0.79, RMSEP = 0.51) compared to the good results obtained for DM (R² >0.95, RMSEP <0.32). They suggest that NIR spectroscopy may be unable to distinguish between the two forms of carbohydrate and is instead more sensitive to total fruit carbohydrate, hence the better prediction models for DM. All three papers used spectra between 700-1100 nm and this range contains a number of overlapping carbohydrate and water absorption bands. This lack of segregation in spectral bands may pose a problem when trying to predict SSC, but be of no consequence when assessing DM. McGlone et al.
(2003) go on to propose that longer NIR wavelengths may be better able to discriminate between the two parameters.

The depth of light penetration is wavelength dependent (Lammertyn et al., 2000), with spectra below 1100 nm passing deeper into biological material than spectra above 1100 nm (Wedding et al., 2013). However shorter wavelengths may be problematic due to secondary correlations between skin properties and the flesh beneath. For instance radiation penetration around 650-700 nm can be drastically reduced due to chlorophyll absorption. Lammertyn et al. (2000) observed a penetration depth of approximately 4 mm when using spectra between 700-900 nm and approximately 2-3 mm at longer wavelengths.

7.2. Some considerations when predicting fruit quality

Assessing fruit quality with NIR spectroscopy is not a straightforward process. There are numerous issues to consider for optimising calibration and prediction model accuracy. Only a few are outlined here. One such consideration is temperature, be it ambient or sample, as it can have a profound influence on spectra. Sample temperature is especially significant as it is the physical properties of the sample that are reflected in the spectra. To counter the effects, Peirs et al. (2003a) proposed the development of a global model robust enough to deal with temperature differences in future samples or individual models specifically for samples of different temperatures.

Biological variability can cause great disparity in fruit internal quality. The protocol outlined in Chapter 2 is an attempt to account for this when sampling. Bobelyn et al. (2010) and Peirs et al. (2003b) found that much of the variability in apple spectra could be
attributed to orchard, season and cultivar influences, as well as changes in fruit internal quality as it senesced in storage. This variability goes on to affect any resulting calibration models and the success of fruit quality prediction. In addition the inherent variability of fruit samples limits the application of a NIR model to its calibration cultivar – the model is unable to extrapolate. However including more variability brings its own problems, as increasing model robustness and complexity can compromise its accuracy (Bobelyn et al., 2010; Nicolaï et al., 2007).

Spectrometers can vary a great deal, not only in terms of wavelength range but also due to differences in optics, detectors and light sources, environmental influences (temperature and humidity) etc. (Nicolaï et al., 2007). These differences make calibration transfer between one instrument and another impossible. This requires repetition of the calibration process for each instrument, making implementation of the technology in a commercial context difficult. Attempts at developing calibration transfer techniques have been made (Greensill et al., 2001; Alamar et al., 2007) but the best method still seems to be ensuring that calibration models are regularly updated and improved with the addition of fresh samples (Nicolaï et al., 2007).

NIR spectroscopy has been widely researched and with a move towards online integration and the development of more accurate instrumentation, together with advances in chemometric methods, there is huge potential for it as an applied technique for predicting internal fruit quality. However success depends on model robustness and their ability to accommodate sample variability, be it seasonal, cultivar or biological. This emphasises the
importance of robust calibration models based on a broad range of data, spanning years, orchards and sampling conditions (Nicolaï et al., 2007).
Chapter 8 - Discussion, conclusions and perspectives

The intention of this chapter is to provide an overview of the main results obtained during this PhD project in relation to the hypotheses outlined in Chapter 1, with reference to the manuscripts 1-4 included in the Appendices and in the context of existing literature. Many other interesting perspectives and questions have arisen during this PhD study and these are also included in this chapter.

This PhD project had two distinct parts: the manipulation of fruit quality (Hypotheses 1, 2 and 3) and its measurement with NIR spectroscopy (Hypothesis 4). The hypotheses are answered first, with some joint aspects mentioned at the end.

8.1. Hypothesis 1.

Root pruning increases preharvest DM in ‘Clara Frijs’ pears but compromises overall harvest fruit quality (Paper 2.).

The effect of root pruning on fruit SSC (Elfving et al., 1991; Ferree, 1992; Schupp and Ferree, 1988) are well documented, but its effect on DM are not. According to the results presented in this thesis, root pruning did increase preharvest DM in fruit (Table 2, Paper 2.). However the mechanism of this increase is complex and related not only to root pruning, but also to crop load, light interception and soil water availability.

As discussed in Chapter 4., root pruning creates an imbalance between the canopy and root system of a tree whilst also forcing a redirection of resources to restore equilibrium. Root pruning stimulated root regrowth in certain soil layers, while also significantly inhibiting shoot growth (Figure 4., Paper 4.) and terminating it two weeks earlier.
compared to NP trees. Furthermore by reducing root volume, root pruning also limits access to stored assimilates and curtails a tree’s ability to take up water and nutrients from the soil (Khan et al., 1998b). This may explain the higher soil water content found at the 30-60 cm layer, as it suggests the trees were unable to access water here due to removal of a large part of their root system (Figure 3., Paper 4.).

However, redirection of new and stored assimilates after root pruning is based on the relative strength of sink organs. Fruit are a strong sink and a high crop load enables more assimilates to be drawn from proximal leaves (Hansen, 1967a), generally to the detriment of shoot and root growth (Geisler and Ferree, 1984; Palmer, 1992; Schupp, 1990). The effect of crop load was evident in RP trees in 2011 with a 30 % increase in total DM allocation to fruit due to carrying nearly twice the number of fruit compared to 2010 (Table 6., Paper 2.).

Conversely a low crop load can be insufficient competition for assimilates Therefore in low cropping trees assimilates are diverted into shoot and root growth early in the season (Palmer, 1992), as evidenced by greater shoot length and number found in NP trees for both years (Table 1., Paper 4.). Furthermore shade during peak cell division (3-5 weeks after full bloom) caused by excessive vegetative growth can result in a deficit in carbon supply to fruit. This deficit is associated with reduced photosynthetic activity of bourse shoots as they try to compete with stronger vegetative sinks (Palmer, 1992; Wünsche and Lakso, 2000a). NP pears over both years were an excellent example of fruit from vigorous non-root pruned trees: large, green with low SSC (Saure, 2007).
RP fruit had a significantly higher preharvest DM on the first day of sampling (51 DAFB) and this disparity was maintained until harvest. Indeed total cumulative DM of RP fruit was more than double that of NP fruit at harvest in 2011 (Table 6., Paper 2.). However the difference in total DM allocation between RP and NP trees between the years seems to be related to crop load rather than an alteration in sink strength. Crop load of NP trees in 2011 was approximately two-thirds of that in 2010 and it was matched by an equivalent reduction (~35 %) in total DM allocation, even though average fruit DM and size was similar for both years. Likewise RP crop load increased by about 30 % in 2011 as did its total DM allocation to fruit for that year. The absence of an apparent difference in fruit sink strength in NP trees between the years, even with a change in crop load, suggests that shoot growth was still vigorous enough to out compete fruit even though the average crop for both years was not excessively low.

One of the main effects root pruning has on quality is a reduction in fruit size (Khan et al., 1998a; Schupp and Ferree, 1989) and therefore, total yield. Root pruning reduced ‘Clara Frijs’ harvest fruit size in both years. Differences in RP preharvest fruit weight in 2011 were evident on Day 51 (Figure 2., Paper 2.) and accompanied by lower fruit expansion rates (Figure 6., Paper 4.), and although crop load played a role, the trend was consistent with the year before when crop load was not a factor. In addition to their smaller size, pears from the RP trees were significantly softer and more yellow than NP fruit and contained less starch at harvest. Differences in fruit quality from both treatments were obvious (Figure 8.1) and indicate that root pruning advanced fruit physiological maturity.
8.2. Hypothesis 2.

Supplementary irrigation of root pruned pear trees has the potential to improve harvest quality and fruit size (Papers 2. and 4.).

Supplementary irrigation primarily improved harvest quality by increasing fruit size. Fully irrigated (FI) pears were significantly larger than non-irrigated (NI) fruit in both years. However the lower crop load of FI trees may have exaggerated the weight difference slightly in 2010. A reduction in fruit size as a result of root pruning is well documented (Marsal et al., 2000; Mpelasoka et al., 2001) and may be related to a loss in water uptake ability as well as a reduction in total root surface area for doing so. Cell expansion relies on water availability (Hsiao and Xu, 2000), so any restriction has the potential to inhibit cell expansion and ultimately fruit size. Pear trees can be prone to water stress and root pruning may exacerbate this, even in a temperate climate. Therefore irrigation of root-pruned trees, especially when crop load is high, could be beneficial to ensure consistent fruit size and yield (Janssens et al., 2011).
The reduction in DM and SSC in FI fruit may be a necessary consequence when irrigating root-pruned trees in order to improve fruit size. Supplementary irrigation may also help delay fruit maturity, as according to the Streif Index, DI and FI fruit were less mature at harvest than NI.

NI fruit had the highest SSC at harvest for both years. Water stress is known to increase fruit SSC (Ebel et al., 1993; Lopez et al., 2011; Mills et al., 1994) and the may be due to advanced maturity (Mpelasoka et al., 2001) or osmotic adjustment, whereby fruit accumulate solutes to decrease their plant water potential in order to maintain cell turgor (Behboudian and Lawes, 1994). However it is hard to know if differences in SSC between treatments are due to the dilution effect of water or to differences in maturity, or a combination of both.

![Figure 8.2. Preharvest DM of root pruned (RP) and non-root pruned (NP) ‘Clara Frijs’ pear trees in 2011.](image)
8.3. Hypothesis 3.

Preharvest DM of pears is an indicator of postharvest fruit quality (Paper 2.).

Preharvest DM was a good indicator of postharvest fruit quality. One of the reasons for this is the relationship of DM to SSC, after starch hydrolysis (Palmer, 2010). Therefore it is to be expected that fruit with high preharvest DM will have more potential SSC. Another reason is its relative stability as a parameter both during development on the tree and during postharvest storage. This is illustrated by steady trend in DM during the latter part of fruit development (Figure 8.2.). This provided a strong indication of harvest DM as early as 31 days before it occurred ($R^2 = 0.84$) and of postharvest DM (after 8 weeks of storage) at harvest ($R^2 = 0.77$) (Table 8, Paper 2.). These attributes make it an ideal indicator of potential harvest DM and postharvest SSC. They also are what make DM an ideal ‘prediction’ parameter in NIR spectroscopy, which will be discussed later in this chapter.

Differences in fruit quality at harvest are generally maintained postharvest (Behboudian et al., 2011; Mpelasoka et al., 2001b). The results suggest that low preharvest DM results in low harvest and postharvest SSC. Without exception, fruit containing the highest levels of DM at harvest had the highest SSC after storage in 2010 and 2011. The fact that the differences in DM were observed as early as 51 DAFB makes discussing the stability of DM as a parameter and as an indicator of postharvest DM and SSC extremely interesting. NP fruit had the lowest preharvest DM for both years and even after storage could not attain a SSC of more than 11.5 °Brix. In contrast, RP fruit are a good example of how variation in DM from year to year, due to a different crop load, can change postharvest SSC. DM of RP fruit was higher at harvest in 2010 than 2011 and these fruit had a SSC of 15 °Brix at the end of storage compared to 12.3 in the 2011 fruit.
Physiological maturity is an important factor in determining whether fruit can ripen well after harvest and achieve good eating quality. RP maturity dictated when fruit from all treatments were harvested and the results for both years clearly show that NP fruit were not physiologically mature when harvested and this may have had some bearing on the results, especially regarding SSC. A separate shelf life experiment conducted in 2011 showed NP fruit could not ripen successfully, even after 4 weeks of cold storage. Out of storage they became quite ‘spongy’ to the touch, losing on average 30-40 % more water than RP fruit. Additional NP trees in the replicate were harvested two weeks later than the main experiment and changes in their quality were comparable to RP fruit, in that they ripened successfully, becoming softer, more yellow and losing a similar amount of water as they ripened and senesced. Successful ripening is determined by certain physiological and chemical processes, including the production of enzymes required for the biosynthesis of ethylene (Villalobos-Acūna et al., 2011).

However DM does not reveal the full picture in terms of internal fruit quality. The interaction of DM with other parameters needs to be accounted for to understand if and how they relate to DM and how this translates into actual eating quality and consumer preference. Although the remit of this PhD project did not extend to consumer testing, there is extensive literature available on the contribution DM makes to eating quality.

Preharvest DM can be considered as an indicator of fruit quality in that it signifies the potential for postharvest SSC once the solubilisation of starch is completed. However according to the results presented in this thesis, there does not appear to be a link between DM and other quality parameters such as fruit colour and firmness. Differences in colour
and firmness between the NP and RP treatments, for example are probably related to fruit maturity at harvest. However other factors are relevant, including improved light interception in root pruned trees (Ferree, 1992; Schupp and Ferree, 1988) and smaller fruit size, which may have a bearing on fruit firmness (Sams, 1999), although the results here do not support this.

The strength of the relationship between DM and SSC should not be used to infer that fruit is more appealing to consumers just because they are sweeter as preference is not based on individual attributes, but on their interaction. Research by Harker et al. (2009) on kiwifruit DM and consumer liking found that fruit with high SSC were preferred only if they were firm too. Their earlier work on consumer acceptability of apples and the relationship between firmness and SSC drew a similar conclusion, with the balance of sugar and acidity also playing a role (Harker et al., 2008). Therefore if high DM in fruit is associated with negative changes in other quality parameters, such a loss of firmness and colour then it is may be too much of a compromise, in terms of consumer preference and storage potential, for it to be worthwhile. Arguably the benefit of increased fruit size and a slight delay in maturation (according to the Streif Index) of the irrigation treatments (DI and FI) compared to the NI, made the reduction in DM and SSC worthwhile.

Consideration should also be given as to how a potential increase in SSC as a result of high DM may alter fruit physiology in terms of its ability to deal with stress, such as desiccation tolerance (Ingram and Bartels, 1996), and the protection of fruit under low temperature conditions (Holland et al., 2002; Palonen, 1999). In addition to its influence on consumer preference, low DM in kiwifruit, for instance, is known to compromise storage quality and
is associated with increased incidences of pitting, fruit softening, storage rot (Ferguson et al., 2003) and chilling rot (Clark et al., 2004).

8.4. Hypothesis 4.

Prediction of apple and pear DM and SSC is possible using NIR spectroscopy and chemometrics (Papers 1. and 3.).

The results presented in Papers 1. and 3. demonstrated that prediction of DM and SSC was possible using NIR spectroscopy. Both papers focused on using longer wavelength spectra for the prediction of fruit quality, rather than the more commonly used Vis/NIR range of between 300-1100 nm.

Apple prediction models based in fruit after a period of postharvest storage (Paper 1.) were reasonable for DM ($R^2 = 0.63-0.86$, $RMSEP = 0.37-0.48$, $LVs = 6-7$, $RPD = 1.6-2.7$) and SSC ($R^2 = 0.76-0.85$, $RMSEP = 0.39-0.46$, $LVs = 6-7$, $RPD = 2.3-2.6$) and on a par with previous work (Table 7.2., Chapter 7.). Nevertheless RPD values were below the minimum threshold of 3 for reliable quantitative prediction.

The results presented in Paper 3. show that models based on longer wavelength spectra were more successful at predicting pear preharvest and harvest DM ($R^2 = 0.77-0.78$, $RMSECV = 0.78-0.8$, $LVs = 6-10$, $RPD = 1.87-1.91$) and SSC ($R^2 = 0.81-0.84$, $RMSECV = 0.44-0.47$, $LVs = 7-10$, $RPD = 2.1-2.3$), although RPD values were still below 3. No previous data was found regarding the prediction of DM in pears, but models were reasonable compared to results for DM of avocado and kiwifruit (Table 7.4., Chapter 7.).
Models for SSC were comparable to those of Peirs et al. (2000), also working with preharvest fruit.

8.5. **Other points for discussion**

8.5.1. **Discrimination between DM and SSC**

This may not be an issue for DM as there is a strong correlation between it and fruit total carbohydrate content (Jordan et al., 2000), but successful prediction of SSC is based on the premise that models can distinguish between the two parameters. However McGlone and Kawano (1998) and McGlone et al. (2002a and 2003) raised the possibility of a lack of distinction when trying to explain why SSC models based on shorter wavelength spectra were more accurate when no starch was present in the fruit. This problem was the basic premise behind the use of longer wavelength NIR spectra and the use of regression coefficients to assess what parts of the spectra were useful when predicting each parameter and whether they were able to differentiate between them (Papers 1. And 3.).

DM and SSC have similar molecular composition and so virtually identical spectra, and their regression coefficients did reveal, albeit subtle, differences between DM and SSC models. CARS variable selection was more helpful than iPLS in doing this, as it selected individual spectra and increased the possibility of finding differences between models. The iPLS regression coefficients did not provide enough evidence to suggest that apple prediction models were actually discriminating between parameters (Figure 4., Paper 1.).
8.5.2. Lack of research on pears and differences in physiology

Prediction of pear quality using NIR spectroscopy is less researched and no specific work seems to exist on the prediction of pear DM or of preharvest quality. Models for pear SSC were far better than expected considering fruit still contained starch (average 5.9 on the SPI). Differences in pear physiology compared to apple, for example, may have contributed to the success of SSC prediction models.

Pears contain lignified parenchyma cells called sclereids, or ‘stone cells’, which are randomly distributed throughout their flesh but concentrated predominantly around the core and just beneath the skin. Stone cells first begin differentiating from parenchyma cells approximately 14 DAFB and most are finished developing between 53-67 DAFB (Cai et al., 2010; Sterling, 1954). Tissue softening during fruit maturation and ripening does not change the size or number of stone cells (Bain, 1961). Pear skin is approximately 15-22 µm thick and longer NIR spectra can penetrate approximately 2-3 mm into fruit (Lammertyn et al., 2000). Stone cells may affect the way starch and other carbohydrates are bound or distributed in pears, so their presence may significantly influence spectra and prediction models. Stone cells are also known to contribute to SSC in ripening fruit (Martin-Cabrejas et al., 1994). In addition pear spectra (Figure 1., Paper 3.) clearly had a large amount of scatter but it is unclear whether this was directly related to stone cells. If research on the prediction of pear quality with NIR spectroscopy is to advance, the role of stone cells needs to be investigated further.
8.5.3. Methodological issues

Some of the methods employed during this PhD project could be improved as they may sometimes yield ambiguous results. A prime example is the starch pattern index (SPI), which is based on the ranking of samples according to a visual scale (Section 2.5.7., Chapter 2.) and by its very design, highly subjective. Starch analysis would be far more accurate if a technique such as Ion Chromatography was used in situations where precise starch values were needed, for example in regression modelling. This method would also be useful for analysis of individual sugars. DM sampling could be improved by freeze-drying samples instead to make the process more consistent and less prone to error from too little or too much oven drying and to reduce the possibility of absorption of atmospheric moisture. The method used during this PhD is standard practice and potential error was minimised by weighing samples as soon as possible after they came out of the oven.

Apart from the desire to produce data that is as accurate as possible, precise measurement of fruit quality parameters is all the more important when the information is being used as reference data for NIR spectroscopy models. NIR spectroscopy is a secondary method and will always remain so, as it will never eliminate the need for conventional laboratory based quality analysis. Therefore the accuracy and strength of prediction models is entirely dependent on the precision of these reference measurements (Osborne et al., 2000).

8.6. Conclusions and perspectives

The intention of this PhD was to contribute to existing knowledge on how root pruning affects fruit quality and to assess the value of supplementary irrigation in mitigating some
of its effects on fruit. A large part of this was to understand how these methods alter fruit DM in particular and if this has a bearing on quality in harvest and postharvest. This project was linked to another at the KU-LIFE looking at the effects of supplementary irrigation on shoot and root growth of root pruned pears. The results of this collaboration are presented in Paper 4.

Results from the root pruning and irrigation experiment (Paper 2.) indicate that root pruning rather than irrigation has the most impact on fruit quality, especially on fruit size, DM and SSC. Supplementary irrigation did alter fruit quality by increasing average fruit size and reducing DM and SSC. However a slight reduction in DM and SSC may be an acceptable trade-off in years where irrigation can help increase average fruit size if it is at risk of it being below marketable standard.

The experiment was designed to minimise the confounding effects of rain when irrigating trees in order to obtain a reasonably independent result. The results do show that supplementary irrigation influenced fruit quality, but the effect of the plastic cover are harder to quantify. These could include reduced evaporation of water from the soil, increased temperature etc. It is an unrealistic set-up and growers are unlikely to want to cover the soil around their root pruned trees, but it does give an indication of the potential changes irrigation can bring about in fruit quality.

To our knowledge, Paper 3. is the first to investigate the prediction of pear preharvest DM using NIR spectroscopy. The results are promising, but little is known of the potential effect pear internal physiology has on prediction models. The SSC results were better than
expected given the presence of starch in the fruit and were in contrast to previous literature. Therefore repeat experiments are required to verify the results presented here.

Another intention was to assess the potential of NIR spectroscopy to accurately predict fruit DM and SSC, whilst also trying to address the problem of carbohydrate distinction in models. However there are still many obstacles in NIR spectroscopy, including a lack of instrument cross-calibration, the need to build models based on data from many seasons and cultivars and the lack of model extrapolation. The ultimate challenge for the future is to bring the technology out of the laboratory and to fully integrate NIR spectroscopy into on-line systems located on farms and packhouses.

To conclude, the work presented here demonstrates fruit DM links many aspects of fruit husbandry. DM can be a reliable indicator of potential fruit quality due to its relative stability, even in preharvest fruit, but it is also possible to manipulate its value by altering tree physiology. Together with the prospect of non-destructive prediction, these attributes combined have the potential to improve fruit production by providing an insight into the status of fruit as they develop on the tree and an idea of their potential quality at harvest and after a period of storage.

8.7. Future research

This PhD project has tested the hypotheses and fulfilled the aims outlined in the introduction. Nevertheless researching and carrying out experiments during the course of this project has raised many questions and highlighted a number of under-investigated
areas in the literature. Listed below are some issues that may be of interest for future research.

- Potential effects of root pruning on early fruit development, during cell division: Research here would involve earlier sampling of fruit. Is timing of root pruning a factor and does it hasten a shift from the main cell division phase to cell expansion? Does it have an effect on preharvest fruit DM?

- Biological basis of fruit DM: low DM brings the risk of storage disorders, whilst high SSC may improve fruit stress tolerance. Investigation into the biological changes and benefits high DM and SSC may bring to fruit storage quality.

- Creation of prediction models based on un-averaged reference data and spectra: averaging the two measurements taken on each fruit (the blush and shade side) is well established by previous authors and seems to be standard protocol. Investigating the influence of location on the repeatability and accuracy of models may be interesting. Previous work is published regarding within fruit biological variability (Peirs et al., 2010), but it may be of practical value to refine existing sampling protocols.

- Global models: with reference to Paper 1., a more practical approach and one that would be more robust is to compare a global prediction model (as in Paper 3.) against creating prediction models based on individual sampling days.

- Further examination of the results set out in Paper 3.: the unusually good prediction models for preharvest pear SSC require additional research for verification. Pears are also relatively under-investigated relative to their economic importance with only a handful of NIR spectroscopy papers published so far. In addition their biology is
different enough from apples to justify this, as no other literature appears to exist concerning prediction of pear DM.

- Pear stone cells: additional research to clarify how pear internal structure may influence prediction models, with particular reference to their role and potential relationship to starch and/or SSC and overall DM. Some investigation into their anatomy and structure may provide some insight.

- Distinction between DM and SSC: Over 280 papers to date have been published concerning NIR spectroscopy and apples, with DM research gaining momentum over the last 10 years. SSC is a standard quality parameter and DM is becoming a more important parameter in commercial fruit production, yet investigation into DM/SSC distinction is under-investigated.
References


Hansen, P. 1970. ^14_C-studies on apple trees. V. Translation of labelled compounds from leaves to fruit and their conversion within the fruit. Physiol. Plant. 23, 564-573.
Hansen, P. 1971. ¹⁴C-studies on apple trees. VII. The early seasonal growth in leaves, flowers and shoots as dependent upon current photosynthates and existing reserves. Physiol. Plant. 25, 469-473.


an indicator of dry matter and ripened soluble solids of kiwifruit. Postharvest Biol. 
Technol. 20, 163-173.

Kader, A.A. 1999. Fruit maturity, ripening and quality relationships, Acta Hort., 485, 203- 
208.

maintaining postharvest quality. Postharvest Technology Research and Information 
Centre. University of California, Davis, CA.


Kavakli, I.H., Slattery, C.J., Ito, H. and Okita, T.W. 2000. The conversion of carbon and 
nitrogen into starch and storage proteins in developing storage organs: an overview. 

233-247.


Khan, Z.U., McNeil, D.L. and Samad, A. 1998a. Root pruning reduces the vegetative and 
reproductive growth of apple trees growing under an ultra high density planting 


Li, J., Huang, W., Zhao, C. and Zhang, B. 2013. A comparative study for the quantitative
determination of soluble solids content, pH and firmness of pears by Vis/NIR
Lin, H. and Ying, Y. 2009. Theory and application of near infrared spectroscopy in
quality indices by visible and near infrared spectroscopy. Food Sci. Technol. 41, 1720-
1725.
Liu, Y., Ying, Y., 2005. Use of FT-NIR spectrometry in non-invasive measurements of
López-Carmelo, A.F. 2004. Manual for the preparation and sale of fruits and vegetables,
in ‘Conference’ pear grown under deficit irrigation: implications for fruit quality at
of apples using near infrared diffuse reflectance. J. Texture Stud.31, 615-630.
Sci. 38, 119-128.
Maggs, D.H. 1964. Growth rates in relation to assimilate supply and demand. I. Leaves and
roots as limiting regions. J. Exp. Bot. 15, 574-583.

Mallardi, A., Hirst, P.M. 2010. Increase in fruit size of a spontaneous mutant of ‘Gala’ apple (Malus x domestica Borkh.) is facilitated by altered cell production and enhanced cell size. J. Exp. Bot. 61, 3003-3013.


McCarthy, A. 2001. Avocado Culture in Western Australia. The Department of Agriculture, Western Australia.


Naschitz, S., Naor, A., Genish, S., Wolf, S., Goldschimdt, E.E. 2010. Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations, Tree Physiol. 30, 715-727.


References


References


Appendices

Paper 1.

ACCEPTED

Predicting apple (cv. Elshof) postharvest dry matter and soluble solids content with NIR spectroscopy
Travers, S., Bertelsen, M.G. and Kucheryavskiy, S.V. (2013)

Journal of the Science of Food and Agriculture
Predicting apple (cv. Elshof) postharvest dry matter and soluble solids content with NIR spectroscopy

RUNNING TITLE

Predicting apple dry matter and soluble solids content with NIR spectroscopy

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ABSTRACT

BACKGROUND: Fruit dry matter (DM) and soluble solids content (SSC) are primarily composed of carbohydrate and are standard parameters for assessing quality. Near infrared spectroscopy provides potential for non-destructive fruit quality analysis but the collinearity between DM and SSC is an issue for prediction. Shorter wavelength spectra have been used for the prediction of fruit DM and SSC, but radiation between 1000-2500 nm may be suitable for distinguishing between the two forms of carbohydrate.

RESULTS: Spectra and DM and SSC samples were taken for a total of 450 ‘Elshof’ apples 30, 58 and 93 days after harvest. Regression models were built using the iPLS method. Prediction models
for DM and SSC for each day yielded $R^2$ values between 0.63-0.86 and RPDs between 1.7-2.7 for DM and $R^2$ 0.76-0.85 and RPDs 2.2-2.6 for SSC.

**CONCLUSION:** Model RPD values were not high enough for general quantitative predictions, although they compare well to previous work. Certain factors affected model success, including changes in fruit physiology over time and the range of reference data. The complexity of absorbance spectra for DM and SSC plus their strong correlation suggests that prediction models cannot easily distinguish between soluble and non-soluble forms of carbohydrate.

**Keywords:** apples; NIR spectroscopy; collinearity; dry matter; soluble solids content

**INTRODUCTION**

Quality does not only relate to a fruit’s sensory properties, it also includes a range of parameters vital for monitoring development, determining optimum maturity before harvest and ensuring maximum storage potential. These parameters include: soluble solids content (SSC), starch, titratable acidity and firmness. Recent research has proposed fruit dry matter (DM) content as an alternative quality parameter.$^{1,2}$ DM is composed of non-soluble carbohydrate, present in cell walls and fibrous tissue. Other parameters containing carbohydrate, such as starch and SSC, are traditionally assessed when determining harvest quality. Therefore DM and SSC are related as two forms of carbohydrate but also via the breakdown of starch by hydrolysis as fruit matures in storage.

Direct measurement of DM and SSC is laborious and time consuming whilst also only allowing a selection of fruit to be analysed. Near infrared (NIR) spectroscopy offers the potential for extensive non-destructive fruit quality analysis. One of the main benefits of spectroscopy is that more than
one parameter can be estimated simultaneously from the same spectra.\textsuperscript{3} The NIR range includes electromagnetic radiation between 780-2500 nm and is particularly suited to analysis of samples containing carbon-hydrogen (C-H), carbon-oxygen (C-O), oxygen-hydrogen (O-H) and nitrogen-hydrogen (N-H) bonds.\textsuperscript{4} This makes it ideal for assessing fruit samples which contain high percentages of water and carbohydrate.

Much research exists on quality prediction of numerous types of fruit using spectroscopy, ranging from apples; mangoes; peaches; apricots and pears.\textsuperscript{5} Research has primarily concentrated on firmness and SSC, as they are two major quality parameters and are considered the most successfully predicted.\textsuperscript{6} The preferred spectral range for predicting apple SSC varies widely, though most research employs wavelengths between approximately 800-1650 nm.\textsuperscript{5,7} Alternatively Park et al.\textsuperscript{8} used radiation between 400-2400 nm; Peirs et al.\textsuperscript{9} between 1080-2000 nm; and Walsh et al.\textsuperscript{10} between 300-1100 nm. Most existing research concerning DM prediction has generally focused on shorter wavelengths, between approximately 300-1100 nm for kiwifruit,\textsuperscript{11,12} avocado\textsuperscript{13,14} and numerous other fruit.\textsuperscript{10} Alternatively Wedding et al.\textsuperscript{15} used wavelengths between 780-2500 nm to predict DM of avocado.

An issue arising when trying to predict both DM and SSC is the need for models to be able to distinguish between the two parameters. Distinction is not necessary for DM prediction alone, but it may be of value when attempting to predict fruit SSC after starch hydrolysis is completed. This is further complicated, when using shorter wavelength radiation, by the presence of overlapping carbohydrate and water absorption bands between 700-1200 nm.\textsuperscript{4,12,16} Even at longer wavelengths, spectra of the two parameters are virtually identical. However subtle differences in the spectral
window between the two major water absorption peaks (1550-1850 nm), which includes the C-H 1st overtone, and in the C-H combination region (2200-2400 nm) may shed some light on the issue. Therefore despite limited light penetration in intact apples, longer NIR wavelengths, such as those between 1000-2500 nm, may contain information relevant to the prediction of fruit carbohydrate and could be suitable for discriminating between soluble and insoluble forms.¹

To the authors’ knowledge, no previous literature exists specifically concerning the distinction between forms of carbohydrate when using NIR spectroscopy. Even when considering research concerned with the prediction of carbohydrate content in other foodstuffs, which has been able to determine different soluble sugars and predict both insoluble soluble and carbohydrate at longer wavelengths, nothing has been found addressing this issue of discrimination.

With this in mind, and given the growing importance of DM as a quality parameter in apples, the work presented here investigated the possibility of predicting postharvest DM and SSC using reflectance spectra between 1000-2500 nm. The primary objective was to assess how well the models could predict apple DM and SSC after different periods of time in storage. Furthermore given the collinearity between DM and SSC, a secondary aim was to examine whether these prediction models, based on longer NIR spectra, had the ability to distinguish between the two parameters.
EXPERIMENTAL

Fruit

450 apples (cv. ‘Elshof’) were harvested from an orchard located at the Department of Food Science, Aarhus University, Aarslev, Denmark (55°31’ N, 10°44’ E, 49 m MSL) on the 11th October 2010. They were stored at 1 °C at 95 % relative humidity until required for sampling.

Quality testing and spectral measurements

Quality tests were performed on fruit 30, 58 and 93 days after harvest (Day 0). 24 hours before each test, the apples were moved to a 16 °C room at 30 % relative humidity to equilibrate. 150 fruit were measured on each quality test day. Two measurements sites were located and marked on each apple at its equator: one on the blush side of the fruit (1) and another on the opposite side (2). The location of measurement 1 dictated the location of measurement 2. All parameters were obtained at or as close to these two points. Spectra were taken immediately before destructive DM and SSC sampling. All spectra and samples were collected on the same quality test day.

General parameters

The peduncle was removed from the apple prior to sampling. All fruit were weighed (FX300i scales, A&D Co., Ltd., 0.01 g accuracy) and the diameter taken with a digital calipers (Whitworth Ltd.). Other parameters taken on the day include firmness, colour and starch content.

Spectral measurements

Spectral data were acquired on each fruit from points 1 and 2 using a semi-portable LabSpec 5000 (ASD Inc., Boulder, CO, USA). The LabSpec is a NIR spectrometer fitted with an InGaAs detector.
with a sampling increment of 1 nm and a spectral range of 1000-2500 nm. The number of spectrum
scans was set at 64. The instrument was fitted with a fibre optic cable and a direct contact probe
working in reflectance mode with a diameter of approximately 10 mm. A white Teflon tile was used
to provide a reference spectrum before and during each sampling session. The instrument was
configured with Indico Pro Data software.

Dry matter

Two 10mm plugs of tissue were taken from the same location on the fruit as the spectra. The core
was removed from each plug and it was weighed on a pre-weighed foil tray. The samples were oven
dried at 70 °C for 20 h until constant weight and weighed again. DM was expressed as percentage
dry weight of the initial fresh sample.

Soluble solids content

SSC was obtained by squeezing flesh samples from the immediate area around the DM plug with a
garlic press to extract the juice. SSC was determined (°Brix) with a temperature compensated
digital refractometer (RFM712, Bellingham Stanley Ltd., UK).

Chemometric and statistical analysis

Chemometric analysis was completed using MatLab (MathWorks, Natick, USA) software coupled
with PLS Toolbox (Eigenvector Research Inc., Wenatchee, USA). As two measurements were
taken per fruit, all measurement, including spectra, were averaged to create datasets based on each
fruit.11,22,23
The partial least squares (PLS) method was chosen to build regression models for predicting DM and SSC values from the measured NIR spectra. In contrast to traditional multiple linear regression (MLR), PLS takes into account only the most relevant part of the variation in the spectra (X-variables).\textsuperscript{24} The spectral data and response variables (Y-variables) data are projected onto a new latent variable space to build the PLS model. The latent variables are selected to give maximum covariance between the projected X and Y data. This makes it possible to construct simple and stable linear models even in cases when X contains hundreds of highly correlated variables, a common characteristic of spectral data.

Before further assessment all spectra were transformed from reflectance (R) to absorbance (Log 1/R) and preprocessed. Preprocessing included Savitsky-Golay spectral smoothing (with no derivative) as well as scatter correction with Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC). The best experimental configuration was based on 25-point Savitsky-Golay spectral smoothing (1\textsuperscript{st} order polynomial and SNV). Spectra and response values were also mean centred.

The datasets (reference values and spectra) for DM for each sampling day were y-sorted and systematically split into two subsets: 100 samples for calibration/validation modelling and 50 for a separate test set. The same procedure was applied to the SSC data. Random 10-segmented cross-validation was used to find the optimum number of latent variables (LVs). The models were then applied to the test set to evaluate their performance.
Samples that were not fitted by a proper PLS model in terms of residual distance and/or leverage were considered as outliers. A plot with squared residual distances ($Q^2$) vs. Hotelling $T^2$ values and corresponding significance limits was used. Objects with values far above the significance limits were excluded from the calibration.

Prediction models were built on the optimal number of latent variables (LVs) suggested by the calibration models using the separate test set. The statistics used to select the best models were: coefficient of determination ($R^2$); root mean square error (RMSE) of calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP); optimal number of latent variables required to build the model (#LVs); residual predictive deviation (RPD) and number of outliers removed (#Outliers). For the purposes of this paper, the most realistic comparison of model calibration statistics was RPD (SD/SEP). It is defined as the ratio of the standard deviation (SD) of the reference values to the standard error of cross-validation or prediction. RPD was used to evaluate the potential of NIR to determine DM and SSC. General summary statistics for DM and SSC were obtained using R (version 2.1.11.).

Interval Partial Least Squares (iPLS) regression was used to improve models by excluding spectra not relevant for modeling, whilst also limiting inclusion of interference. iPLS functions by creating numerous models based on sub-intervals of spectra (of equal width) and comparing their predictive performance - with each other and with the original spectral range. It gives an overview of spectra and highlights wavelengths that are most useful for modeling.
RESULTS

Reference data

Table 1. Summary statistics for DM and SSC reference data on Days 30, 58 and 93. Number of fruit (Number); Minimum (Min.); mean; maximum (Max.) and standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Min.</th>
<th>Mean</th>
<th>Max.</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 30</td>
<td>150</td>
<td>12.9</td>
<td>14.9</td>
<td>17.5</td>
<td>0.9</td>
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<tr>
<td>Day 58</td>
<td>150</td>
<td>12.2</td>
<td>14.9</td>
<td>17.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Day 93</td>
<td>150</td>
<td>11.8</td>
<td>14.8</td>
<td>17.7</td>
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<table>
<thead>
<tr>
<th>SSC, °Brix</th>
<th>Number</th>
<th>Min.</th>
<th>Mean</th>
<th>Max.</th>
<th>SD</th>
</tr>
</thead>
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<tr>
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<td>12.3</td>
<td>14.2</td>
<td>16.3</td>
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</tr>
<tr>
<td>Day 58</td>
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<td>11.4</td>
<td>14.3</td>
<td>17.1</td>
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<tr>
<td>Day 93</td>
<td>150</td>
<td>11.3</td>
<td>14.3</td>
<td>16.7</td>
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</table>

Table 1 shows minimum, mean, maximum and standard deviation values for DM and SSC for each of the three sampling days. The range of values was quite small, both between and within the parameters. Individual histograms showed that SSC and DM measurements for all three days were normally distributed around the mean (data not shown). There was no relationship between DM or SSC and fruit weight or firmness on a per fruit or per tree level. A strong linear relationship was found between DM and SSC over the three days (Fig. 1).

Figure 1. Correlation between apple DM and SSC reference measurements on Days 30, 58 and 93.
Features of spectra

Preliminary analysis of transformed spectra using iPLS made clear that the spectral range from 1000-1800 nm provided the best potential for modelling. Comparing models with full and reduced ranges proved this experimentally. Fig. 2 shows absorbance (Log 1/R) spectra before preprocessing for each sampling day with iPLS results. Although iPLS suggested that spectra between 1000-1100 nm be excluded, this did nothing to improve model performance.

Figure 2. Transformed (Log(1/R) spectra (light blue), average spectra (dark blue) and iPLS results (grey bars) for DM (left) and SSC (right) on Days 30, 58 and 93. The weight of each grey bar corresponds to the RMSECV of a local interval model.
The spectra are composed of multiple absorption peaks for the different chemical components and typically have broad undulations rather than sharp distinct peaks. Water absorbance peaks and overtone bands of the OH⁻ bonds dominate the spectra. A strong OH⁻ overtone band is visible at 1450 nm and the small peak at approximately 1150 nm is a mixture of carbohydrate (CH, CH₂ and CH₃) second overtones. Other carbohydrate absorbance bands are located at around 1400 and 1600 nm. Spectra for all three days are similar in shape but clear differences in absorbance are visible.

Calibration models for quality parameters

Correlation between the spectral information and quality data was assessed. Table 2 summarises the predictive ability of the best fitting calibration models for apple DM and SSC 30, 58 and 93 days after harvest. Comparison of models from each of the three days indicated that their predictive potential varied significantly. All models included a maximum of 20 latent variables (LVs) but the optimum number estimated using cross-validation was between 6 and 7.

Dry matter prediction

The predictive ability of models for DM varies according to sampling day (Table 2). Day 58 clearly produces the best model for DM (R² = 0.86; RMSEP = 0.37; RPD = 2.7), with Day 30 having the lowest predictive ability (R² = 0.63; RMSEP = 0.48; RPD = 1.7).
Table 2. Model statistics for apple DM and SSC on Days 30, 58 and 93. All models were cross-validated and predicted with a test set. Spectra were transformed to absorbance (Log 1/R); selected variables 1000-1800 nm; 25-point Savitsky-Golay spectral smoothing (1st order polynomial); standard normal variate (SNV).

Statistics: number of fruit (Number); coefficient of determination (R²); root mean square error (RMSE); residual prediction deviation (RPD); number of latent variables (#LVs); and number of outliers (#Outliers).

<table>
<thead>
<tr>
<th>Day</th>
<th>Model</th>
<th>Number</th>
<th>R²</th>
<th>RMSE</th>
<th>RPD</th>
<th>#LVs</th>
<th>#Outliers</th>
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<td>Cross-validation</td>
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<td>0.38</td>
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<td>Prediction</td>
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<td>0.48</td>
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<td>0.37</td>
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<td>0.91</td>
<td>0.29</td>
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Soluble solids content

SSC was marginally better predicted on Day 58 (R² = 0.85; RMSEP = 0.4; RPD 2.6) compared to Day 30 (R² = 0.81; RMSEP = 0.39; RPD 2.4) and Day 93 (R² = 0.76; RMSEP = 0.46; 7 LVs; RPD 2.2).
Figure 3. Predicted vs. measured plots for DM (left) and SSC (right) on Days 30, 58 and 93. Cross validation (CV); test set (Test).

The R² values for SSC prediction models are similar on Day 30 and 58, except that Day 58 requires an extra LV. On the other hand, DM prediction on Day 58 is better compared to that of Day 30. However by Day 93 there is a marked drop in the predictive ability of models for both parameters.
This is visible in the predicted versus measured plots (Fig. 3) for each day and parameter where the linear correlation for Day 58 is better than the other two days. The standard of models for DM and SSC were similar on Days 58 ($R^2 >0.84$; RMSEP <0.40) and 93 ($R^2 >0.75$; RMSEP <0.50). SSC prediction on Day 30 was similar to that for Day 58.

Figure 4. Regression coefficients for DM and SSC models on Days 30, 58 and 93. The absorbance spectra are outlined in grey.
Regression coefficients

Regression coefficients, plotted according to sampling day (Fig. 4), show consistently strong peaks and troughs at certain wavelengths for all three sampling days – close to 1100 nm (CH₃), 1200 nm (CH, CH₂), 1250 nm (CH), 1450 nm (OH⁻) and 1600 nm (CH₃).

DISCUSSION

Predictions and the relationship between DM and SSC

DM is considered a relatively stable parameter compared to SSC. This is of particular importance when starch is still present in fruit. DM is a combination of both insoluble and soluble solids and results from McGlone et al.¹ show approximately 2% difference between the two parameters. The relatively small difference between DM and SSC reference values may be due to removal of the core when the DM samples were being prepared.

The narrow range of DM and SSC references values and the fact they did not change much over the period the fruit were in storage may have been an issue for modelling. With reference to Table 1, Day 30 had the smallest data range, for both DM and SSC whilst two days were appreciably wider. It follows that the statistics for Days 58 and 93 are generally better as by extending the data range of the parameter of interest, the calibration statistics can be improved.¹⁰ Furthermore RMSECV values did not vary significantly (0.32-0.45) throughout the experimental period. However other factors previously mentioned, as well as apple biological variability, may have hindered DM and SSC prediction on Day 93.
The DM and SSC prediction models presented here are on a par with previous research, as are the $R^2$ and RMSEP values and the number of LVs used.$^{1,11,15}$ The predictive ability of DM models varied depending on sampling day, with Day 58 being the best (Table 2). On the other hand SSC prediction remained more consistent than DM across all three sampling days (Table 3). This model consistency and the strong linear relationship between the two parameters suggest that starch hydrolysis was complete or near complete before sampling commenced.$^{27}$ On the first sampling day (Day 30) all samples were between 7 and 10 on the starch-iodine chart (10 = no starch present), with an average reading of 8.4 (data not shown). It is not surprising that prediction statistics for SSC on Day 30 were only marginally different to the other two days, as most starch had been hydrolysed.

Given the close relationship between DM and SSC, their regression coefficients (Fig. 4) may provide some insight into the ability of prediction models to distinguish between parameters. However assignment of spectral bands using regression coefficients is risky as they hide collinearities and complications that hinder accurate interpretation of raw spectra. They are best viewed in conjunction with spectra, as weights that apply to spectra at certain points. For instance, there is a shift in the regression coefficient peak for SSC at approximately 1250 nm on Day 30, at first glance this would seem significant and a sign of distinction between models of the two parameters. However the amplitude of the absorbance spectra here is relatively low compared to longer wavelengths. The smaller positive peak (in contrast to the negative DM one) found at approximately 1750 nm is probably more significant for SSC modelling on that day than DM given the greater amplitude of the absorbance spectra here. Overall the regression coefficients demonstrate that DM and SSC models are based on the same spectral features since they are very
similar in their general pattern – three strong peaks at approximately 1250, 1450 and 1600 nm – but they do not provide enough evidence to show the models could actually distinguish between the two parameters. Furthermore this lack of distinction, coupled with the strong collinearity of the DM and SSC, creates uncertainty about what parameter the models are actually predicting.

RPD values are useful for evaluating how well a regression model can predict chemical data. The higher the value, the more probable the model can predict the composition of samples outside the calibration set. The RPD values for the models presented here were always less than 3, which is below the minimum threshold required to consider the model strong enough for general quantitative predictions. However they compare favourably to those of Bobelyn et al., who had RPD values for apple SSC of between 1.36-2.89 ($R^2 = 0.46-0.88$, RMSECV = 0.6-0.87, LVs = 6-8), to Paz et al. with a RPD range of 1.38-1.82 for pear SSC ($R^2 = 0.61-0.79$, RMSECV = 0.74-1.21) and to Park et al. where $R^2 = 0.93-0.96$, RMSEP = 0.28-0.34, LV = 10. The results for DM were not as strong as the models for apple made by McGlone et al. where $R^2 > 0.95$ (RMSEP < 0.32) but they were better than those of Wedding et al. for avocado where $R^2 = 0.76$ (RMSEP = 1.53, LVs = 5).

Changes in fruit quality

‘Elshof’, a clone of ‘Elstar’, is still considered marketable even when less firm, unlike other cultivars such a ‘Golden Delicious’ for example. By Day 93, average firmness was 4.2 kg cm$^{-2}$ compared to 5.9 kg cm$^{-2}$ and 4.6 kg cm$^{-2}$ on Days 30 and 58 respectively (data not shown). This does not however explain the reduction in model performance for both DM and SSC by Day 93. It is possible that other factors including changes in the apples as they aged hindered radiation
penetration and increased scattering, significantly compromising the predictive ability of the models.

Fruit softening over time and the resultant weakening of cellular structures influences the ability of light to pass through the flesh. Likewise as cell walls become less turgid and overall cell volume is reduced, there is an increase in light scattering over a smaller area. These changes in internal and external fruit structure may explain the higher reflectance and lower absorbance of spectra the longer the fruit remained in storage. However despite alterations in fruit quality, fruit mean DM content did not change significantly over the three sampling days. The non-soluble forms of carbohydrate that comprise apple DM are mainly structural components and so are relatively physiologically stable. ‘Elshof’ is also known for its ability to maintain quality during storage, so a relatively small change over the period of the experiment is not surprising.

The loss of predictive ability for DM and SSC models on Day 93 could also be related to alterations in apple skin interfering with radiation penetration and causing scattering. Even though ‘Elshof’ skin is relatively thin and smooth, it becomes wrinkly and tough as it ages. Skin can also be an issue with longer wavelength reflectance spectra, as used here - sampling depth is estimated to be between 1-2 mm and 2-3 mm. Such small distance brings the risk of skin dominating the spectra. Nevertheless compared to other fruit, apples tend to produce better calibration models, as their skin is relatively thin (approximately 1 mm) and smooth and their flesh reasonably homogeneous in structure.
CONCLUSION

Model RPD values were insufficient for general quantitative predictions, although they compare well to previous authors using shorter wavelengths or a combination of visible and NIR spectra. Certain factors affected model success, including changes in fruit physiology over time and the range of reference data. The complexity of absorbance spectra for DM and SSC, plus their strong correlation suggests that prediction models cannot easily distinguish between soluble and non-soluble forms of carbohydrate.

REFERENCES


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(Pyrus communis L.) pear quality

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The effect of root pruning and supplementary irrigation on ‘Clara Frijs’ (*Pyrus communis* L.) pear quality

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Abstract

Orchard trees are root pruned to regulate tree vigour in favour of reproductive growth and to promote canopy light interception for optimal fruit yield and quality. Root pruning encourages flower bud initiation but also affects fruit quality and maturity. The work here investigated the impact of root pruning on pear preharvest development and harvest and postharvest quality, particularly on fruit dry matter (DM) content. Furthermore the potential of supplementary irrigation to help counter some of the consequences of root pruning were also assessed. Fruit dry matter (DM) is a useful indicator of quality due to its relative stability as a parameter and its close relationship with postharvest soluble solids content (SSC). Root pruning increased crop load and advanced harvest maturity the following year. It also reduced individual fruit size and increased harvest DM and SSC. Indeed the impact of root pruning on DM content was evident 51 days after full bloom (DAFB). Supplementary irrigation helped counter the effects of root pruning on fruit size, DM and SSC but did not influence maturity or the following year’s crop load. Non-root pruned trees showed a typical biennial bearing crop load the following year, producing large fruit with low DM and SSC. The quality of these fruit did not improve in storage. The results suggest that high harvest DM was a good measure of postharvest pear quality, due to its link with potential SSC. Furthermore preharvest DM was found to be a good indicator of DM at harvest and after storage.

Keywords: pears, dry matter, quality, root pruning, irrigation, postharvest
1. Introduction

Root pruning is routinely used in Denmark as a non-chemical alternative to plant growth regulators (PGRs) for controlling vegetative growth in orchard trees. Regulating tree vigour is an essential aspect of fruit production to maintain the balance between vegetative and reproductive growth whilst also ensuring maximum canopy light penetration to optimise fruit yield and quality. The use of chemical PGRs in Danish commercial pear production was prohibited in 2002, mainly due to concern over their effects on human health. However other European countries, including the UK and Ireland, still use PGRs, such as Regalis (Prohexadione-Calcium), in apple production.

The impact of root pruning on vegetative growth varies according to its timing. Research has shown that root pruning was most successful in reducing canopy volume when completed before bud break in early spring (February-March) (Khan et al., 1998a; McArtney and Belton, 1992) or slightly later (March-May) when shoot growth was at its peak (Schupp and Ferree, 1987). Other studies found no significant effect on shoot growth when root pruning was performed during dormancy, full bloom or in mid-June (Ferree, 1992). Schupp and Ferree, 1987 also reported that late root pruning during ‘June drop’ increased preharvest fruit abscission but had no influence on shoot growth. Apart from the timing of root pruning, tree age and crop load influence the response with growth more inhibited in older trees with a high crop load than in younger ones with little or no fruit (Schupp and Ferree, 1987).

The primary function of root pruning is to reduce root volume in the upper soil layer and limit water and nutrient uptake (Green and Clothier, 1999; Green et al., 2003). Roots also function as a carbohydrate store during dormancy and reducing a tree’s capacity to take up, store and access
assimilates throughout the growing season compromises its storage pool the following winter (Khan et al., 1998b). End of season assimilate storage also depends on other factors such as crop load, seasonal canopy light interception and/or the severity of root pruning.

Therefore root pruning essentially restricts carbohydrate mobilisation and alters the intricate carbohydrate source-sink relationship between photosynthetic source leaves and vegetative and reproductive sinks (Wünsche and Ferguson, 2005). Carbohydrate mobilisation is particularly vital in early spring for shoot growth, fruitlet survival and flowering induction as trees rely on stored resources before photosynthesis starts (Naschitz et al., 2010; Wünsche and Ferguson, 2005). Vegetative growth is a strong sink early in the season but this changes as extension growth terminates and fruit take over as the dominant assimilate sink. Furthermore root pruning causes a tree to shift from vegetative to more reproductive growth the following year through the promotion of flowering spurs (Khan et al., 1998a). In practical terms, root pruning could be an effective and economic way of reducing excessive tree volume, improving flowering and minimising orchard pruning labour costs.

Root pruning also has an effect on fruit quality. In apples it primarily reduces fruit size (Ferree, 1992; Khan et al., 1998a; Schupp and Ferree, 1988), though timing does not seem to be a factor (Ferree, 1992). It also increases soluble solids content (SSC), firmness and improves fruit colour in apples (Elfving et al., 1991; Ferree, 1992; Schupp and Ferree, 1988). However McArtney et al. (1992) and Vercammen et al. (2005) report softer fruit and elevated SSC in pears from root-pruned trees due to early maturation of fruit compared to non-root pruned trees. There is also evidence to suggest that these differences in harvest quality persist during storage (Elfving et al., 1991).
Supplementary irrigation may help counter some of the effects of root pruning on fruit quality. Rather than simply providing unrestricted amounts of water, techniques such as deficit irrigation, which limit the water supply to the trees, may improve fruit quality (Behboudian and Mills, 1997). Deficit irrigation during fruit development involves certain periods where water is supplied below the evaporative demand of the tree to achieve a plant/soil water deficit. The technique was originally designed to control vegetative growth, as this is more sensitive to water deficit than fruit growth (Ebel et al., 1995; Forshey and Elfving, 1989).

The effects of deficit irrigation on apple and pear quality are similar to those for root pruning. Deficit irrigation reduces harvest fruit size for apple (Ebel et al., 1993; Ebel et al., 1995; Mpelasoka et al., 2001a) and pear (Marsal et al., 2000). However the results are inconsistent as others find only a marginal reduction in pear (Kang et al., 2002) or apple size (Leib et al., 2006). Higher firmness, SSC and DM, improved colour (Behboudian and Lawes, 1994; Ebel et al., 1993; Kilili et al., 1996; Mpelasoka et al., 2001a, 2001b) and delayed starch degradation (Ebel et al., 1993; Elfving et al., 1991; Mpelasoka et al., 2001b) were reported in apples following deficit irrigation. Ebel et al. (1993) and Mpelasoka et al. (2001a and 2001b) also found that harvest differences in apple SSC and TSS due to deficit irrigation were maintained during storage. However Mpelasoka et al. (2001a and 2001b) noted that deficit-irrigated fruit softened faster compared to non-deficit irrigated fruit.

Fruit quality assessment is based on a number of parameters, but recent research has focused on DM as a reliable indicator of quality at harvest and during storage (Harker et al., 2009; McGlone et al., 1998). DM is primarily composed of soluble carbohydrates such as those comprising SSC and starch, which are routinely assessed when determining fruit quality and of non-soluble
carbohydrates, present in fruit cell walls and fibrous tissue. Consideration of DM as a measure of postharvest quality is due to its stability as a parameter and its close relationship to SSC, via starch hydrolysis (Palmer et al., 2010) and the fact that DM and SSC are two parameters positively linked to consumer acceptance of fruit (Harker et al., 2008; Harker et al., 2009). McGlone et al. (2003) and Palmer et al. (2010) and others have investigated the relationship of harvest DM to postharvest quality, but to the author’s knowledge no work exists regarding preharvest estimation of pear DM and its possible relationship to harvest and postharvest quality.

The most common pear cultivar in Denmark is ‘Clara Frijs’. It is a medium sized fruit with an average weight of 130 g (60-70 mm diameter). Harvest is recommended when fruit firmness reaches ~5 kg cm\(^{-2}\) and SSC ~11 °Brix. The fruit has a smooth skin with a bright green ground colour that turns a uniform yellow during ripening and is sometimes accompanied by a slight blush. ‘Clara Frijs’ pears are considered at their best when consumed crisp and green, not long after harvest.

The main aims of this study were to examine the effects of root pruning on pear development and on harvest and postharvest fruit quality, particularly DM content and to assess whether irrigation could mitigate some of its side effects. Aside from Patterson et al. (2009), who found root pruning increased DM content in kiwifruit, little research exists regarding the effects of root pruning on the DM of other fruit, such as pears. We hypothesised that pears containing higher levels of DM at harvest were better able to maintain quality postharvest and that harvest DM content could be predicted from preharvest DM content. Therefore correlations between preharvest, harvest and postharvest DM content were investigated for the possibility of predicting pear quality when
implementing strategies such as irrigation or root pruning.

2. Materials and Methods

2.1. Experimental design

Experiments were conducted in 2010 and 2011 at the Department of Food Science, Aarhus University, Aarslev, Denmark (55° 18’ N, 10° 27’ E, 49 m MSL). The pears (*Pyrus communis* L. cv. Clara Frijs) were from 11-year-old field grown trees grafted onto Quince A rootstock, with a planting distance of 3.5 m between rows and 1.5 m within rows (running north to south). The soil was classified as sandy loam with a soil water content (%, vol.) of 26.5 % at field capacity and 7.5 % at wilting point. An on-site Danish Meteorological Institute (DMI) weather station collected field climate data.

The experiment was established as a block design, spanning five rows of trees with every row containing five plots of nine trees each. One plot was assigned per treatment per row and experimental trees were chosen based on number of flower clusters and their location towards the middle of each plot. The trees were in full bloom on 17 May 2010 and on 28 April 2011. Fertiliser was applied to the rows in early spring of each year at a rate of 50 kg N ha⁻¹. It was in solid form in 2010 (NH₃NO₃+CaCO₃) and in liquid form in 2011 (Nitro 30, 4.5 % Urea N, 25.5 % Methylene Urea).

2.2. Treatments

There were five experimental treatments. Two uncovered treatments: root pruned (RP) and non-root pruned (NP), which received no supplementary irrigation and were dependent on rainfall. Three
irrigation treatments, where the soil was covered shortly after root pruning in spring: no irrigation (NI), with no supplementary water supplied; deficit irrigation (DI), irrigation to a soil water content (SWC) of 21%; and full irrigation (FI), irrigation to field capacity (26.5%). The irrigation treatments commenced once the SWC dropped below 21% SWC for FI and 13% for DI at the start of the season. In 2010, FI and DI treatments started 47 DAFB and 60 DAFB, respectively and in 2011, 25 and 34 DAFB, respectively. Thereafter SWC (% vol) was measured once a week prior to irrigation and the volume of water required then calculated. SWC was measured to a depth of 1.0 m with a Trime Time Domain Reflectometry system with a T3 tube access probe (IMKO Micromodultechnik GmbH, Ettlingen, Germany).

Apart from the NP treatment, all trees in the experimental field were root pruned on 14 April 2010 (24 days before full bloom) and 4 April 2011 (33 days before full bloom), 0.4 m from the trunk on both sides of the row and to a depth of 0.3 m with a tractor-mounted subsoiler blade. For the irrigation treatments, the soil around the trees (1.2 m beyond where they were root pruned), was covered with thick translucent white plastic sheeting each spring and arranged so any excess rainfall could run-off between the rows, beyond the immediate root zone of the trees.

2.3. Quality testing and data analysis

Preharvest DM content, whole fruit weight and diameter were recorded for 25 randomly selected fruit per treatment (5 per replicate) 51, 67, 81 and 96 DAFB in 2011. Harvest date was based on firmness (~5 kg cm$^{-2}$) of the first mature fruit from any of the treatments. In addition to firmness, harvest quality testing also included SSC, colour and starch measurements. All treatments were harvested on 31 August 2010 (106 DAFB) and 18 August 2011 (112 DAFB). In 2010 and 2011
changes in postharvest fruit quality were measured after 4, 6 and 10 weeks and 2, 4 and 8 weeks, respectively, of cold storage at 0.5 °C and 95 % relative humidity (RH). Twenty-four hours before each postharvest quality test, the pears were moved to a room (~16 °C) to equilibrate. Colour, DM and SSC for all sampling days were obtained from two regions located on the widest part of the fruit: one on the ‘shoulder’ side (Point 1) and another directly opposite (Point 2). Firmness was taken between Points 1 and 2. All measurements were completed on the same day. The peduncle was removed from the pear prior to measuring. Fruit were weighed using an FX300i scales, A&D Co., Ltd. (0.01 g accuracy) and the diameter recorded with a digital calipers (Whitworth Ltd.).

For DM determination, two 10 mm diameter plugs of tissue were taken perpendicular to the fruit surface. After the core was removed each plug was weighed on a pre-weighed foil tray. The samples were oven dried at 70 °C for 20 h until constant weight and re-weighed. DM was expressed as percentage dry weight of the initial fresh sample. SSC was obtained using a temperature compensated digital refractometer (RFM712, Bellingham Stanley Ltd., UK). Samples were taken from an area adjacent to the DM plug; the flesh was squeezed with a garlic press to extract juice and SSC determined (°Brix).

Two skin colour readings were taken on Points 1 and 2 prior to DM and SSC sampling using a colorimeter (Chroma Meter, CR-400, Konica Minolta Sensing Inc., Japan) to measure L*, a*, b*, C with a focus on hue angle (h). Two firmness measurements were obtained using a penetrometer (GS20 Fruit Texture Analyser, Güss Ltd., South Africa) equipped with an 8 mm probe, after the skin was removed. Firmness values were expressed in kg cm⁻². The starch pattern index (SPI) was determined by dipping a cross-section of flesh (~3 cm from the calyx) in a standard iodine solution
for 30 s and after 2 mins ranked on a visual scale from 1 (black, starch present) to 10 (white, no starch present). Pear maturity was calculated using the Streif Index: Firmness/(SSC*SPI).

All statistical analyses were completed using R (Version 2.1.11.). Fruit measurements were averaged to create datasets on a per tree level for each sampling day. Statistics for the irrigation treatments and the uncovered treatments were calculated separately. Analyses included: two-way analysis of variance (ANOVA); minimum, mean and maximum values; standard error; and the Duncan test for statistical significance (P>0.05). Standard error was calculated using Excel for Mac (Version 14.0.0).

### 3. Results

#### 3.1. Field conditions

Average air temperature from full bloom to harvest in 2010 was 16.2 °C (9.3-25.2 °C range) and total precipitation was 251 mm (Fig. 1). In 2011 average temperature was 15.1 °C (6.0-21.25 °C) and total precipitation was 372 mm. Average soil temperature between 10-30 cm was 16 °C (8.8-20.2 °C) in 2010 and 15 °C (8.8-19.7 °C) in 2011 (Fig. 1).

Fig. 1.

Total irrigation volume supplied from the start of the treatment until harvest was 203 mm and 140 mm under DI in 2010 and 2011, respectively (Table 1). The FI treatment received 512 mm in 2010 and 658 mm in 2011.

Table 1.
3.2. Pear quality and analysis

Pear quality results are presented in three sections: preharvest results for 2011, including weight, diameter and DM; harvest results for 2010 and 2011 comprising weight, diameter, crop load, DM, SSC, firmness, colour and starch; and postharvest results for 2011 including DM, SSC, firmness and colour.

3.2.1. Preharvest quality 2011

Although not always significant, FI fruit were heavier than NI fruit on all preharvest sampling days by 10-12 %. The gap had widened to a 20.3 % difference by harvest. There was a significant difference in fruit weight between the NP and RP fruit from Day 67 (NP fruit were 20.1 % larger). This difference increased to 27.1 % by Day 96 and was 32.2 % by harvest.

By 51 DAFB, DM content for all treatments was between 23-25 %. This had fallen to less than 19 % by Day 67 and remained between 14-18 % until harvest.

NI fruit had significantly higher DM content than FI fruit on all sampling days (Table 2). RP fruit had significantly more DM content than NP fruit on every sampling day: 8.7 % more DM on Day 51, 17.8 % on Day 96 and 24.4 % at harvest.

No clear trend emerges in the irrigation treatments for total cumulative preharvest DM content (g (%DM/100*fruit weight*harvest crop load) of fruit over the sampling days (Table 3). However NP fruit on 51 DAFB have approximately half the total DM compared to RP - a difference that is maintained throughout the preharvest sampling period.
3.2.2. Harvest quality 2010 and 2011

Average crop load in 2010 was reasonably uniform across all five treatments as the experimental trees were selected based on a similar number of flower buds (Table 4). The 2011 crop load for all three irrigation treatments was higher than the previous year. The crop load on NP trees was less than half of the RP.

Table 4.

Irrespective of treatment, average fruit weight was higher in 2010 than in 2011, except for NP, which remained the same. In both years FI fruit were significantly heavier than NI fruit (Table 5). NP fruit were heavier than RP fruit in both 2010 and 2011, but the difference was only significant in 2011, where NP fruit weighed 32.2% more.

Table 5.

Fruit had a higher DM and SSC across all treatments in 2010 than 2011. DM and SSC in irrigated fruit followed the same trend in 2010 and 2011 in that NI fruit had significantly more DM and SSC than FI fruit (Table 5). In 2010 and 2011, pears from RP trees had significantly higher DM and SSC compared to NP. RP DM content was 29.4% higher compared to NP in 2010 and 24.4% in 2011.

Table 6.

Total DM partitioning to fruit in 2010 was relatively consistent between treatments, with NI having significantly more DM and SSC than FI fruit (Table 6). In 2011 general allocation across the treatments was higher and the difference between NI and FI treatments was again present except that FI had more DM and SSC. One major change in 2011 was DM allocation to the fruit of NP trees, which dropped dramatically compared to its value in 2010 and with respect to RP fruit.
Variation in firmness between irrigation treatments over the two years was consistently significant. Pears from NP trees were significantly firmer than RP in both years. There was no positive correlation between firmness and fruit size across or within any of the treatments for either year, even when similar sized fruit from each treatment were compared (data not shown).

Pears were greener (had a higher hue angle) across all treatments at harvest in 2011 compared to 2010 (Table 5). NP pears were significantly greener than RP fruit in both 2010 and 2011.

The results for starch were not consistent over the two years for any treatment (Table 5). The Streif Index showed that most fruit, excluding NP, had a lower value at harvest in 2010 compared to 2011 (Table 5). The higher values for 2011, confirms the general trend of fruit being more immature at harvest compared to the previous year. Consistent with fruit firmness measurements, the Streif Index indicated that NP fruit were less mature at harvest than RP for both years.

A strong relationship was found between harvest DM and harvest SSC for all treatments in both 2010 and 2011 (Fig. 3). The plots show the distinction in DM and SSC values between RP and NP fruit for the two years. It also illustrates the generally higher DM and SSC values present in NI fruit compared to the other two irrigation treatments.

Fig. 3.

3.2.3. Postharvest quality 2010 and 2011

Only the RP and NP treatments maintained harvest firmness and colour differences in storage over both years (Table 7). There was an issue with the DM measurements 4 weeks after harvest in 2011 and these data were omitted from analysis.
Table 7.

Linear regression analysis showed a strong correlation between DM content from different sampling dates in 2011 (Table 8). Correlations improved after Day 67 and were particularly strong for Day 112 (harvest) when correlated with Days 81 (R² = 0.84) or 96 (0.79). After 2 and 8 weeks of cold storage there was a similar and strong correlation between DM content in the stored fruit and preharvest DM content from Days 81, 96 and 112.

Table 8.

4. Discussion

4.1. Crop load

The relative uniformity of the increase in crop load across the irrigation and RP treatments in 2011 (Table 4) suggests that root pruning rather than irrigation was the influencing factor. The drop in harvest fruit weight between years due to the increase in crop load is in agreement with Behboudian et al. (2011) and Lopez et al. (2011). However the trend in weight difference between irrigation treatments remained consistent for each year. Therefore variation in harvest fruit weight across and between the irrigation treatments over the two years could be attributed to the interaction between crop load and irrigation (Mpelasoka et al., 2001c). NP trees appeared to be in biennial bearing by 2011 as their crop load fell by approximately a third on the previous year and to less than half that of the RP trees. Root pruning is known to not only reduce biennial bearing, but also affect potential crop load by promoting flower bud initiation (Asín et al., 2007; Ferree, 1992).

4.2. Preharvest pear development and quality

Total DM allocation to RP and NP fruit did not vary significantly in 2010 (Table 6), when crop load was not a factor, the difference in harvest fruit weight and DM can be attributed to differences in
fruit water content – perhaps due to the effect of root pruning on soil water uptake (Deckers et al., 2011) and differences in transpirational pull. However in 2011, when crop load was a factor, the disparity was evident from 51 DAFB, with RP fruit weighing 32.2 % less and having a 24.4 % higher DM content at harvest. Even though root pruning limits access to soil nutrients and water and to assimilates stored in the severed roots (Khan et al., 1998a), the greater crop load of RP trees ensured their fruit were stronger sinks for carbohydrate as their cumulative total DM was double that of NP fruit throughout preharvest development.

Withholding irrigation reduced preharvest weight and increased DM of NI fruit at harvest. This trend for smaller fruit and higher DM in fruit under deficit irrigation conditions is in agreement with Lopez et al. (2011) and Mpelasoka et al. (2001a). Regular drip irrigation had an impact on preharvest FI fruit size compared to NI fruit. Furthermore the plastic cover ensured minimal soil moisture evaporation throughout the season.

4.3. Harvest quality

Although the pears were harvested two weeks earlier in 2011 they spent more days on the tree (112 days) than in 2010 (106 days). The general lack of maturity across the treatments in 2011 could be the effect of a cooler wetter season.

Significant differences in DM, SSC, firmness and colour between RP and NP fruit for both years, irrespective of season, fruit size or crop load, indicated that root pruning advanced pear maturity. Elfving et al. (1991) reported delayed starch hydrolysis in fruit from root-pruned trees, as evident in 2010. However harvest starch levels in NP fruit were inconsistent across the two years. It is possible
that in 2011, the reduction in NP crop load, combined with general fruit immaturity, as seen in the
firmness and colour data, maintained harvest starch levels.

Lower DM and soluble solids allocation to NP fruit compared to RP in 2011 (Table 6) is typical of
non-root pruned trees with access to abundant water and a low crop load. Redirection of assimilates
into vegetative rather than reproductive growth, and the excess shading caused, ensures that fruit
from these trees tend to be large, green and low in SSC (Saure, 2007). The crop load for NP trees in
2011 was half that of 2010, but there was no dramatic change in fruit size, DM or SSC between the
years, even though the fruit were quite large for the year compared to the other treatments. In
addition there was a major reduction in total assimilate partitioning to NP fruit in 2011 (Table 6),
which is probably also related to the fall in crop load.

Deficit irrigation has been shown to advance fruit maturity (Ebel et al., 1993; Lopez et al., 2011;
Mills et al., 1994). However the absence of consistent significant differences in firmness and colour
between NI and FI fruit for both years (Table 5) suggests that irrigation, or the lack of it, did
nothing to delay or advance fruit maturity in root pruned pear trees. Indeed the similarity between
NI and RP fruit quality further supports this assertion. Increases in SSC and DM in NI fruit seems
more related to reduced fruit size and lower water content rather than maturity *per se*. There was a
small but significant decrease in total DM and SSC allocation to FI fruit in 2010, which was
possibly related to a slightly lower crop load that year. Although results were inconsistent, the
slower starch degradation observed in NI fruit in 2010 and indicated by the lower SPI values may
be related to the reduced water supply combined with lower rainfall that season (Ebel et al., 1993;
Mpelasoka et al., 2001b).
4.4. Postharvest quality

Differences in fruit quality at harvest persisted in storage. Storage quality here was related to maturity, that is colour and firmness of fruit at harvest. Even after 8-10 weeks of storage the pears would have been considered marketable as fruit from all treatments were above a firmness threshold of ~3.5 kg cm$^{-2}$. The maintenance of harvest differences in storage is in agreement with Mpelasoka et al. (2001b) Behboudian et al. (2011) but in contrast to Lopez et al. (2011). Although fruit with higher harvest DM may have the prospect of improved quality due to more potential SSC, Harker et al. (2009) found in relation to kiwifruit that consumers preferred sweet fruit only if they were firm too. Our results here do not demonstrate any strong relationship between DM and firmness. Therefore higher DM may provide the potential for more SSC and sweeter fruit, but not necessarily equate with firmer fruit or deliver improved eating or storage quality, especially for ‘Clara Frijs’ where a crisp, green pear is preferred.

NP fruit were the least mature pears of the entire experiment and contained the least amount of DM and SSC (and starch in 2010) at harvest for both years. Even after more than 2 months in storage they could not attain more than 11.5 and 10.9 °Brix in 2010 and 2011, respectively. Based on consumer tests, Vangdal (1980) proposed a lower threshold limit of 11.3 °Brix as acceptable for SSC in pears. No sensory tests were run to assess flavour or eating quality, but this difference in SSC between NP and the other treatments would have been apparent. Our results suggest that low NP harvest DM content was a good indicator of postharvest SSC. In addition separate shelf life experiments (data not shown) indicated that NP fruit lost water at a faster rate once removed from cold storage. So although they remained firmer than RP fruit their texture was very spongy.
Vigorous, non-root pruned trees are known to produce fruit with low storage ability related to lower cellular Ca\(^{2+}\) content (Saure, 2007).

4.5. Relationship between DM and SSC

The relationship between harvest DM and postharvest SSC, after starch hydrolysis is complete is well established (McGlone et al., 2003; Palmer et al., 2010). However our results show a strong correlation between DM and SSC at harvest for both years, despite the presence of starch (Fig. 3). The plots illustrate the spread of data and the relatively large differences observed in pear DM and SSC across all treatments.

4.6. Preharvest and postharvest DM

The strengthening correlation between DM measurements after Day 81 (Table 8) indicates the relative stability of DM values after this date. It also confirms that preharvest treatment differences in DM were maintained until harvest and continued during storage. Furthermore the strength of the correlation on Day 81\((R^2 = 0.84)\) would have made it possible to reasonably predict harvest DM content 31 days before harvest, but also DM content 8 weeks after harvest \((R^2 = 0.70)\).

Conclusion

The results presented here clearly demonstrate the effects of root pruning and irrigation on pear quality. They also show that fruit quality can be manipulated under field conditions even during a cool, wet summer. Indeed it was possible to repeat the experiment over two years and see consistent treatment effects on DM during fruit development in the second year persisting until harvest and on into storage. Root pruning increased crop load but produced smaller fruit with higher DM and SSC
and advanced harvest maturity, at least compared with NP fruit. On the other hand irrigation mitigated some effects of root pruning on fruit size, DM and SSC by increasing fruit water concentration, but it did not affect maturity. Root pruning rather than irrigation prompted the change in crop load between the two years. On the other hand, not root pruning the trees compromised fruit DM and SSC and drastically reduced crop load the following year. The strong relationship between DM and SSC and the influence these two parameters exert on harvest and postharvest fruit quality, makes early estimation of DM content a useful prospect. Further research is required to confirm this preharvest-harvest-postharvest relationship in order for it to be of practical benefit.

References


Danmarks Meterologiske Institut (DMI) 2010, 2011. www.dmi.dk


Naschitz, S., Naor, A., Genish, S., Wolf, S., Goldschimdt, E.E., 2010. Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations, Tree Physiology, 30, 715-727.


Table 1. Soil water content (SWC) on different days after full bloom (DAFB) in 2011. Measured to a depth of 90 cm before irrigation. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Soil field capacity was 26.5 % and wilting point 7.5 %.

<table>
<thead>
<tr>
<th>Soil water content (%)</th>
<th>34</th>
<th>47</th>
<th>61</th>
<th>74</th>
<th>89</th>
<th>129</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>18.6</td>
<td>17.9</td>
<td>16.3</td>
<td>15.7</td>
<td>15.3</td>
<td>16.3</td>
</tr>
<tr>
<td>DI</td>
<td>18.7</td>
<td>20.5</td>
<td>19.4</td>
<td>19.9</td>
<td>20.4</td>
<td>18.8</td>
</tr>
<tr>
<td>FI</td>
<td>20.1</td>
<td>20.4</td>
<td>20.0</td>
<td>20.8</td>
<td>22.2</td>
<td>21.7</td>
</tr>
<tr>
<td>RP</td>
<td>21.6</td>
<td>23.1</td>
<td>20.3</td>
<td>23.0</td>
<td>23.8</td>
<td>25.4</td>
</tr>
<tr>
<td>NP</td>
<td>20.2</td>
<td>21.3</td>
<td>19.5</td>
<td>21.3</td>
<td>21.4</td>
<td>20.3</td>
</tr>
</tbody>
</table>
Table 2. Pear preharvest dry matter content (%) on sampling days after full bloom (DAFB) in 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Standard error (SE) indicated by ±. Superscript letters beside values denote significance (P>0.05) between treatments for each sampling date. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th>Dry Matter, %</th>
<th>51</th>
<th>67</th>
<th>81</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>25.5 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.4 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.8 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DI</td>
<td>24.2 ± 0.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.7 ± 0.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.6 ± 0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.9 ± 0.30&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>FI</td>
<td>24 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.4 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.3 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RP</td>
<td>25 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.7 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NP</td>
<td>23 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.9 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.9 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 3. Cumulative total preharvest DM (g) allocation to fruit per tree on each sampling day after full bloom (DAFB) in 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Standard error (SE) indicated by ±. Superscript letters beside values denote significance (P>0.05) between treatments for each sampling but not between samplings. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th></th>
<th>DAFB</th>
<th>Cumulative DM, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51</td>
<td>67</td>
</tr>
<tr>
<td>NI</td>
<td>740±66b</td>
<td>1132± 89ab</td>
</tr>
<tr>
<td>DI</td>
<td>811±73a</td>
<td>1108± 68b</td>
</tr>
<tr>
<td>FI</td>
<td>769±62ab</td>
<td>1182± 66a</td>
</tr>
<tr>
<td>RP</td>
<td>688±59a</td>
<td>979± 69a</td>
</tr>
<tr>
<td>NP</td>
<td>315±39b</td>
<td>468± 47b</td>
</tr>
</tbody>
</table>
Table 4. Pear harvest crop load (average number per tree) for 2010 and 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Standard error (SE) indicated by ±. Superscript letters beside values denote significance (P>0.05) between treatments for each year but not between years. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th>Crop Load (n)</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>155 ± 14(^a)</td>
<td>249 ± 17(^a)</td>
</tr>
<tr>
<td>DI</td>
<td>149 ± 10(^{ab})</td>
<td>248 ± 16(^a)</td>
</tr>
<tr>
<td>FI</td>
<td>122 ± 15(^b)</td>
<td>248 ± 19(^a)</td>
</tr>
<tr>
<td>RP</td>
<td>141 ± 10(^a)</td>
<td>237 ± 20(^b)</td>
</tr>
<tr>
<td>NP</td>
<td>156 ± 7(^a)</td>
<td>102 ± 10(^a)</td>
</tr>
</tbody>
</table>
Table 5. Pear harvest quality results for 2010 and 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Standard error (SE) indicated by ±. Superscript letters denote significance (P>0.05) between treatments for each year but not between the years. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>114.0 ± 4.08b</td>
<td>92.0 ± 3.50b</td>
</tr>
<tr>
<td>DI</td>
<td>123.9 ± 3.88b</td>
<td>98.1 ± 5.93ab</td>
</tr>
<tr>
<td>FI</td>
<td>146.2 ± 6.64a</td>
<td>110.7 ± 5.32a</td>
</tr>
<tr>
<td>RP</td>
<td>110.5 ± 5.17a</td>
<td>94.3 ± 3.77b</td>
</tr>
<tr>
<td>NP</td>
<td>123.4 ± 4.15a</td>
<td>124.6 ± 4.0a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Dry Matter (%)</th>
<th>Soluble Solids (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
</tr>
<tr>
<td>NI</td>
<td>17.8 ± 0.52a</td>
<td>16.4 ± 0.68a</td>
</tr>
<tr>
<td>DI</td>
<td>15.5 ± 0.61ab</td>
<td>15.7 ± 0.32ab</td>
</tr>
<tr>
<td>FI</td>
<td>15 ± 0.28b</td>
<td>14.4 ± 0.26b</td>
</tr>
<tr>
<td>RP</td>
<td>16.8 ± 0.67a</td>
<td>15.3 ± 0.24a</td>
</tr>
<tr>
<td>NP</td>
<td>13.1 ± 0.41b</td>
<td>12.6 ± 0.17b</td>
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<table>
<thead>
<tr>
<th></th>
<th>Firmness (kg cm⁻²)</th>
<th>Colour (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
</tr>
<tr>
<td>NI</td>
<td>4.8 ± 0.17b</td>
<td>4.9 ± 0.04b</td>
</tr>
<tr>
<td>DI</td>
<td>5.0 ± 0.04a</td>
<td>5.1 ± 0.19a</td>
</tr>
<tr>
<td>FI</td>
<td>4.9 ± 0.15ab</td>
<td>5.0 ± 0.23ab</td>
</tr>
<tr>
<td>RP</td>
<td>4.7 ± 0.14b</td>
<td>5.0 ± 0.10b</td>
</tr>
<tr>
<td>NP</td>
<td>5.4 ± 0.06a</td>
<td>6.0 ± 0.11a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Starch Pattern Index</th>
<th>Streif Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
</tr>
<tr>
<td>NI</td>
<td>6.3 ± 0.27b</td>
<td>7.4 ± 0.19ab</td>
</tr>
<tr>
<td>DI</td>
<td>7.4 ± 0.59a</td>
<td>7.2 ± 0.30b</td>
</tr>
<tr>
<td>FI</td>
<td>7.1 ± 0.26ab</td>
<td>7.9 ± 0.26a</td>
</tr>
<tr>
<td>RP</td>
<td>7.3 ± 0.29b</td>
<td>7.4 ± 0.19a</td>
</tr>
<tr>
<td>NP</td>
<td>9.3 ± 0.12a</td>
<td>7.3 ± 0.15a</td>
</tr>
</tbody>
</table>
Table 6. Total DM and soluble solids allocation (g) to fruit in 2010 and 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Standard error (SE) indicated by ±. Superscript letters beside values denote significance (P>0.05) between treatments for each sampling but not between samplings. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th></th>
<th>DM, g</th>
<th>Soluble solids, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
</tr>
<tr>
<td>NI</td>
<td>3015 ± 260&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3732 ± 247&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DI</td>
<td>2970 ± 136&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3773 ± 140&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>FI</td>
<td>2728 ± 250&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3954 ± 344&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RP</td>
<td>2617 ± 239&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3388 ± 259&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NP</td>
<td>2498 ± 49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1623 ± 202&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>
Table 7. Pear postharvest quality for 2010 and 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Fruit were stored at 0 °C at 95% RH and were evaluated after 24 h at 16 °C. Standard error (SE) indicated by ±. Superscript letters beside values denote significance (P>0.05) between treatments for each sampling but not between samplings. Significance values were calculated for irrigation and root pruned treatments separately.

<table>
<thead>
<tr>
<th></th>
<th>2010 Dry Matter (%)</th>
<th>2011 Dry Matter (%)</th>
<th>Soluble Solids (°Brix)</th>
<th>Soluble Solids (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
<td>6 weeks</td>
<td>10 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>NI</td>
<td>17.2 ± 0.18a</td>
<td>17.7 ± 0.14a</td>
<td>17.9 ± 0.63a</td>
<td>13.8 ± 0.17a</td>
</tr>
<tr>
<td>DI</td>
<td>16.2 ± 0.52ab</td>
<td>16.6 ± 0.29b</td>
<td>17.0 ± 0.41ab</td>
<td>13.2 ± 0.25ab</td>
</tr>
<tr>
<td>FI</td>
<td>15.6 ± 0.34b</td>
<td>16.5 ± 0.31b</td>
<td>16.6 ± 0.45b</td>
<td>12.7 ± 0.33b</td>
</tr>
<tr>
<td>RP</td>
<td>17.7 ± 0.45a</td>
<td>17.9 ± 0.44a</td>
<td>18.5 ± 0.55a</td>
<td>14.0 ± 0.41a</td>
</tr>
<tr>
<td>NP</td>
<td>13.5 ± 0.55b</td>
<td>14.0 ± 0.20b</td>
<td>13.9 ± 0.32b</td>
<td>10.6 ± 0.23b</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Firmness (kg cm⁻²)</th>
<th>Colour (h)</th>
<th>Firmness (kg cm⁻²)</th>
<th>Colour (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
<td>6 weeks</td>
<td>10 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>NI</td>
<td>4.6 ± 0.15b</td>
<td>4.6 ± 0.10ab</td>
<td>4.6 ± 0.09b</td>
<td>112.5 ± 0.18a</td>
</tr>
<tr>
<td>DI</td>
<td>4.8 ± 0.06ab</td>
<td>4.5 ± 0.06b</td>
<td>4.2 ± 0.07b</td>
<td>110.5 ± 0.13a</td>
</tr>
<tr>
<td>FI</td>
<td>5.0 ± 0.12a</td>
<td>4.9 ± 0.12b</td>
<td>4.7 ± 0.20a</td>
<td>110.5 ± 0.13a</td>
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<tr>
<td>RP</td>
<td>4.9 ± 0.03a</td>
<td>4.6 ± 0.08b</td>
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<td>110.5 ± 0.13a</td>
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<tr>
<td>NP</td>
<td>5.3 ± 0.18a</td>
<td>5.1 ± 0.09a</td>
<td>4.7 ± 0.12a</td>
<td>113.1 ± 0.13a</td>
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</table>

<table>
<thead>
<tr>
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<th>2011 Dry Matter (%)</th>
<th>2011 Dry Matter (%)</th>
<th>Soluble Solids (°Brix)</th>
<th>Soluble Solids (°Brix)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
<td>8 weeks</td>
<td>4 weeks</td>
<td>8 weeks</td>
</tr>
<tr>
<td>NI</td>
<td>17.4 ± 0.85a</td>
<td>15.8 ± 0.55a</td>
<td>12.7 ± 0.60a</td>
<td>12.6 ± 0.41a</td>
</tr>
<tr>
<td>DI</td>
<td>16.4 ± 0.84ab</td>
<td>15.4 ± 0.42ab</td>
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</tr>
<tr>
<td>FI</td>
<td>15.7 ± 0.30b</td>
<td>14.7 ± 0.21b</td>
<td>11.3 ± 0.23b</td>
<td>11.5 ± 0.11b</td>
</tr>
<tr>
<td>RP</td>
<td>15.7 ± 0.30b</td>
<td>15.1 ± 0.25a</td>
<td>11.8 ± 0.23a</td>
<td>11.8 ± 0.10a</td>
</tr>
<tr>
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<td>14.1 ± 0.15b</td>
<td>13.8 ± 0.48b</td>
<td>10.9 ± 0.06b</td>
<td>10.8 ± 0.19b</td>
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</table>
Table 8. Correlation between DM on all sampling days in 2011. Day refers to number of days after full bloom (DAFB). Harvest occurred 112 DAFB. Weeks refer to number of weeks in cold storage after harvest at 0°C and 95% RH.

<table>
<thead>
<tr>
<th></th>
<th>Day 51</th>
<th>Day 67</th>
<th>Day 81</th>
<th>Day 96</th>
<th>Harvest</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>8 weeks</th>
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<tr>
<td>Day 51</td>
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<td>0.64</td>
<td>0.54</td>
<td>0.52</td>
<td>0.44</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td>Day 67</td>
<td>-</td>
<td>-</td>
<td>0.68</td>
<td>0.60</td>
<td>0.62</td>
<td>0.69</td>
<td>-</td>
<td>0.69</td>
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**Fig. 1.** Average air temperature, precipitation and soil temperature (10-30 cm below ground) during the experimental period in 2010 and 2011. Numbers on the horizontal axis refer to sampling days after full bloom (Day 0).

* Indicates days when soil water content (SWC) was measured. Fruit were harvested 106 days after full bloom in 2010 and 112 days after full bloom in 2011.
Fig. 2. Pear preharvest fruit weight on sampling days after full bloom (DAFB) in 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP). Bars above columns indicate standard error. Letters above columns denote significance (P>0.05) between treatments on each sampling day.
\[ y = 0.5502x + 4.1366 \]
\[ R^2 = 0.81709 \]

\[ y = 0.7063x + 1.391 \]
\[ R^2 = 0.95333 \]
Fig. 3. Per fruit correlation ($R^2$) between pear DM and SSC at harvest in 2010 and 2011. Treatments: no irrigation (NI); deficit irrigation (DI); full irrigation (FI); root pruned (RP); non-root pruned (NP).
Predicting pear (cv. Clara Frijs) preharvest dry matter and soluble solids content with NIR spectroscopy

Travers, S., Bertelsen, M.G., Petersen, K.K. and Kucheryavskiy, S.V.

LWT – Food, Science and Technology
Predicting pear (cv. Clara Frijs) dry matter and soluble solids content with near infrared spectroscopy

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Abstract
Regression models for predicting preharvest dry matter (DM) and (SSC), based on two spectral ranges (680-1000 nm and 1100-2350 nm), were compared. Models based on longer NIR spectra were more successful for both parameters (DM/SSC: R² = 0.78-0.84; RMECV = 0.78/0.44; LVs = 6/7). SSC prediction was better than expected considering the presence of starch in fruit. Generally poor SSC prediction in the presence of starch could be related to the inability of models to distinguish between forms of carbohydrate. Variable selection and regression coefficients highlighted the contribution certain wavelengths made to DM and SSC models, especially for SSC prediction between ~1500-1800 nm. Pear physiology may influence the accuracy of DM and SSC prediction due to the presence of stone cells just below the skin. These cells contain lignin and are a source of soluble solids in ripening fruit. Further research is needed to qualify and build on the results presented here.
1. Introduction

Fruit physiological maturity at harvest is essential for optimising fruit postharvest storage potential and future eating quality (Kader, 2002). Determining fruit maturity, including that of pears, requires a level of assessment prior to harvest and the two standard parameters used are firmness and soluble solids content (SSC). Apart from being destructive, measurement of these parameters is laborious. Extensive research and advances in NIR spectroscopy now offer the possibility of measuring these internal attributes non-destructively and with reasonable accuracy Nicolaï et al. (2007). NIR spectroscopy is suitable for analysis of fruit, with its high water and carbohydrate contents, as absorbance is high for samples containing C-H, C-O and O-H molecules (Williams and Norris, 1987).

Relatively little NIR spectroscopy research exists concerning preharvest measurement of fruit quality. There is some concerning apple (Peirs et al., 2000 and 2005; Zude et al., 2006), apricots (Camps and Christen, 2009 and Bureau et al., 2009) and mango (Delwiche et al., 2008; Nagle et al., 2010). Most has focused instead on harvest and postharvest assessment of apples (Bobelyn et al., 2010; Lammertyn et al., 2000; Liu and Ying, 2005; Park et al., 2003) and kiwifruit (Clark et al., 2004; McGlone and Kawano, 1998), but there is also some harvest and postharvest research on pears (Liu et al., 2008; Nicolaï et al. 2008; Paz et al., 2009).

Research into fruit dry matter (DM) content and the need for a reliable quality parameter based on fruit physiology runs parallel to the interest in non-destructive quality assessment. DM is essentially
composed of carbohydrate, in soluble (sugars) and insoluble forms (starch and various structural carbohydrates) (Suni et al., 2000; Gibson, 2012). Harvest DM content is considered a reliable indicator of postharvest SSC once starch hydrolysis is completed (Palmer et al., 2010). Pear flesh differs slightly to apple, for example, in that it also contains lignin in the form of brachysclereids or ‘stone cells’ (Tao et al., 2009).

Preharvest fruit DM content reaches its maximum level around the start of the cell expansion phase, but levels off as development progresses, remaining relatively stable until harvest (Schechter et al., 1993). Prediction of fruit DM content with NIR spectroscopy has concentrated so far on harvest and postharvest assessment of apples (Fan et al., 2009; McGlone et al., 2003), kiwifruit (McGlone et al., 2002b; Wedding et al., 2010) and avocados (Clark et al., 2003). To the authors’ knowledge, no previous work exists on the prediction of pear DM at any stage of fruit development. However the plateauing of fruit DM content before harvest, coupled with its relationship to postharvest SSC and previous success in predicting harvest and postharvest SSC prediction is generally poor when starch is still present in fruit and this lack of success may be attributed to the inability of models to distinguish between forms of carbohydrate (McGlone et al., 2002a; McGlone et al. 2003). Distinction is not necessary for DM prediction, but it may be of use when trying to estimate fruit maturity through the assessment of preharvest or harvest SSC when starch is still present in the fruit.

Water is a strong absorber in the NIR region therefore spectra from samples with high water content (>80 %), such as pears, are dominated by its signature (Büning-Pfaue, 2003). Fruit DM (Clark et
al., 2003; Delwiche et al., 2008; McGlone et al., 2003; McGlone and Martinsen, 2004; Peirs et al.,
2003) and SSC (Fan et al., 2009; Walsh et al., 2004; Zude et al., 2006) prediction has tended to
focus on shorter wavelength NIR spectra (~300-1100 nm), often including some of the visible
range, mainly due to deeper sample penetration. However the presence of a number of overlapping
carbohydrate and water absorption bands between 700-1200 nm (Williams and Norris, 1987) and a
chlorophyll absorption peak ~680 nm, can compromise model accuracy. Longer NIR spectra,
between 1000-2500 nm, have been successfully employed in carbohydrate analysis of liquids and
powders. Despite less sample penetration, these spectra contain a number of carbohydrate
absorption bands and so may help regression models pick up subtle differences between DM and
SSC (Osborne et al., 1993).

The main aim of this research was to compare the ability of models based on spectra between 300-
1100 nm and 1000-2500 nm to predict pear preharvest DM and SSC. A secondary aim was to
investigate the potential improvement in model accuracy by using variable selection. As the
distinction between DM and SSC prediction could be an issue for preharvest prediction, a tertiary
aim, through examination of model regression coefficients, was to assess whether models based on
longer wavelengths were better able to distinguish between either parameter.

2. Materials and Methods

2.1. Fruit

A total of 375 pears (Pyrus communis L. cv. Clara Frijs) were sampled from an orchard located at
the Department of Food Science, Aarhus University, Aarslev, Denmark (55° 31’ N, 10° 44’ E, 49m
MSL). The pears came from a field experiment assessing the effect of root pruning and irrigation on
fruit quality and were chosen for their broad range of DM and SSC. Sampling constituted spectra collection and destructive quality testing for the reference dataset. DM and SSC measurements on all sampling days were taken at two sites located on the widest part of the fruit at its equator: one on the ‘shoulder’ side (Point 1) and another directly opposite (Point 2). Samples for starch analysis were obtained below the equator. All measurements for each day were completed in one session. Spectral data and DM samples were collected 96 and 106 days after full bloom (DAFB) and at harvest (112 DAFB). SSC and starch content data were obtained on Day 106 and at harvest.

2.2. Spectral measurements

Spectra were taken immediately before destructive sampling from Points 1 and 2 on each fruit using an AgriQuant (Q-Interline, Tølløse, Denmark) FT-NIR spectrometer. The AgriQuant was fitted with SI and InGaAs detectors with a sampling increment of 4 nm and a spectral range of 300-1100 nm and 1000-2500 nm, respectively. The number of spectrum scans was set at 64. The instrument was fitted with a fibre optic cable and a direct contact probe working in reflectance mode with a diameter of approximately 10 mm. A white Teflon tile was used to provide a reference spectrum before and during every sampling session. The instrument was configured with PLCATS (Version 4.4) and InfraQuant 2.5 software.

2.3. Quality testing

Fruit were weighed, minus their stem, using a FX300i scales, A&D Co., Ltd. (0.01 g accuracy) and the diameter recorded with a digital calipers (Whitworth Ltd., UK). For DM determination two ~10 mm diameter plugs of tissue were taken at Points 1 and 2, perpendicular to the fruit’s surface. The core was removed from each plug and it was weighed on a pre-weighed foil tray. The samples were
then oven dried at 70 °C for 20 h until constant weight and weighed again. DM was expressed as percentage dry weight of the initial fresh sample. Another flesh sample was obtained from an area immediately surrounding the DM plug and squeezed with a garlic press to extract the juice. °Brix was determined from this juice using a temperature compensated digital refractometer (RFM712, Bellingham Stanley Ltd., UK). Fruit starch content (starch pattern index, SPI) was determined by dipping a cross-section of flesh (~3 cm from the calyx) in a standard iodine solution for 30 s and after 2 mins ranking it on a visual scale from 1 (black, starch present) to 10 (white, no starch present).

2.4. Chemometric and statistical analysis

All chemometric analysis was completed using MatLab (MathWorks, Natick, USA) software coupled with PLS Toolbox (Eigenvector Research Inc., Wenatchee, USA). All models were based on original reflectance spectra (R). Spectra were acquired from the SI and InGaAs detectors for DM on Days 96, 106 and 112. The datasets for all three days were combined to create a single larger set, one for each spectral range. As two measurements had been taken per fruit, the data in each of these sets were averaged to provide a per fruit dataset containing 375 samples for analysis. SSC and spectral data from Days 106 and 112 were treated the same way and provided a dataset containing 250 samples. For each spectral range and parameter, two PLS models were built: a standard model without variable selection and another incorporating the CARS method of variable selection. Outlier screening was carried out using Q² residuals vs. Hotelling T² values plot and resulted in 16-18 outliers for DM models and 12-14 for SSC (~5 %).
2.4.1. **SI spectra (300-1100 nm)**

Preliminary analysis revealed noise at both ends of the range of the detector caused by the narrow band pass of the fibre optic probe. Therefore the spectra were cut to 680-1000 nm to exclude it. Calibration models were based on cut spectra preprocessed with 11-point Savitzky-Golay spectral smoothing (1st order polynomial, no derivative) for noise reduction and standard normal variate (SNV) to correct potential scatter effects.

2.4.2. **InGaAs spectra (1000-2500 nm)**

Again spectra were cut to 1100-2350 nm due to a weak signal at both ends of the detector’s range. Savitzky-Golay spectral smoothing was applied (3-point, 1st order polynomial, no derivative) and SNV.

2.4.3. **PLS modelling**

Using data from all three sampling days for DM and from the two later days for SSC, PLS models were calibrated and validated using 4-segmented cross-validation with random subset selection. Cross-validation was repeated four times to achieve robust results. Models were based on the optimal number of latent variables (LVs) suggested by the calibration and cross-validation results. Model performance was evaluated according to: root mean square error (RMSE) of calibration (RMSEC), of cross-validation (RMSECV); coefficient of determination ($R^2$); residual predictive deviation (RPD); and optimal number of LVs required to build the model (nLV). The RPD value is the most realistic comparison and evaluation of model calibration statistics and of the potential of the model to determine DM and SSC. It is defined as the ratio of the standard deviation (SD) of the reference values to the standard error of cross-validation (SD/SECV) and the higher the value the
stronger the model. General summary statistics for DM and SSC were obtained using R (version 2.1.11.).

2.4.4. Competitive adaptive reweighted sampling (CARS)

Variable selection was by way of the CARS algorithm. CARS optimises the predictive performance of multivariate linear regression models, such as PLS regressions, by selecting wavelengths with large absolute regression coefficients (Li et al., 2009). Absolute regression coefficients function as a useful evaluator of wavelength importance. Therefore depending on the importance of each wavelength, CARS sequentially selects $N$ subsets of wavelengths from $N$ Monte Carlo (MC) sampling runs in an iterative and competitive manner. For each sampling run, a fixed ratio (e.g. 80%) of samples is randomly selected to establish a calibration model. Then, depending on the regression coefficients, a two-step procedure including exponentially decreasing function (EDF) based enforced wavelength selection and adaptive reweighted sampling (ARS) based competitive wavelength selection is adopted for selecting key wavelengths. Finally, cross-validation (CV) is applied to choose the subset with the lowest root mean square error of cross-validation (RMSECV).

3. Results

3.1. Reference data

Table 1. illustrates minimum, mean, maximum and SD values of reference data for DM, SSC and SPI of pears 96, 106 DAFB and at harvest (112 DAFB). The SPI values clearly indicate that starch was present in pears on all sampling days and also, by default, Day 96, which was not measured.
3.2. Analysis of spectra

Figure 1. shows typical SI and InGaAs reflectance spectra minus noise, before and after SNV scatter reduction. The trend of spectra is similar for each of the two ranges, but baseline shifts and bias caused by light scatter and variation in sample concentration are visible, especially in the InGaAs spectra.

The SI spectra are dominated by a large chlorophyll absorbance peak at ~680 nm (although only half the peak is visible in the cut spectra) and there is a small bump of an O-H \(^{2}\)nd overtone at ~950nm. In contrast the InGaAs spectra show two broad peaks at ~1450 nm and ~2000 nm which contain numerous overlapping water and carbohydrate absorption bands. The smaller peaks at ~1200 nm and ~1750 nm are related to carbohydrate.

3.3. Partial least squares (PLS) regression analysis

Separate global prediction models were built for both DM and SSC for each spectral range, with or without the use of CARS variable selection.

3.3.1. PLS models for SI spectra (680-1000 nm)

Variable selection slightly improved the predictive ability of the SI calibration or cross-validation models for either parameter. It also made the models simpler, by reducing the number of LVs required (Table 2): from 10 to 8 for DM and from 10 to 5 for SSC. The RMSE values for DM were almost twice that of SSC, irrespective of variable selection.
Cross-validation RPD values show that models were slightly better at predicting pear SSC than DM. The predicted versus measured plots results for variable selected and non-selected PLS models for SI spectra show barely a difference (Figure 2).

Without CARS variable selection, the regression coefficients for both DM and SSC show a similar pattern of peaks and troughs (Figure 3). Variable selection revealed differences in the regression coefficients for each parameter, particularly on the border between visible and NIR wavelengths. For instance the region between 680-800 nm suggests it was relevant for the DM model but not for SSC one. There is a strong chlorophyll peak located in this region and O-H and C-H overtones at ~760 nm. Alternatively, wavelengths between ~800-900 nm (C-H, 3rd overtone; O-H, 2nd overtone) appeared more relevant for SSC prediction. The region between 900-970 nm is important for both parameters, something which is evident in the regression coefficients after variable selection.

Variable selection did not significantly alter model predictive ability when using longer wavelength NIR spectra, but it again made models simpler. However the reduction in the number of LVs required for the InGaAs models when predicting SSC was not as great compared to the SI models (Table 3).

Cross-validation RPD values show that models were better at predicting SSC than DM. The predicted versus measured plots results for variable selected and non-selected PLS models for InGaAs spectra are shown in Figure 4. The number of LVs required and the $R^2$ values of the variable selected InGaAs models for both DM and SSC were similar (Table 3). However the RMSE
values for calibration and cross-validation of DM were almost double that of SSC. Cross-validation RPD values for all models demonstrate that SSC was better predicted irrespective of spectral range or CARS variable selection.

*Figure 4.*

CARS highlighted subtle differences in DM and SSC PLS models that were obscured without variable selection (Figure 5). The regression coefficients showed that the region located between the two major water absorption bands, ~1450-1850 nm, was relevant for predicting SSC but not DM and included spectra at ~1600 nm (C-H, 1st overtone), 1700 nm (CH) and ~1800 nm (C-O, 2nd overtone).

*Figure 5.*

4. Discussion

One intention of this work was to compare prediction models for pear preharvest DM and SSC based on two different spectral ranges and to assess the value of variable selection. Another was to investigate whether prediction models based on longer wavelengths could distinguish between the two parameters.

4.1. DM and SSC Prediction – the two spectral ranges

To the author’s knowledge, there is no previous research concerning the prediction of DM in pear with NIR spectroscopy. Models for DM based on short wave spectra (680-1000 nm), with or without variable selection, were comparable to previous work by Clarke et al. (2003) on avocado at harvest using a similar range ($R^2 = 0.47-0.78$, RMSEP = 1.8-2.8, LVs = 13-20) but were not as accurate as those of McGlone et al. (2003) ($R^2 = 0.95$ and RMSEP = 0.27, LVs = 8) on apple DM at harvest. Prediction models for SSC using shorter wavelength spectra were reasonable, considering
the inclusion of fruit containing starch in the reference dataset, and were in line with harvest prediction models for apples by McGlone et al. (2002a) \((R^2 = 0.63, \text{RMSEP} = 0.72, \text{LVs} = 10)\) and Ventura et al. (1998) \((R^2 = 0.58, \text{RMSEP} = 0.105)\). The results of McGlone et al. (2003) \((R^2 = 0.79, \text{RMSEP} = 0.53, \text{LVs} = 10)\) and Fan et al. (2009) \((R^2 = 0.98 \text{ and } \text{RMSEP} = 0.38, \text{LVs} = 4)\) were more successful.

Models based on longer wavelength spectra \((1100-2350 \text{ nm})\), with or without variable selection, were more able to predict pear DM than shorter wavelengths. They compare well to Lü et al. (2010) when predicting harvested but unripe kiwifruit DM \((R^2 = 0.74, \text{RMSEP} = 0.49, \text{LVs} = 11)\) and to Wedding et al. (2013) on avocado \((R^2 = 0.92, \text{RMSEP} = 1.49, \text{LVs} = 7)\), both using a similar spectral range. The results presented here are on a par with work by Jiang and Zhu (2013) \((R^2 = 0.82, \text{RMSEP} = 0.4, \text{LVs} = 9)\) and Paz et al. (2009) \((R^2 = 0.85-0.98, \text{RMSECV} = 0.51-0.68)\) on postharvest fruit. There is little research on preharvest prediction of either parameter, with the exception of Peirs et al. (2000) on preharvest SSC \((R^2 = 0.89, \text{RMSEP} = 0.59, \text{LVs} = 5)\), using an intermediate radiation range \((780-2000 \text{ nm})\). RPD values for all models, irrespective of wavelength range, were always less than 3, making them unsuitable for general quantitative predictions (Williams and Sobering, 1996).

4.2. The value of variable selection

McGlone et al. (2002a) and McGlone et al. (2003) raised the possibility that Vis/NIR spectroscopy \((300-1100 \text{ nm})\) may not easily distinguish between soluble and insoluble forms of carbohydrate. There are numerous reasons for this, including similarities in molecular composition of DM and SSC; collinearity of the two parameters, plus the problems fruit starch causes when trying to predict
SSC. Prediction of SSC may require clear separation of carbohydrate and water absorption bands in order for distinction between the two parameters to be possible.

Longer NIR spectra produced better prediction models, but they reveal nothing regarding their ability to discriminate between DM and SSC. Regression coefficients may provide some clarity regarding this. However they can also mask collinearities and overlaps in spectra, so are best viewed in combination with spectra as weights that apply at particular points. Regression coefficients of unselected short (SI) or long (InGaAs) wavelength spectra (Figures 3. and 5.) showed a similar series of positive and negative peaks. They also demonstrated that prediction of both DM and SSC is based on similar spectral characteristics, making it hard to discern much difference in the prediction of either parameter.

Variable selection highlighted the contribution certain wavelengths made to either the DM or SSC prediction models. A chlorophyll absorbance peak at ~680 nm (Figure 3., bottom), which changes as fruit mature, appears relevant to the DM prediction model. On the other hand, SSC models did not employ spectra below 800 nm, implying that chlorophyll was not relevant for preharvest and harvest SSC prediction. This is in general agreement with Nicolaï et al. (2008) and Slaughter and Crisosto (1998) who demonstrated that spectra between ~800-1100 nm were most sensitive to SSC. However harvest SSC models of McGlone et al. (2002a) included spectra below 800 nm implying that information on fruit maturity, provided by chlorophyll, was relevant to SSC prediction when starch was still present in fruit.
The spectral region between ~900-970 nm was relevant for both parameters, but some differences between models were present. Unlike SSC, DM required additional wavelengths in this region (~950 nm) for optimal prediction. The slight peak at ~950 nm, whose importance is only highlighted in the regression coefficients, is composed of overlapping absorbance bands of carbohydrates, such as starch and cellulose between ~900-930 nm (Williams and Norris, 1987) and sucrose (~900-920 nm) (McGlone and Kawano, 1998), and water (~940-960 nm).

The regression coefficients for longer wavelength spectra again show some common elements (Figure 5.), but differences in DM and SSC prediction are also evident. Spectra located between the two water peaks (~1500-1800 nm), and the small inflections at ~1200 and 1350 nm, containing carbohydrate absorbance bands, helped optimise the SSC model but not the DM one (Figure 5., bottom right). The importance of NIR spectra located between these two water peaks in revealing subtle differences between forms of carbohydrate concurs with work by Dull and Giangiacomo (1986) who determined individual sugars in aqueous solutions. Although not undertaken on actual fruit, their results were promising regarding the specific determination of soluble sugars ($R^2$, 0.99 and SEP, 0.49-0.69).

4.3. Pear structure and the prediction of SSC

Pear SSC predictions were surprisingly good (also in comparison to those of DM) considering they were based on a reference dataset where average starch content was 5.9 according to the SPI (Table 1). The presence of stone cells, a physical and structural characteristic of pears, may help shed some light on this issue. These cells are modified parenchyma cells with thick lignified walls (Jiang and Zhu, 2013; Sterling, 1954) and aside from other forms of carbohydrate, this deposited lignin can
comprise approximately 30% of their dried content (Tao et al., 2009). There is no pattern in their
distribution, but large groups of stone cells are concentrated near the core of the fruit and in sub-
epidermal tissue (Bain 1961; Martin-Cabrejas et al., 1994b). Therefore stone cells add to overall
fruit carbohydrate content and are known to contribute to SSC content in ripening fruit (Martin-
Cabrejas et al., 1994b).

Radiation penetration is wavelength dependent, with shorter NIR spectra (700-900 nm) going ~4
mm deep and longer spectra (900-1900 nm), between ~2-3 mm (Lammertyn et al., 2000). Pear skin
is composed of the cuticle and one layer of epidermal cells and its thickness varies from between
15-22 µm (Nguyen et al., 2004). The concentration of stone cells just beneath pear skin may have
some bearing on the reasonable predictions achieved for SSC at both wavelength ranges, even when
starch was present in fruit. Reflectance spectra before preprocessing (Figure 1.) clearly had
significant scatter, which may be due to this particular characteristic of pear flesh.

Research into the prediction of pear DM and SSC is still in its early stages and there are very few
papers published in comparison to other fruit, such as apple. If non-destructive prediction of pear
quality is to progress, a deeper understanding of pear biology is necessary.

5. Conclusion

Assessment of SSC at harvest and just prior to it is still an essential part of maturity testing in
commercial fruit production and more accurate estimation of SSC when starch is still present in
fruit is desirable. The prediction of pear DM with spectroscopy was previously untested. The results
presented here are promising regarding the prediction of both parameters non-destructively,
particularly at longer NIR wavelengths. Variable selection was successful in highlighting differences in predictions models of both parameters using both wavelength ranges. However if SSC models are to be improved, greater understanding of the impact pear physiology can have on NIRSpectroscopy measurements and models is needed. Furthermore distinguishing between the two parameters when predicting SSC could help produce more accurate prediction models even when starch hydrolysis is ongoing.

References


Table 1. Summary statistics for DM reference data 96, 106 and 112 DAFB and for SSC and Starch 106 and 112 DAFB. Number of fruit sampled that day (Number); minimum (Min.); mean; maximum (Max.); and standard deviation (SD).

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Table 2. SI model statistics based on data for all sampling days for pear DM and SSC with (v.s) or without (no v.s) variable selection. Reflectance spectra (R) including selected variables 680-1000 nm; Savitzky-Golay spectral smoothing (11-point, 1st order polynomial); standard normal variate (SNV). Statistics: root mean square error (RMSE); coefficient of determination (R²); residual predictive deviation (RPD); number of latent variables (nLV).

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Table 3. InGaAs model statistics based on data for all sampling days for pear DM and SSC with (v.s) or without (no v.s) variable selection. Reflectance spectra (R) including selected variables 1100-2350 nm; Savitzky-Golay spectral smoothing (3-point, 1st order polynomial); standard normal variate (SNV). Statistics: root mean square error (RMSE); coefficient of determination ($R^2$); residual predictive deviation (RPD); number of latent variables (nLV).

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Figure 1. SI (680-1000 nm) (left) and InGaAs reflectance spectra (1000-2350 nm) (right) for all sampling
days, before (top) and after (bottom) scatter reduction with SNV preprocessing.
Figure 2. Predicted vs. measured plots for PLS prediction models based on SI spectra for all sampling days without variable selection (top) and with it (bottom), for DM (left) and SSC (right) for all 3 sampling days.
Figure 3. Regression coefficients for PLS prediction models based on SI spectra for all sampling days, without variable selection (top) and with it (bottom), for DM (left) and SSC (right).
Figure 4. Predicted vs. measured plots for PLS prediction models based on InGaAs spectra and data for all sampling days without variable selection (top) and with it (bottom), for DM (left) and SSC (right).
Figure 5. Regression coefficients for PLS prediction models based on InGaAs spectra for all sampling days without variable selection (top) and with it (bottom), for DM (left) and SSC (right).
Effects of root pruning and irrigation regimes on pear trees: growth, yield and yield components

Wang, Y., Travers, S., Bertelsen, M.G., Thorup-Kristensen, K., Petersen, K.K. and Liu, F.

Horticultural Science
Effects of root pruning and irrigation regimes on pear tree: growth, yield and yield components

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Abstract

The effects of root-pruning (RP) were compared to those of non-root pruning (NP), together with the potential of supplementary irrigation in alleviating the negative effects of root pruning on fruit growth, yield and yield components in a pear orchard between 2010 and 2011. Results showed that total shoot length and number of shoots per tree decreased by 72 % and 43 % in RP and NP trees respectively. However lateral root growth was stimulated by the RP treatment in the upper soil layers (30-40 cm). Full irrigation (FI) and deficit irrigation (DI) treatments stabilized return bloom and improved fruit yield and size compared to the non-irrigated (NI) treatment, without stimulating vegetative growth. In conclusion, the results indicate that root pruning is effective in controlling excessive shoot growth, while supplementary irrigation can improve fruit yield and quality in root pruned trees. Therefore, a combination of root pruning and irrigation could be a promising alternative to control tree size and secure a stable fruit yield in pear orchards.

Keywords: Fruit yield; growth control; irrigation; return bloom; root pruning
Growth control is one of the important elements in pear orchard management, as excessive vigour often causes a decrease in light penetration, a reduction in fruit yield and quality and an increase in pruning costs (Miller 1995). Chemical growth retardants such as Cycocel (Chlormequat Chloride) have been widely used previously for controlling canopy growth in pear orchards. However, due to environmental concerns, their use has been prohibited in Danish and European pear production since 2002. Therefore, in recent years great efforts have been made to develop sustainable alternatives to replace the use of chemical growth regulators in pears.

Root pruning is a proven alternative for controlling vegetative growth of fruit trees (Geisler, Ferree 1984; Schupp, Ferree 1990). Poni et al. (1992) reported that root pruning was very effective in reducing shoot growth in potted grapevine, apple and pear trees. Ferree (1992) noted that root pruning reduced terminal shoot growth of apple trees by approximately 20%. Similarly, Khan et al. (1998) found the height of apple trees was reduced by 12% in the second season and shoot length, shoot number and fruit diameter all decreased as a consequence of root pruning. Asín et al. (2007) reported that root pruning significantly decreased shoot length in pear trees by 25% compared to a non-root-pruned control. The literature concerning the effects of root pruning on alternate bearing and flower induction is conflicting. Some authors found root pruning reduced the occurrence of alternate bearing (Ferree 1992) and increased flowering spurs (Khan et al. 1998) in apple. Others found little or no impact on return bloom (Asín et al. 2007) or alternate bearing (McArtney, Belton 1992; Schupp, Ferree 1987).

Despite the aforementioned advantages of root pruning in controlling canopy size, there are several obvious negative effects associated with this practice. Early studies have shown that root pruning could
reduce the yield of fruit trees due to reduction in leaf area index and so a reduced photo-assimilate supply (Ferree 1989; Khan et al. 1998). The capacity for water and nutrient uptake of fruit trees is often negatively affected under root pruning due to severe damage of the root system (Vercammen et al. 2005). Therefore root-pruned trees are more likely to suffer from drought stress and nutrient deficiency, which may constrain fruit growth, fruit yield and quality, resulting in smaller fruit. There is an urgent need to research and develop field strategies to mitigate the negative effects caused by root pruning.

Among other possible management strategies, supplementary irrigation may counteract the negative effects of root pruning by supplying water directly to the cut root system to avoid tree water stress. The impact of no or deficit irrigation on fruit trees is similar to that of root pruning in that it restricts vegetative growth (Ebel et al. 1995; Forshey, Elfving 1989) and can reduce harvest fruit size in apple (Mpelasoka et al. 2001) and pear (Marsal et al. 2000). It is hypothesised that an improved tree water status in root-pruned trees after supplementary irrigation may enhance fruit growth and yield without compromising the effect on vegetative growth. However this possibility has not been previously investigated in pear orchards in a Nordic climate where water deficits are unlikely to occur during most of the growing season.

Root pruning has become common practice in Denmark for controlling canopy size in pear orchards. ‘Clara Frijs’ is the most important pear cultivar, being widely planted in the country. The objectives of the present study were twofold: 1) to examine the value of root pruning compared to non-root pruning for growth control in pear trees; 2) to investigate the effect of supplementary irrigation on fruit yield and quality and vegetative growth in root pruned pear trees.
MATERIALS AND METHODS

The experiment was conducted during the growing seasons of 2010 and 2011 at Aarhus University, Department of Food Science, Aarslev, Denmark (10°27′ E, 55°18′ N). The pear trees (Pyrus communis L. cv. Clara Frijs, grafted onto Quince A rootstock) were planted in March 1999 at a spacing of 3.5 m between rows and 1.5 m within rows in a north-south row orientation. One ‘Conference’ pear tree was planted as pollinator between each plot. The trees were not root-pruned between 2005 and 2009. The soil was classified as sandy loam having soil a water content (% vol.) of 26.5 % at field capacity and 7.5 % at permanent wilting point in the 1.0 m soil profile. Plant available water in the 1.0 m soil profile was 181.4 mm. Nitrogen was applied at the rate of 50 kg ha⁻¹ each year before treatments commenced. The orchard was managed according to local practices, including pest management and weed control. Air temperature, global radiation, and potential evapotranspiration (ETp) during the experimental periods in 2010 and 2011 were recorded by a Danish Meteorological Institute (DMI) weather station located at the University (Fig. 1). Average temperature and total precipitation during the growing season were 16.4 °C and 331 mm in 2010, respectively; and 15.3 °C and 339 mm in 2011, respectively. Full bloom occurred on 17th May 2010 and 28th April 2011.

The experiment was a complete randomised block design consisting of five treatments (Fig. 2): root pruning treatment (RP), where the trees were root-pruned and relied only on rainfall; 2) non-root pruning treatment (NP), where the trees were not root-pruned and relied only on rainfall. NP and RP treatments were used to compare the effects of root pruning. For the root-pruned trees, three different irrigation strategies were included: 3) full irrigation (FI), where the rootzone of the trees were irrigated to field capacity (i.e. a volumetric soil water content of 26.5 %); 4) deficit irrigation (DI), where the
plots were irrigated to 70% of the plant available water (i.e., a volumetric soil water content of 20.8%); 5) non-irrigation (NI), where the plots received no irrigation or rainfall throughout the treatment period. FI, DI and NI treatments were used to compare the effect of irrigation on root-pruned trees. For each tree, the irrigation area was $1.5 \times 0.8 \, \text{m}^2$ to a depth of 1.0 m. Immediately after root pruning, the plots of the three irrigation treatments were covered with white woven waterproof plastic to exclude rainfall from around the trunk area and approximately 1.2 m away from the root pruning cut. The covering was mounted on a wire along the rows of the trees at a height of 60 cm and arranged so that all rainwater would be channelled to the centre of the alleyway away from the trees.

Each treatment had five replicates (plots) and each plot consisted of nine trees demarcated by two pollinating trees. For each treatment, data was collected from one experimental tree within each plot and the sampling trees were selected based on a similar number of flower clusters (132-140 per tree) and trunk diameter in April 2010. The trees were root pruned on 14th April 2010 and 4th April 2011 using a tractor mounted root-pruning knife that cut to a depth of approximately 30 cm and a distance of 40 cm from the truck on both sides of row.

A drip irrigation system (Durable multi-season integral drip line, John Deere Water Company, California, USA) was installed and the amount of irrigation water applied was monitored using water meters. Volumetric soil water content was measured with a Trime Time Domain Reflectometry system with T3 tube-access probe (IMKO Micromoduletechnik GmbH, Ettingen, Germany). The tubes were positioned vertically within the rows to a depth of 1.0 m and soil water content readings were taken every 10 cm. The amount of irrigation (mm) for FI and DI treatments was 512 mm and 203 mm in
2010, respectively, and 658 mm and 140 mm in 2011, respectively.

Shoot length and maximum diameter of fruit during the growing season were measured weekly, using a steel ruler and a digital calipers, on fifty randomly selected shoots and fifty fruit at different heights and exposures on the sampling trees of each treatment. Final shoot length was measured during the winter of 2010 and 2011. Return bloom was determined as the number of flower clusters per tree and was evaluated in the sampling trees in April 2011 and 2012. To observe root growth at different soil layers in the NP and RP treatments during the growing seasons in 2010 and 2011, three mini-rhizotron glass tubes (70 mm outer diameter and 1.5 m long) were installed on 25th May 2010 parallel to the row at approximately 50 cm from the trunk at an angle of 30°. Root intensity was measured following the method described by Thorup-Kristensen (1998).

Harvest commenced when fruit colour reached commercial specification (i.e. on 31st August 2010 and 18th August 2011). Fruit were counted and weighed for each tree and average fruit fresh weight was obtained. Fruit from each tree were graded using a commercial grader (Aweta Rollerstar, The Netherlands). The output from the machine included individual fruit weight and diameter. The size distribution was determined based on fruit diameter categories of < 50 mm, 50-60 mm, 60-70 mm, and > 70 mm.

Means values from measured parameters in the root-pruned irrigation treatments (DI, FI and NI) were compared by analysis of variance (ANOVA) at $P < 0.05$ using SPSS 13.0 (SPSS Inc. Chicago, USA). Independent $t$-test at $P < 0.05$ was used to compare measured parameters of the RP and NP treatments.
RESULTS AND DISCUSSION

For each treatment, the soil water content showed a similar pattern of development for both years (Fig. 3). RP and NP treatments had similar soil water content at 0-0.3 m and 0-6-0.9 m soil layers, whereas at the 0.3-0.6 m soil layer, soil water content was significantly higher in the RP treatment. In 3-10 year old apple trees, the majority of fine roots (< 0.2 mm) are found in the upper 30 cm soil layer within a radius of approximately 1 m from the trunk (Gong et al. 2006; Sokalska et al. 2009). However depending on soil type, irrigation practice, rootstock and other factors, a substantial number of fine roots may also be found in lower soil layers (Sokalska et al. 2009). In the present study, root pruning removed a large number of the fine roots in the upper 0 – 0.3 m soil layer thereby reducing water uptake. This may have increased the downward movement of water in the RP treatment resulting in the observed increased soil water content in the 0.3-0.6 m soil profile compared to the NP treatment. After commencement of the FI and DI treatments, soil water content was the highest for the FI treatment in the 0-0.3 and 0.3-0.6 m soil layers, intermediate for DI, and lowest for NI. Soil evaporation was reduced in these treatments due to the plastic cover. When irrigation was supplied, a downward movement of water was found in the FI treatments in both years and the DI treatment in 2011.

Root pruning significantly inhibited shoot growth of pear trees. Shoot growth terminated two weeks earlier in the RP compared to the NP treatment (Fig. 4). Consequently, total shoot length and number of shoots per tree decreased by more than 72 % and 43 % in the RP and NP treatments in 2010 and 2011, respectively (Table 1). This was in good agreement with earlier findings (Schupp, Ferree 1990; Khan et al. 1998; Vercammen et al. 2005; Asín et al. 2007). Even though root pruning stimulated root growth in certain soil layers, e.g. 30-40 cm (Fig. 5), it did not seem to corresponded to increased soil water
extraction from that layer (Fig. 3). The higher soil water content in the 30-60 cm soil layer (particularly in 2011) indicated that partial loss of the root after root pruning reduced water uptake and is likely to have had a similar effect on nutrient uptake. In addition, root pruning re-established the equilibrium between root and shoot growth, involving the mobilisation of stored carbohydrates to provide energy for new root growth (McArtney, Belton 1992). Early in the growing season, the greater proportion of root carbohydrate reserves are used during metabolism associated with new root growth and activity, resulting in a decline in available carbohydrates (McArtney, Belton 1992), therefore inhibiting shoot growth following root pruning. No significant differences in shoot growth termination, total shoot length, number of shoots per tree and the percentage of shoots shorter than 30 cm were found among the FI, DI and NI treatments (Table 1), implying that the suppression of shoot growth following root pruning might have not been due to water stress, other factors such as reduced carbohydrates availability or lowered nutrient uptake maybe involved. Consistent with this, Asín et al. (2007) found that with a 50 % reduction in irrigation water during the period from April to May, shoot length and number of shoots per pear tree remained unaffected.

The number of flower clusters per tree, is an important predictor of fruit number and yield at harvest. At the start of the experiment, trees carried a uniform number of flower clusters that were sufficient for a full and uniform crop to develop. After onset of the treatments, the root-pruned trees had remarkably higher return bloom than that in the NP treatment in 2011 (Table 2), indicating that there was a significant positive effect of root pruning on return bloom. Similar positive results after root pruning have been reported by Asín et al. (2007) and McArtney and Belton (1992). Changes in hormone concentration, such as in cytokinins in the xylem, during root regeneration after pruning may be
involved in increasing return bloom in the RP treatment (Webster et al. 2003). Nonetheless, both NP and RP treatments showed significant alternate return bloom between the years and the NP trees exhibited a large fluctuation in flowering compared to the RP. In the current study, we found that FI and DI treatments had higher return bloom as compared with the NI treatment, though not always statistically significant (Table 2), indicating that irrigation can stabilise flower bud formation at a level sufficient for a high regular crop load of the root-pruned pear trees.

It has long been recognized that root pruning reduces fruit size (Schupp, Ferree 1987; Ferree 1989; Mika, Krzewinska 1995; Khan et al. 1998). In accordance with this, we observed that fruit expansion rates were lower and fruit size smaller in the RP trees during the two experimental years (Fig. 6, Tables 3 and 4). The mechanisms behind this may be twofold. Khan et al. (1998) proposed that the decrease in fruit size as a consequence of root pruning might be partly due to the reduced availability of photo-assimilates because of a reduction in size and number of leaves during fruit growth. Hsiao and Xu (2000) stated that fruit cell expansions relies on water availability and on solute uptake and is facilitated by turgor pressure, which is generated by differences in osmotic potential. Therefore restrictions in water and solute uptake have the potential to alter cell expansion and ultimately fruit size. In the present experiment, we did see an increase in fruit size in response to irrigation thus supporting the latter argument. The NP treatment had higher crop load in 2010 compared to the RP trees, and this resulted in 25% higher marketable yield (Table 3) with a better size distribution (Table 4). However, in 2011 the number of fruit was 56% higher in the RP than in the NP treatment. Despite a reduced average fruit size and a large increase in the number of small fruit (Table 4) in the RP treatment, marketable yield increased significantly (Table 3). The three irrigation treatments had similar fruit
expansion rates in both years during most of the experimental period, only on a few occasions did the
FI trees have significantly higher fruit expansion rate than the DI and NI trees. There was no difference
in fruit set between the FI, DI and NI treatments, but average fruit size and fruit size distribution was
reduced in the NI treatment. Marketable yield obtained at harvest was significantly better in the FI than
in the NI treatment, with the DI showing intermediate results (Table 3). Therefore, supplementary
irrigation (FI and DI) increased fruit size, improved fruit size distribution and marketable yield
compared to the NI treatment.

CONCLUSIONS

The results of this study indicate that root pruning is an effective method for controlling shoot growth
and improving return bloom of pear trees during two experiment years. However it also produces
smaller fruit. Irrigation of root-pruned trees can stabilise return bloom and improve fruit yield and
quality without stimulating additional shoot growth. Therefore, a combination of root pruning and
irrigation could be a promising alternative for controlling the tree size and for mitigating the negative
effects of root pruning on pear fruit yield and quality.

Acknowledgements

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‘Sustainable Future for Danish Fruit’ (J. nr: 3412-09-02385) and by Plan Danmark.
References


Table 1.

Total shoot length, number of shoots per tree and percentage shoot length < 30 cm in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments in 2010 and 2011. ** and ns indicate significant differences between treatments at $P < 0.01$ and no significance, respectively.

<table>
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<tr>
<th>Treatment</th>
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<th>Number of shoots /tree</th>
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<tr>
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<td>74</td>
<td>58</td>
<td>130</td>
</tr>
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<td>14</td>
<td>74</td>
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<tr>
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<td>***</td>
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<tr>
<td>FI</td>
<td>24</td>
<td>19</td>
<td>91</td>
</tr>
<tr>
<td>DI</td>
<td>28</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>NI</td>
<td>26</td>
<td>20</td>
<td>90</td>
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<tr>
<td>ns sig.</td>
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Table 2.

Return bloom of pear trees in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments. *, ** and ns indicate significant differences between treatments at $P < 0.05$, $P < 0.01$ and no significance, respectively.

<table>
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<th>Return bloom</th>
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<td>120</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>RP</td>
<td>138</td>
<td>208</td>
<td>93</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>NI</td>
<td>132</td>
<td>212</td>
<td>81</td>
<td>ns</td>
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<tr>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
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</table>
Table 3. Average fruit weight, number of pears per tree, diameter and marketable yield (diameter > 50 mm) of pears in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments in 2010 and 2011. *, ** and ns indicate significant differences between treatments at $P < 0.05$, $P < 0.01$ and no significance, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average fruit weight (g)</th>
<th>Number of pears/tree</th>
<th>Diameter (mm)</th>
<th>Marketable yield (kg/tree)</th>
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<tbody>
<tr>
<td>NP</td>
<td>109.8</td>
<td>118.7</td>
<td>181</td>
<td>82</td>
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<tr>
<td>RP</td>
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<td>89.9</td>
<td>138</td>
<td>186</td>
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<td>sig.</td>
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<td>FI</td>
<td>105.9</td>
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<td>103.1</td>
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<td>NI</td>
<td>95.5</td>
<td>86.8</td>
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<td>192</td>
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<td>sig.</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
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</table>


**Table 4.**

Fruit number distribution (%) of different size categories at harvest in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments in 2010 and 2011. *, ** and ns indicate significant differences between treatments at $P < 0.05$, $P < 0.01$ and no significance, respectively.

<table>
<thead>
<tr>
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<tr>
<td>NP</td>
<td>4.1</td>
<td>6.2</td>
<td>58.1</td>
<td>42.7</td>
</tr>
<tr>
<td>RP</td>
<td>8.7</td>
<td>21.4</td>
<td>68.8</td>
<td>64.4</td>
</tr>
<tr>
<td>sig.</td>
<td>*</td>
<td>**</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>FI</td>
<td>6.8</td>
<td>17.4</td>
<td>62.7</td>
<td>64.5</td>
</tr>
<tr>
<td>DI</td>
<td>7.2</td>
<td>19.5</td>
<td>67.7</td>
<td>63.7</td>
</tr>
<tr>
<td>NI</td>
<td>11.8</td>
<td>25.9</td>
<td>72.1</td>
<td>60.9</td>
</tr>
<tr>
<td>sig.</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>
Fig. 1. Seasonal temperature variation, global radiation, potential evapotranspiration \((\text{ET}_p)\) and vapour pressure deficit (VPD) during the experimental period in 2010 and 2011. Data was recorded by the Danish Meteorological Institute (DMI) at Aarhus University, Årslev less than 1 km from the experimental pear orchard.
(a) Layout of individual plots

X = Clara Frijs trees
O = Pollinating tree Conference
X = measuring tree Clara Frijs
• = tube for measuring soil water content
♦ = tube for measuring root in NP and RP

(b) Layout of the field site with plot locations

Fig. 2. Diagram showing experimental plots for non-root pruning (NP), root pruning (RP), deficit irrigation (DI), full irrigation (FI) and non-irrigation (NI) treatments.
Fig. 3.
Fig. 3. Changes in volumetric soil water content in the 0-90 cm soil profile in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments during the experimental period in 2010 (Fig. 3A) and 2011 (Fig. 3B). Values are means ± S.E.
Fig. 4. Percentage of shoot growth termination in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments in 2010. Values are means ± S.E. * and ** indicate significant differences between treatments at $P < 0.05$ and $P < 0.01$, respectively.
Fig. 5. Root intensity at a depth of 30, 40, 50, 75, 100, 125 and 150 cm in non-root pruning (NP) and root pruning (RP) treatment in 2010 and 2011. Values are means + S.E.
Fig. 6. Fruit expansion rate in non-root pruning (NP), root pruning (RP), full irrigation (FI), deficit irrigation (DI) and non-irrigation (NI) treatments and average temperature in 2010 and 2011. Values are means ± S.E. * and ** indicate significant differences between treatments at $P < 0.05$ and $P < 0.01$, respectively.
Prediction of postharvest dry matter, soluble solids content, firmness and acidity in apples (cv. Elshof) using NMR and NIR spectroscopy – a comparative study

Møller, S.M., Travers, S., Bertram, H.C. and Bertelsen, M.G.

European Food Research and Technology
Prediction of postharvest dry matter, soluble solids content, firmness and acidity in apples (cv. Elshof) using NMR and NIR spectroscopy – a comparative study.

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Abstract

Prediction of dry matter (DM), soluble solids content (SSC), firmness and acidity by proton nuclear magnetic resonance (NMR) T$_2$ relaxometry and near infrared (NIR) spectroscopy were investigated on a total of 390 apples (cv. Elshof). The fruit came from four different pre- or postharvest treatments and covered a large range of DM (11.4-20.0 %) and SSC values (10.5-18.3 °Brix). NIR was superior in predicting DM ($R^2 = 0.82$) and SSC ($R^2 = 0.80$), compared to NMR ($R^2 = 0.50$ and $R^2 = 0.58$). However, NMR relaxometry was able to detect multiple water populations assigned to different water pools in the apples and variation in the water distribution between different pre- or postharvest treatments. Differences in the mobility of the vacuole water (population T$_{24}$) were consistent with changes in fruit firmness. In conclusion, even though NIR is superior in predicting DM and SSC, NMR provides useful information about the intrinsic water state and its distribution in the fruit.
1.0 Introduction

Postharvest fruit and vegetable quality is of great importance and can be investigated using a variety of mainly destructive methods, including the measurement of dry matter (DM), soluble solids content (SSC), firmness and acidity. However some spectroscopic methods are non-destructive and provide the potential for extensive and rapid quality prediction. Near infrared (NIR) spectroscopy has been applied in several studies on different types of fruit and shown promise for prediction of different quality parameters such as sensory attributes, SSC and firmness (Fraser et al., 2003; Lammertyn et al., 1998; Lammertyn et al., 2000; Mehinagic et al., 2004; Rahim et al., 2010; Slaughter, 1995; Ventura et al., 1998). Another spectroscopic method investigated for the rapid determination of fruit and vegetable quality is Nuclear Magnetic Resonance (NMR) Relaxometry. This method has shown promise for evaluating juiciness and firmness of apples (Barreiro et al., 2002) and SSC in apples and strawberries (Marigheto et al., 2006). The ability of these two methods to evaluate and predict fruit quality, including that of apples, is much researched. However to the authors’ knowledge, no direct comparison of the two methods for evaluating and predicting quality in apples after a period in storage has been reported. Consequently, the objective of this study was to compare the ability of NIR and NMR relaxometry to predict apple DM, SSC, firmness and acidity, and to investigate water state and dynamics in apples using NMR relaxometry.

2.0 Materials and methods

2.1 Experimental design

Four groups of apples, exposed to four different pre- or postharvest treatments (Table 1) were investigated after removal from cold storage and placed in 20 °C room with a relative humidity of 90 %. Samples were taken for analysis the day after placement began and after a further 8, 15 and 22 days. The different treatments and time spent at 20 °C was introduced to obtain a larger range of DM, SSC, firmness and acidity reference values. Approximately 25 fruit from each treatment were analysed (~100 apples in total) on each day. DM, SSC, firmness, $^1$H T$_2$ relaxation and NIR measurements were calculated on a per fruit basis, while acidity (malic acid) was based on 5-pooled samples.
2.2 DM, SSC, firmness and acidity reference data

The DM samples were oven dried at 70 °C for 20 h until constant weight. DM was expressed as percentage dry weight of the initial fresh sample. SSC (°Brix) was determined with a temperature compensated digital refractometer (RFM712, Bellingham Stanley Ltd., UK). Flesh firmness (kg cm⁻²), after skin removal was measured with a penetrometer (GS20 Fruit Texture Analyser, Güss Ltd., South Africa) fitted with a 8 mm probe. Titratible acidity was measured by blending 200 g of diced flesh (Wareing Laboratory blenders, UK) with 100 g of deionised water for 2 mins. The puree was titrated with 0.1 M NaOH to pH 8.1 using an autotitrator (7195-Titrino, Metrohm, Switzerland). Results were expressed as mg/g of malic acid equivalent (MAE).

2.3 NMR measurements

¹H T₂ relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) operating at 23.2 MHz and equipped with an 18 mm variable temperature probe, using a CPMG sequence. The acquisition settings included a delay between the 90 ° and the 180 ° pulse (tau spacing) of 800 μs, a recycle delay of 7 s with 8 scans acquired. Cylindrical samples (approx. 4 cm long and 1 cm in diameter) were analysed at 25 °C. The obtained T₂ relaxation data were analysed using distributed exponential fitting analysis as described by Møller et al. (2012), and relative population size (Size) and relaxation time (Time), for each T₂ population found were calculated.

2.4 NIR measurements

Prior to the NMR and reference data sampling, spectra were acquired using an AgriQuant (InfraQuant) FT-NIR spectrometer fitted with an InGaAs detector with a sampling increment of 4 nm and a spectral range of approximately 1000-2500 nm. The number of spectrum scans was set at 64. The instrument was fitted with a fibre optic cable and a direct contact probe working in reflectance mode with a diameter of 10 mm. A white Teflon tile was used to provide a reference spectrum before and during every sampling session. The instrument was configured with PLCATS (version 4.4) and InfraQuant 2.5 software.
2.5 Data analysis

Principal component analysis (PCA) was applied on intensity-normalised $^1$H T$_2$ relaxation data and on SNV pre-processed NIR absorbance data minus noise (943-2474 nm) in order to investigate any cluster patterns, applying segmented cross-validation according to treatment (four segments). Partial least squares (PLS) regression analysis was selected to build models for predicting apple quality using the NIR spectra or intensity-normalised NMR relaxation decays. The datasets for DM and SSC were y-sorted and systematically split into a calibration/validation set for modelling with 20% of the dataset reserved for a separate test set. To evaluate the models, the number of principal components (PCs), test set coefficient of determination ($R^2_{\text{test set}}$) and root mean square error of prediction (RMSEP) were obtained. For statistical analysis, a Students t-test was performed in SAS 9.1 (SAS Institute Inc, Cary, NC).

3.0 Results and discussion

3.1 Prediction of DM, SSC, firmness and acidity

PLS regressions did not reveal any correlation between analysed NMR and firmness or acidity, while NIR spectra showed only a weak correlation to firmness and none to acidity (data not shown). According to Table 2, NIR is clearly a superior method in the prediction of DM and SSC, explaining 80-82% of the variation in these parameters, whereas the NMR models explain only 50-58%. The NIR results are in good agreement with previous research (Liu et al., 2008; McGlone et al., 2002; Peirs et al., 2000), while for the NMR results higher correlations to sucrose concentration have been obtained by using water suppression by diffusive attenuation in apples and strawberries (Marigheto et al., 2006).

3.2 Water state and dynamics in apples investigated using NMR relaxometry

NMR relaxometry is known for its ability to probe water state and dynamics in food. Thus, even though NMR is inferior to NIR in the prediction of DM and SSC, NMR relaxometry is able to provide information concerning water state and dynamics in apples, which can be affected by pre-
or postharvest treatments and storage time. According to Fig. 1, distributed analysis of the NMR $T_2$ relaxation data revealed four populations of protons present in the apples: $T_{21}$ (population size approx. 5 %; relaxation time 10 ms); $T_{22}$ (population size approx. 3 %; relaxation time 64 ms); $T_{23}$ (population size approx. 9 %; relaxation time 284 ms); and $T_{24}$ (population size approx. 83 %; relaxation time 1.2 s). The presence of these four relaxation populations is consistent with the findings of Marigheto et al. (2008) who observed four populations of protons in tissue of ‘Red Delicious’ apples. These four populations can be assigned to the different cell compartments in the apple tissue. $T_{21}$ is assumed to arise from the more rigid components of the cell wall, $T_{22}$ and $T_{23}$ may be associated with water in the cytoplasm and extra-cellular compartments and the major population of $T_{24}$ is assumed to be associated with water in the vacuole (Hills & Remigereau, 1997; Marigheto et al., 2008; Snaar & Van As, 1992).

For each proton population, population size and relaxation time were obtained and statistical analysis revealed significant differences between the treatments for several of the NMR relaxation parameters (Table 3).

Apples treated with MCP postharvest clearly differed in water state and dynamics compared to the three preharvest treatments (Table 3). The relaxation time of the water populations $T_{22}$, $T_{23}$ and $T_{24}$ were significantly longer, indicating that water in the cytoplasm and extra-cellular compartments, and water in the vacuole were less restricted in apples treated with MCP compared to apples from the three preharvest treatments. Apples from the Early N+K treatment had a significantly smaller $T_{24}$ population demonstrating a lower amount of water in the vacuole of apples from this treatment. These apples also displayed differences in firmness, where MCP treated apples displayed the highest firmness and apples from the Early N+K treatment the lowest firmness, although not significantly different to the Early N/Late K apples. These findings suggest that changes in firmness could be related to the amount of water located in in the vacuole, where a low firmness is associated with a high amount of vacuolar water. Further studies in order to verify these findings could be of great interest.
4.0 Conclusion

The present study revealed that NIR was superior in predicting DM ($R^2 = 0.82$) and SSC ($R^2 = 0.80$) compared to NMR ($R^2 = 0.50$ and $R^2 = 0.58$, respectively). However, NMR relaxometry was able to detect multiple water populations in apples that were assigned to different water pools and differences in water distribution between the different pre- and postharvest treatments were demonstrated. Furthermore, NMR revealed that differences in the mobility of vacuole water (population $T_{24}$) were consistent with changes in apple firmness.

Acknowledgements

The authors wish to thank the Danish Ministry of Food, Agriculture and Fisheries for financially supporting the project ‘Integrated characterization of quality and microbial safety of foods’ (project no. 3304-FVFP-07-784-01) and project ‘Sustainable future for Danish fruit’ (project no. 3412-09-02385). The technical assistance of Elisabeth Kjemtrup and Karin Henriksen is gratefully acknowledged.
References


Table 1
Overview of the four pre- and postharvest apple treatments.

<table>
<thead>
<tr>
<th>Preharvest treatments</th>
<th>Nitrogen</th>
<th>Potassium</th>
<th>Potassium</th>
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<tr>
<td>Group</td>
<td>April-Sept</td>
<td>April-June</td>
<td>July-Sept</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early N+K</td>
<td>70</td>
<td>25</td>
<td>25</td>
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<tr>
<td>Early N/Late K</td>
<td>35</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

Postharvest treatment

- MCP: Smartfresh™ (1-Methylcyclopropene)
PLS regression results calculated from intensity-normalised NMR decay data or SNV filtered NIR absorbance data (x-variables) and dry matter (DM) or soluble solids content (SSC) (response variable). Models were calculated using segmented cross-validation according to treatment and tested using an independent test-set (20% of the original dataset). Statistics: principle components (PCs); test set coefficient of determination ($R^2_{\text{test set}}$); root mean square error of prediction (RMSEP).

<table>
<thead>
<tr>
<th>X-variables</th>
<th>NMR relaxation decay</th>
<th>PC</th>
<th>$R^2_{\text{test set}}$</th>
<th>RMSEP</th>
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<td><strong>Response variable</strong></td>
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<tr>
<td>DM (%)</td>
<td>2</td>
<td>0.58</td>
<td>1.06</td>
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<tr>
<td>SSC ('Brix')</td>
<td>2</td>
<td>0.50</td>
<td>1.07</td>
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<table>
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<tr>
<th>X-variables</th>
<th>NIR absorbance (923-2474 nm)</th>
<th>PC</th>
<th>$R^2_{\text{test set}}$</th>
<th>RMSEP</th>
</tr>
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<tr>
<td><strong>Response variable</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DM (%)</td>
<td>4</td>
<td>0.82</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>SSC ('Brix')</td>
<td>4</td>
<td>0.80</td>
<td>0.70</td>
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Table 3

LSmeans for population size (Size) and relaxation time (Time) for the four different relaxation populations (T21, T22, T23 and T24) obtained by distributed exponential fitting, and dry matter (DM), soluble solids content (SSC), firmness and acidity from apples from four different treatments (Control, Early N+K, Early N/Late K and MCP).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Control</th>
<th>Early N+K</th>
<th>Early N/Late K</th>
<th>MCP</th>
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<tbody>
<tr>
<td>Size T21 (P = 0.031)</td>
<td>5.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.07&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Time T21 (NS)</td>
<td>9.68</td>
<td>9.45</td>
<td>9.64</td>
<td>9.23</td>
<td></td>
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<tr>
<td>Size T22 (P = 0.015)</td>
<td>2.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Time T22 (P = 0.002)</td>
<td>61.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Size T23 (P = 0.005)</td>
<td>9.13&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>9.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Time T23 (P&lt;0.001)</td>
<td>278.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>280.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>281.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>296.77&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Size T24 (P&lt;0.001)</td>
<td>82.68&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>83.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Time T24 (P&lt;0.001)</td>
<td>1163.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1132.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1163.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1194.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DM (%) (P&lt;0.001)</td>
<td>16.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.42&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>SSC (°Brix) (P&lt;0.001)</td>
<td>14.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Firmness (kg cm&lt;sup&gt;-2&lt;/sup&gt;) (P&lt;0.001)</td>
<td>4.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Acidity (mg/g) (P&lt;0.001)</td>
<td>5.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.27&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a-c Different letters within each row indicate significant difference (P<0.05)</sup>

Values in bold denote significant difference between treatments (column) within the same parameter (row) (P<0.05).
Fig. 1. Distributed $T_2$ relaxation times obtained from an apple from the Early N+K treatment, analysed 1 day after storage commenced.