Project title:	Application of chlorophyll fluorescence for prediction of harvest maturity in broccoli
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Date project commenced:	1 April 2014
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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

A non-destructive measurement of broccoli heads using chlorophyll fluorescence (CF) has been identified and is currently under development. It has potential to assess consignments of broccoli at the point of harvest and to predict the storage potential.

Background

It is difficult to pinpoint exactly when broccoli is at the right harvest maturity for good storage behaviour and shelf-life. Areas of a crop with seemingly identical heads, harvested at the same time, can show widely differing keeping qualities – which creates an obvious problem for managing the schedule of a crop that, thanks to variability in weather and consumer demand through the season, may need to be stored for up to three weeks to balance supply and demand.

However, the technology of chlorophyll fluorescence could potentially be used to monitor the maturity and health of broccoli heads. This project, is investigating two key questions: can chlorophyll fluorescence be used to assess heads at harvest for their subsequent keeping quality; and can the technology be used to inform crop management decisions in the field and after harvest?

Chlorophyll fluorescence

Green plant tissues contain chloroplasts, the microscopic organs within the cells where photosynthesis takes place. The chlorophyll molecules in the chloroplasts absorb sunlight. Most of the energy received is used to drive photosynthesis which in turn supplies energy to the plant, but a portion is unused and re-emitted by the chlorophyll as fluorescence. The more active the chloroplasts the more energy is released as fluorescence.

For decades scientists have used this as a tool to study some fundamental aspects of photosynthesis, for example, it can indicate both the concentration and the activity or health of chloroplasts within plant tissue. Chloroplasts are very sensitive, rapidly losing activity if the tissues become stressed, so measuring chlorophyll fluorescence has been used to assess crop health in the field and, in particular, disease load for arable crops. Changes in fruit and vegetable maturity are also associated with changes in chloroplast function and concentration. The ripening of most fruit involves very significant loss of green colour and

that's down to a loss of chloroplasts. It is already known, for example from work in project TF 142, that chlorophyll fluorescence can be a valuable tool to assess maturity of tree fruit.

Summary

As a technique that can measure both the concentration and the activity/health of chloroplasts within plant tissues, chlorophyll fluorescence has been used to assess maturity and health for a wide range of crops. Specifically chlorophyll fluorescence has been use to map changes in the health of broccoli during storage and shelf-life (FV 395) where a decline in the number of active chloroplasts is correlated with a reduction in head quality leading to senescence.

The overall objectives of this project are:

1) To optimise an existing chlorophyll fluorimeter for use on broccoli heads in collaboration with the manufacturer (Hansatech Instruments Limited)
2) To relate chlorophyll fluorescence profiles of broccoli to maturation in the field as estimated by the effective day degrees after transplant and morphological characteristics
3) To identify biochemical changes (antioxidants and isothiocyanates) during broccoli head maturation
4) To develop strategies for predicting the shelf-life of broccoli consignments at harvest in order to improve scheduling of broccoli marketing
5) To model broccoli head maturity, including biochemical and morphological changes in terms of chlorophyll fluorescence profile.

During the first year of the project it was found that measurement chlorophyll fluorescence characteristics of broccoli heads at harvest could provide a prediction of quality after storage. The evidence was not yet strong enough to suggest that it could be used to grade individual heads, but it could be used to predict the overall behaviour of consignments. As an illustration of this, Figure A shows a plot between the predicted and actual Maturity Index for four consignments of broccoli.

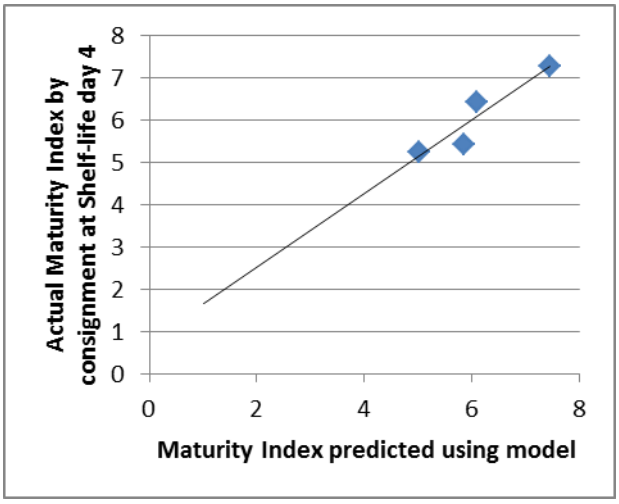

Figure A. 2014 Actual v predicted Maturity Index after 4 days shelf-life using the model developed using head diameter and chlorophyll fluorescence

During the second year of the project trials were conducted to test the CF measuring protocol in order to optimise the design of a specialised probe, and to test and refine the CF predictive model for broccoli harvested over a wider range of conditions.

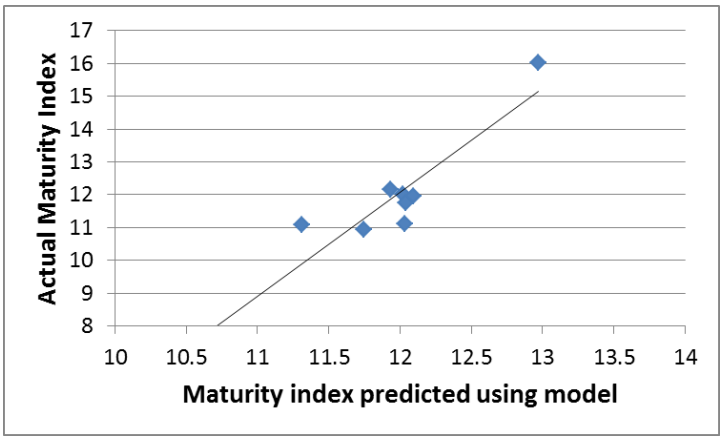

Figure B. 2015 Actual v predicted Maturity Index after 4 days shelf-life using the model developed using head diameter and chlorophyll fluorescence

Figure B shows a similar plot between the predicted and actual Maturity Index as shown in Figure A, but this time for eight consignments of broccoli in 2015.

As a result of the trials conducted this year, it has been shown that a more rapid measurement protocol can be used, using a single pulse, rather than the previous double pulse protocol. There is an indication that two sources of variability arise due to variable positioning of the probe on the broccoli head, and due to external light interfering with the chlorophyll fluorescence measurement. Both of these sources of variability can be overcome by design of a specialised measuring head. This will be investigated in the final season of the project.

Financial Benefits

The potential financial benefits from this project will arise as a result of growers being able to predict the storage potential of consignments, so that they can optimise scheduling of harvesting and the order of distribution of consignments.

Action Points

No specific change in practices is recommended at this stage of the project. However, in order to ensure that the technology development is focused as effectively as possible to industry needs, the researchers welcome input from growers on the way in which they would envisage using the technology.

SCIENCE SECTION

Introduction

Broccoli is a hardy cool season Brassica that is grown predominantly in East Anglia/Lincolnshire and the East of Scotland. UK production figures for Broccoli and Cauliflower combined in 2013 estimate production of 155,000 tonnes with a total value of greater than £100 M although a decrease in production was recorded in 2014 with a total value of only £79 M.

To ensure continuity in the supply of broccoli to the retail sector it is increasingly important to be able to predict the time required for broccoli heads to reach the required market size. Unpredictable climate conditions during the growing season have meant that both time of head initiation and rate of head growth can be variable. While recent studies on improving the storage life of Brassicas (FV 395) have yielded some promising results in improving the quality of stored broccoli, allowing for peaks and troughs in demand and supply to be smoothed out, such benefits are strongly dependent on the quality of the harvested crop. Models, such as the "Wellesbourne Cauliflower Model" predict the time taken to reach the required head size (7-20 cm) incorporating the effect of solar radiation and temperature to estimate the effective day-degrees during the growing season (Wurr *et al* 1991, 1992, FV 57a). While these models help to manage crop productivity it has been observed that a range of physiological maturities exist between commercially harvested heads leading to variability in the storage and shelf-life characteristics (AHDB Field Crops Technical Panel, personal communication). Moreover, variation in temperature or excessive rainfall during the growing season often translates into poor storage and shelf-life potential of the crop.

The objective of this project is to develop sensors adapted to field or postharvest use that will enable the assessment of broccoli head maturity and plant health. This will afford the opportunity for field operatives to make an assessment of optimum harvest date for particular field sites and to predict storage and shelf-life potential of heads once harvested.

Chlorophyll Fluorescence (CF) analysis, is a technique that can measure both the concentration and the activity/health of chloroplasts within plant tissue. The technique has been used to assess health for a wide range of crops and specifically to map changes in the health of broccoli during storage and shelf-life (FV 395) where a decline in the number of active chloroplasts is correlated with a reduction in head quality leading to senescence.

As plant tissues such as broccoli age, cell membranes become leaky leading to the onset of senescence. The aging process includes loss of photosynthetic function and the shrinkage and breakdown of chloroplasts (Krupinska 2006). As broccoli heads age this is clearly seen

as loss in green colour. Previous studies have correlated changes in colour of broccoli with the quantity of chlorophyll and carotenoid pigments using colour meter data (L*,a*,b*) (Fernández-León *et al* 2012). However, while a relationship between chlorophyll content and green colour clearly exists, CF can assess chlorophyll concentration more accurately than colour (Gitelson et al 1999), and moreover is an indication of chloroplast function therefore providing a stronger, more robust relationship with maturity. CF has the potential to correlate the health of tissues with storage and shelf-life. Importantly CF can provide an earlier indication of the onset of senescence than visual or colour meter assessment.

The importance of broccoli over other green vegetables is in part due to its phytonutrient content, as it is an abundant source of vitamin C, antioxidants and other phytonutrients such as isothiocyanates. Any assessment of harvest maturity and shelf-life should therefore consider the nutrient content. Broccoli is an excellent source of phytonutrients made up of ascorbic acid, phenolic acids, flavonoids (querticin and kaempferol). Querticin and kaempferol are reported to accumulate with developmental stage of broccoli, peaking just after commercial harvest maturity (Krumbein *et al* 2007) and may provide a biochemical indicator of physiologically maturity that can be correlated with chlorophyll fluorescence signals.

In addition, broccoli is an important source of isothiocyanates that are derived from the hydrolysis of glucosinolates (GLS) which show protective effects against cancer (Keck and Finely 20042002). In general the complement of intact glucosinolates (glucoraphanin, sinigrin, and glucobrassicin) peak approximately 40 days after transplant followed by a decline as broccoli heads reach maturity giving rise to corresponding isothiocyanates (sulforaphane, allyl isothiocyante and idole-3-carbinol) that peak in over-mature heads prior to a decline with the onset of senescence (Botero-O'mary *et al* 2003).

The overall objectives of this project are:

1) To optimise an existing chlorophyll fluorimeter for use on broccoli heads in collaboration with the manufacturer (Hansatech Instruments Limited)
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4) To develop strategies for predicting the shelf-life of broccoli consignments at harvest in order to improve scheduling of broccoli marketing
5) To model broccoli head maturity, including biochemical and morphological changes

in terms of chlorophyll fluorescence profile.

In order to achieve these objectives the specific objective for this phase of the project was to identify a measurement at harvest that could predict the subsequent keeping qualities of broccoli heads. In the first year of the project a CF characteristic Fβ was identified that when measured immediately after harvest in the laboratory, could provide a prediction of quality following storage and shelf-life. During the second season trials were conducted with the following specific objectives;

To test the CF measuring protocol in order to optimise the design of a specialised probe

To test and refine the CF predictive model for broccoli harvested over a wider range of conditions.

Materials and methods

Field trials

Five trials were carried out in 2015 using field sites in Kent and Lincolnshire as summarised in Table 1

Table 1. Summary of field/storage trials conducted during 2015

Trial	Harvest date	Varieties	Growing location
A	Purchased 14 July 2015	Unknown	Purchased from local supermarket
B	30 July 2015	Steel	Kent
C	14 and 21 September	Parthenon, Iron Man, Steel	Kent
D	19 October	Parthenon, Iron Man	Lincolnshire
E	16 November	Iron Man	Kent

Broccoli was grown in Kent on clay soils near Preston, Kent CT3, and in Lincolnshire on Weston Marsh, . at Holbeach Hurn, and at Sandholme. The commercial varieties; Iron Man, Steel and Parthenon were used, grown as commercial crops using standard practices.

Chlorophyll fluorescence (CF) measurement

Chlorophyll fluorescence (CF) measurements were made using a Handy Pea Chlorophyll Fluorimeter (Hansatech Instruments Ltd, King's Lynn, UK). Chlorophyll fluorimeters can be built with a modulated excitation light so that the effects of external light can be filtered out electronically. However, for these trials in order to be able to measure the full range of chlorophyll fluorescence characteristics it was necessary to use a non-modulated fluorimeter, which means that any external light entering the measuring head will interfere with the measurement. The measuring head was fitted with a plate to restrict the measuring area to 4 mm diameter so that the area measured is exposed to a constant excitation light intensity from the light emitting diodes in the head (the plate can be observed as a white disc in Figure 1 (right hand photograph).

Figure 1. Handy Pea Chlorophyll fluorimeter (Hansatech Instruments Ltd, King's Lynn, UK). RH picture: Measurement from a broccoli head using an adapted leaf clip.

The fluorescence transient was measured immediately following the first and second pulse of a double pulse sequence (2s pulse 2000 $\mu E.m^{-2}.s^{-1}$, 3s delay, 2s pulse 2000 $\mu E.m^{-2}.s^{-1}$). Models to interpret fluorescence transients assume that plant material is dark adapted (usually for at least 15 minutes), so for practical measurements, this double pulse protocol was developed during earlier trials to standardise the state of the chloroplasts at the start of the transient and therefore allow measurements without prior dark adaptation. The rationale for using this pulse sequence with a 3s delay was tested in trial A (see below)

Figure 2 shows a typical fluorescence trace (fluorescence transient) obtained from photosynthetic tissue. The fluorescence yield at several points on the trace are measured: Fo (minimum fluorescence yield), Fm (maximum fluorescence yield), Fv (variable fluorescence = Fm-Fo), F1, F2, F3, F4, F5 (fluorescence yield after 10, 30, 100 µs, 1, 3, ms respectively). In addition Tfm (time to reach Fm) and Area above the curve, indicated in the figure are calculated. Models of the functioning of the photosynthetic system have been used to relate the fluorescence characteristics to specific physiological aspects of chloroplasts. These are described in detail at (www.hansatech-instruments.com) and in Strasser et al. 2004.

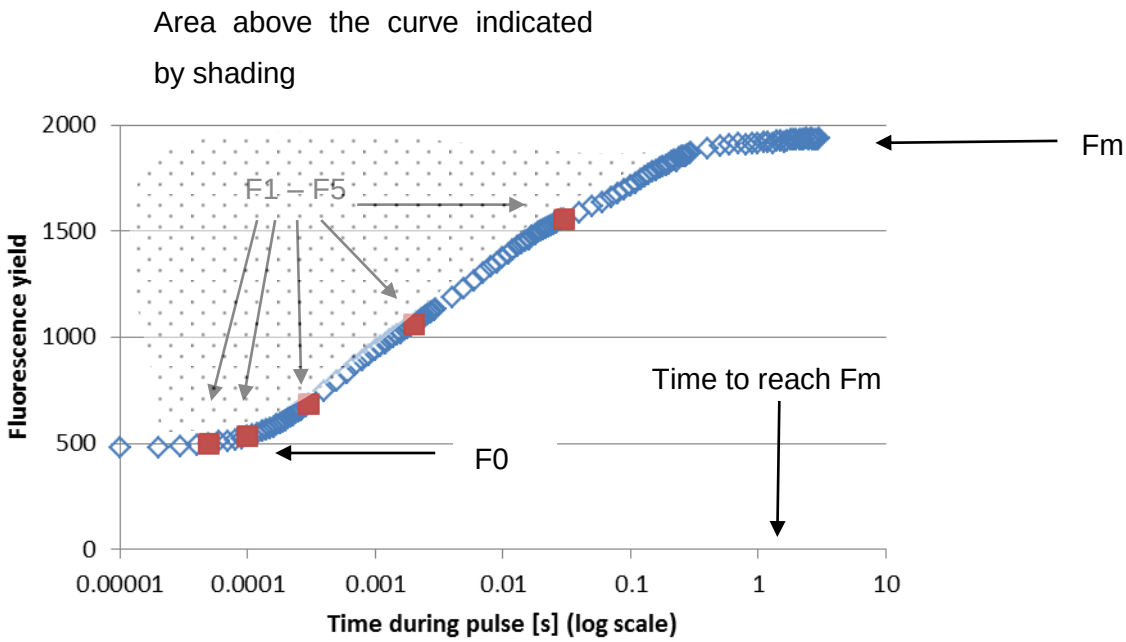


Figure 2. A typical trace of fluorescence yield from a broccoli head exposed to a 3 s light pulse obtained using a non-modulated fluorimeter such as the Handy PEA (Hansatech Instruments Ltd, UK). Some of the parameters used to calculate the fluorescence characteristics are indicated on the figure including Fo (initial fluorescence yield), F1 – F5 (Fluorescence yield at 50 µs, 100 µs, 300 µs, 2 ms and 30 ms respectively), Fm (maximum fluorescence yield), time to reach Fm.

Season 2 (2015) field trials.

Trial A. To optimise double pulse protocol 14 – 17 July

This trial was carried out to check the rationale for using the CF double pulse protocol and checking that the interval between pulses was appropriate.

10 broccoli heads were purchased from a local supermarket. The heads were stored under ambient conditions and assessed for CF characteristics and for maturity indices (see below) over 5 days.

For the CF measurements four protocols were used. The heads were initially fully dark adapted for at least 30 minutes and then were assessed using a single pulse. This is the standard protocol for which models to interpret CF transients have been produced (www.hansatech-instruments.com and in Strasser et al. 2004). The heads were then left for 30 minutes in standard lighting within the laboratory (which is typically less than 10% of outdoor light levels, less than 1% full sunlight) after which all heads were measured using a pulse interval of 1 second. Then all heads were measured again firstly using a pulse interval of 3 seconds, and then again using a pulse interval of 5 seconds.

After the CF measurements on each day heads were assessed for maturity indices.

Standard trial protocol for trials B - E

Except where indicated a standard trial protocol was used for trials B, C, D and E. Heads were transported by car, covered with black plastic to provide a degree of dark adaptation, to the Jim Mount Building at East Malling Research. On arrival, heads were labelled individually, the diameter in two perpendicular directions was measured and each head was weighed. Machine colour and CF characteristics were measured without further dark adaptation, and each head was assessed for maturity. Heads were then stored for 2 weeks at high humidity at 1°C, then moved to high humidity at 18°C for shelf-life assessment.

Repeat assessments of weight, machine colour, CF characteristics and maturity were carried out after 7 and 14 days storage at low temperature and then weight and maturity indices were recorded daily (day 15 – 18) during the shelf-life period.

Trial B. To compare preharvest and postharvest CF measurement and to determine impact on shelf-life of low temperature storage period.

For 30 broccoli heads CF measurements were carried out pre- dawn in the field. 60 broccoli heads including the 30 measured were then harvested and the normal protocol followed,

except that instead of a low temperature storage period of 14 days, half the heads were stored for 11 and the other half for 18 days. The 60 heads were harvested in sequence along a single row. In order to remove field positional effects from the storage period trial, alternate heads were assigned to the two storage periods.

Trial C, D and E Storage/Shelf-life trials on a range of varieties, growing locations and season in order to identify measurable characteristics at harvest capable of predicting storability

Table 2. A summary of the broccoli consignments used for Trials C, D and E

Trial		Growing location	Variety	Harvest date
C	C1	Kent	Iron Man	21/9/15
	C2	Kent	Parthenon	14/9/15
	C3	Kent	Steel	21/9/15
D	D1	Weston Marsh, Lincs	Iron Man	19/10/15
	D2	Holbeach Hurn	Iron Man	19/10/15
	D3	West Marsh, Lincs	Parthenon	19/10/15
	D4	Sandholme	Parthenon	19/10/15
E	E	Kent	Iron Man	16/11/15

Table 2 provides a summary of the broccoli consignments harvested and stored for trials C, D and E. The objective was to test the CF model on consignments that would have a range of growing conditions and therefore a range of storage properties. For trials C, D and E, 36, 30 and 24 heads respectively were harvested for each variety.

Colour measurements

Colour measurement using a Minolta colour meter set to measure in L *a *b* mode provided a measure of loss of green background (*a scale) and the increase in yellowing (*b scale).

Figure 3. the L* a*b*, colour space and Minolta colour meter used to measure machine colour values.

Chlorophyll Fluorescence measurement protocol

CF characteristics were measured using the Hansatech Handy Pea, using a double pulse protocol in four positions across the head positioned on the centre of a whorl wherever possible (outer, inner, inner, outer whorl).

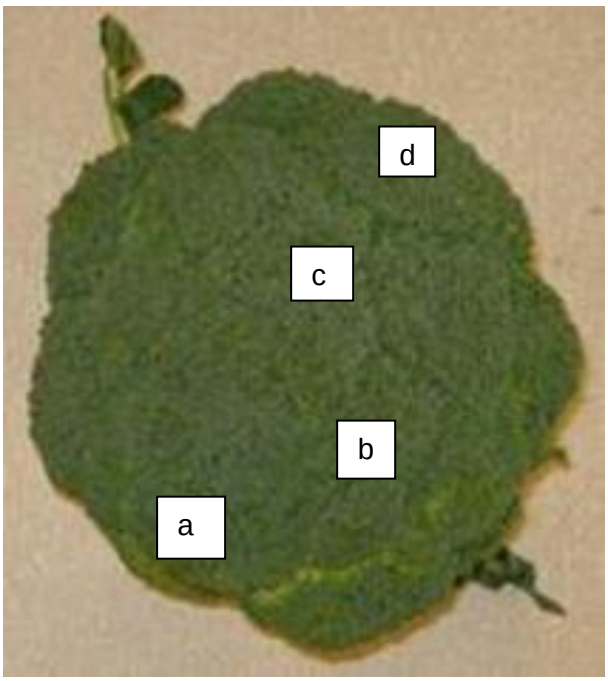


Figure 4 A broccoli head showing the position of the four measurements (a, b, c, d) of CF characteristics.

Maturity assessment

Each head was assessed visually using a scoring system adapted from Wurr *et al* 1991.

- Stem turgor (Turgid – slightly flaccid, very flaccid) 0, 1, 2
- Head colour Blue-Green 1, Green 2, Light green 3, 10% yellowing 4, 20% yellowing 5, 30% yellowing 6, 40% yellowing 7, 50% yellowing 8, 60% yellowing 9, 70% yellowing 10
- Bud compactness (closed – open +yellow petals – open + green and white sepals) 0, 1,
- Bud elongation (Flat head with no elongation – increasing unevenness as buds elongate – individual buds extending) 0, 1, 2

  Floret loosening (firm – florets beginning to loosen – florets wide apart) 0, 1, 2

Maturity Index (MI) = head colour score + stem turgor score + bud compactness score + bud elongation score + floret loosening score

Results

Trials C, D, E Storage/Shelf-life trials on a range of varieties, growing locations and season in order to identify measurable characteristics at harvest capable of predicting storability

Although the trials were conducted chronologically in the order A–E, C-E are presented first in this section as this provides a more logical presentation of findings.

Three trials were conducted covering three varieties grown in Kent and Lincolnshire and harvested on four dates through September, October and November 2015 in order to provide consignments of broccoli with a range of keeping qualities to enable an investigation of which characteristics measured at harvest could predict storability most accurately.

The quality changes of the consignments are shown in figures 5 – 9 in terms of visual assessments; colour score and maturity index (MI = head colour score + stem turgor score + bud compactness score + bud elongation score + floret loosening score), by % weight loss and by % heads considered saleable (a head is considered unsaleable when the colour score is 7 or above, or the stem turgor score reaches 2). Figure 8 shows changes in estimated chlorophyll concentration measured using a novel chlorophyll meter supplied by Hansatach Instruments Ltd. In this case measurements during the whole storage period were only taken for trial C.

It is notable that the range in rates of quality loss, as determined by the parameters measured, was not very great between whole consignments except for consignment E that exhibited a notably rapid loss in quality.

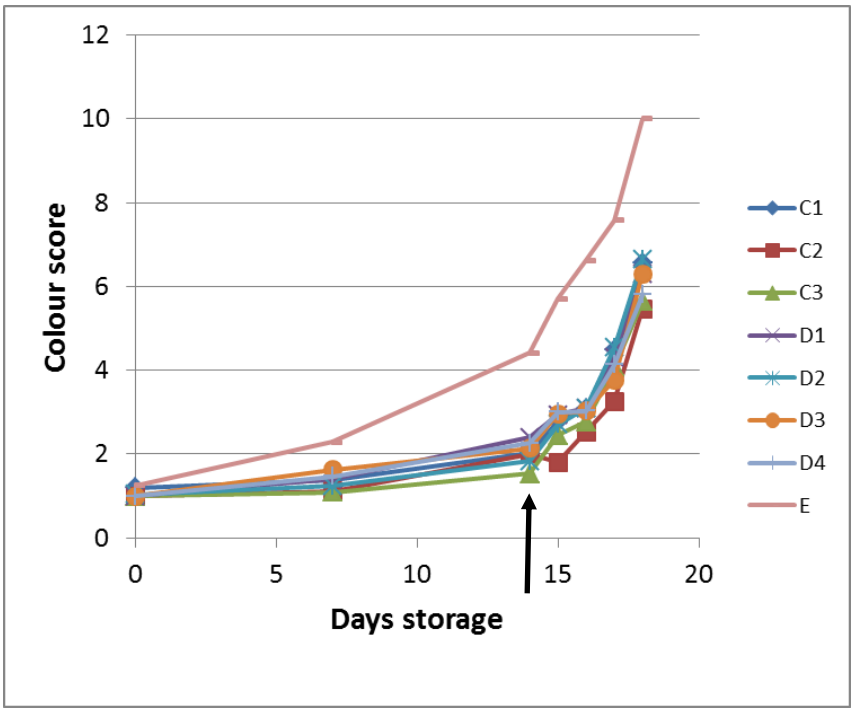


Figure 5. Quality of broccoli consignments in terms of visual colour score during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2. The arrow indicates the start of shelf-life.

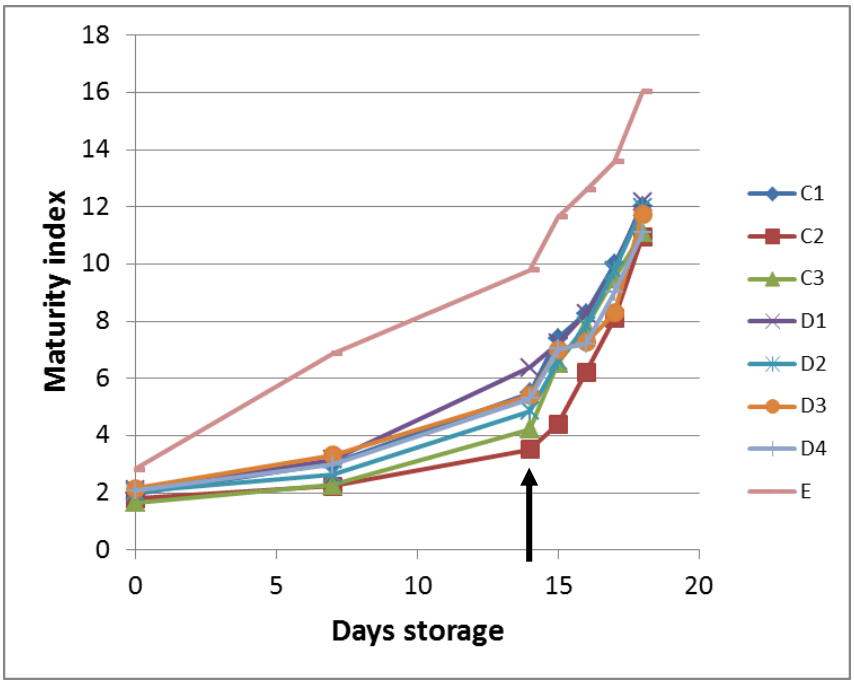


Figure 6. Quality of broccoli consignments in terms of Maturity Index during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2. The arrow indicates the start of shelf-life.

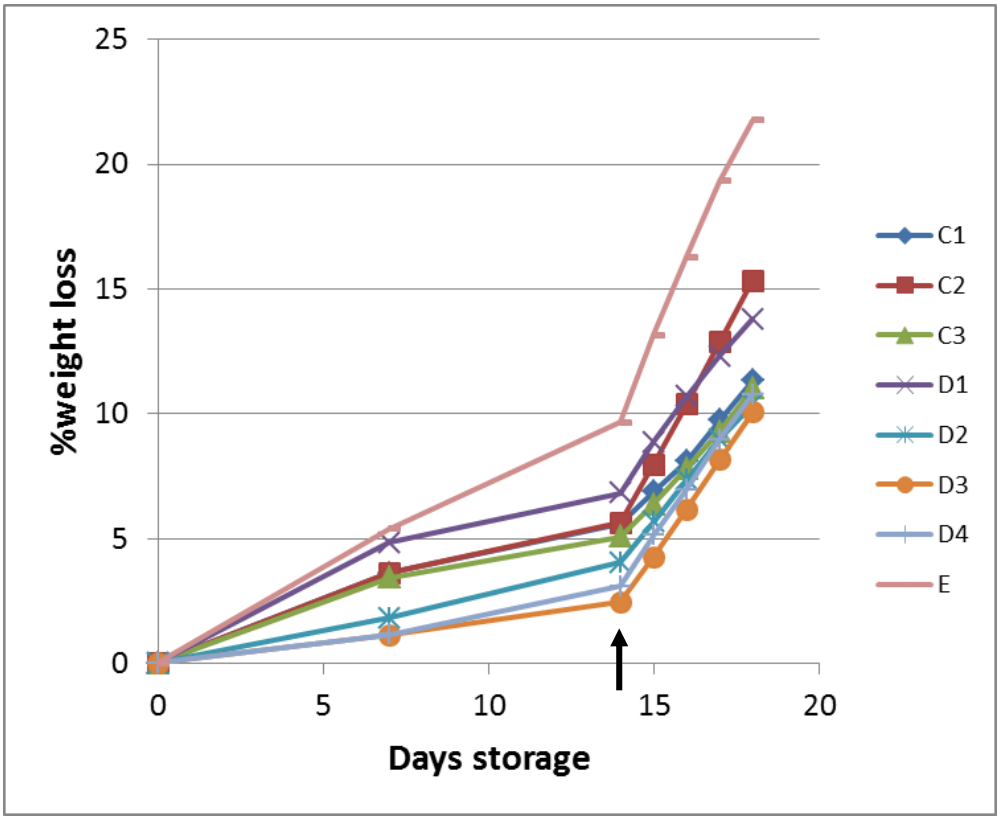


Figure 7. Quality of broccoli consignments in terms of % weight loss during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2. The arrow indicates the start of shelf-life.

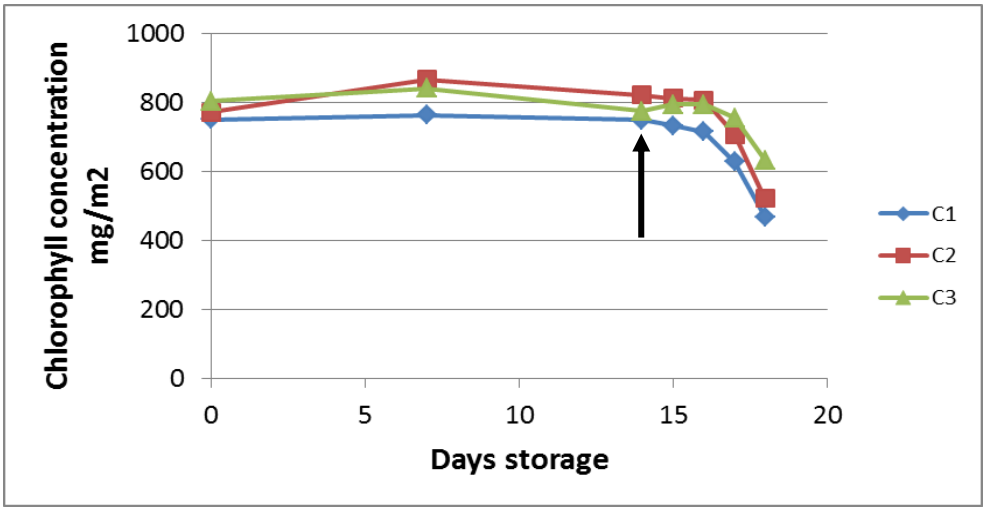


Figure 8. Quality of broccoli consignments in terms of chlorophyll concentration (mg/m^2) measured by a novel chlorophyll meter under test from Hansatech Instruments limited during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2. The arrow indicates the start of shelf-life.

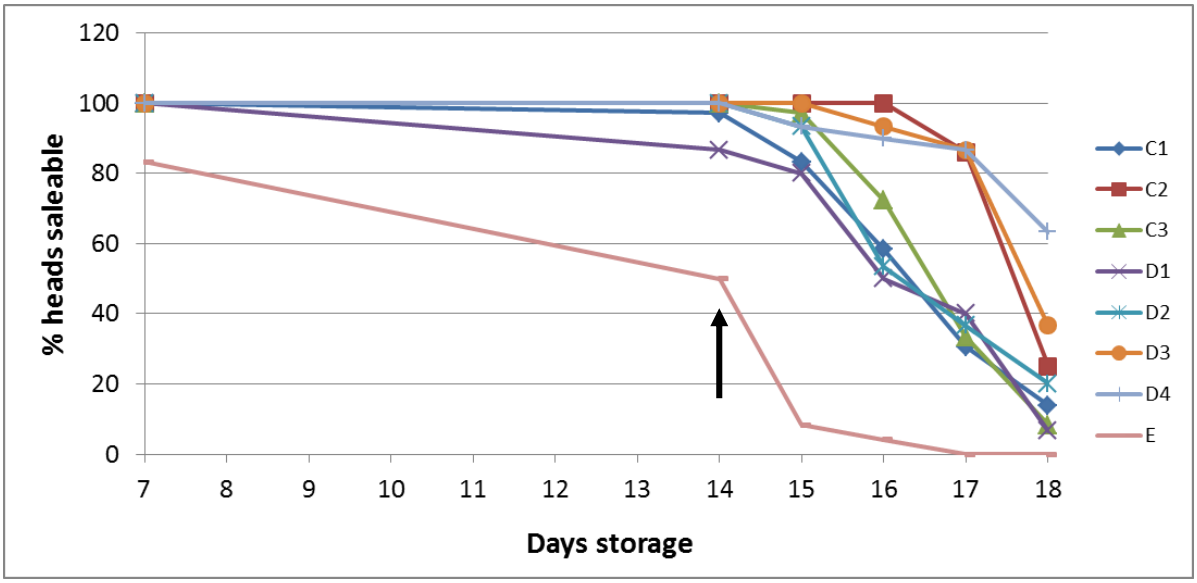


Figure 9. Quality of broccoli consignments in terms of % heads considered saleable during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2. The arrow indicates the start of shelf-life

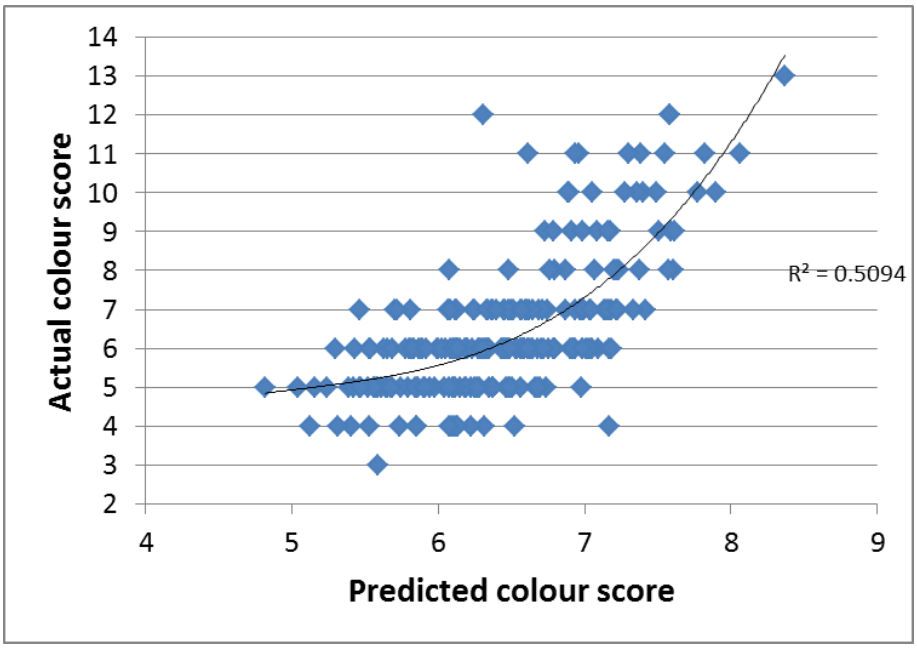
The main objective of these trials was to determine the potential for using measurements at harvest, and particularly chlorophyll fluorescence (CF) characteristics, to predict keeping quality. A very wide range of CF characteristics were measured. A double pulse protocol was used, as described in the Methods section. For each pulse the shape of the rise in fluorescence emission (fluorescence transient) was recorded and the CF characteristics extracted from this information. This means that there were two sets of characteristics for each measurement. Each characteristic was tested for its correlation with quality indicators at each day of shelf-life assessment. The results for the characteristics of most interest are given in Table 3. The strength of correlation is indicated by the magnitude of the "r" value; the higher the r value, the stronger the correlation. A correlation can be positive, in which case the two characteristics increase together, or negative, in which case as one characteristic increases the other decreases. In all cases the CF characteristics for the first pulse showed stronger relationships than for the second pulse, and so it is these that are included in the table.

On the basis of the correlations shown in Table 3, models to predict Colour score and MI on days 17 and 18 were developed in terms of $F\alpha$ (fluorescence yield at a specific time point in the fluorescence transient) and Head size. Figures 10 a) and b) show actual quality scores on day 18 plotted against predicted quality scores for each for all 252 heads used in this set of trials.

Table 3. Correlation, r, between CF characteristics at harvest and quality characteristics using 252 heads. *Fα, Fβ and FΩ are fluorescence yields at specific points on the fluorescence transient, but have not been identified for reasons of commercial confidentiality.*

	Colour score				MI				Shelf-life
	Day 15	Day 16	Day 17	Day 18	Day 15	Day 16	Day 17	Day 18	
Head size	0.187	0.282	0.347	0.525	0.148	0.175	0.290	0.510	0.063
Fo	-0.531	-0.471	-0.433	-0.427	-0.564	-0.475	-0.408	-0.435	0.358
Fα	-0.513	-0.468	-0.431	-0.441	-0.533	-0.467	-0.406	-0.450	0.357
FΩ	-0.469	-0.449	-0.423	-0.438	-0.468	-0.446	-0.406	-0.455	0.338
Fβ	-0.199	-0.228	-0.121	-0.196	-0.063	-0.068	-0.055	-0.216	-0.004
Fv/Fm	0.484	0.380	0.346	0.318	0.556	0.411	0.315	0.308	-0.318
PI	0.373	0.347	0.327	0.326	0.376	0.357	0.321	0.346	-0.272
RC/CS	-0.348	-0.314	-0.286	-0.297	-0.369	-0.313	-0.275	-0.289	0.244

a)



b)

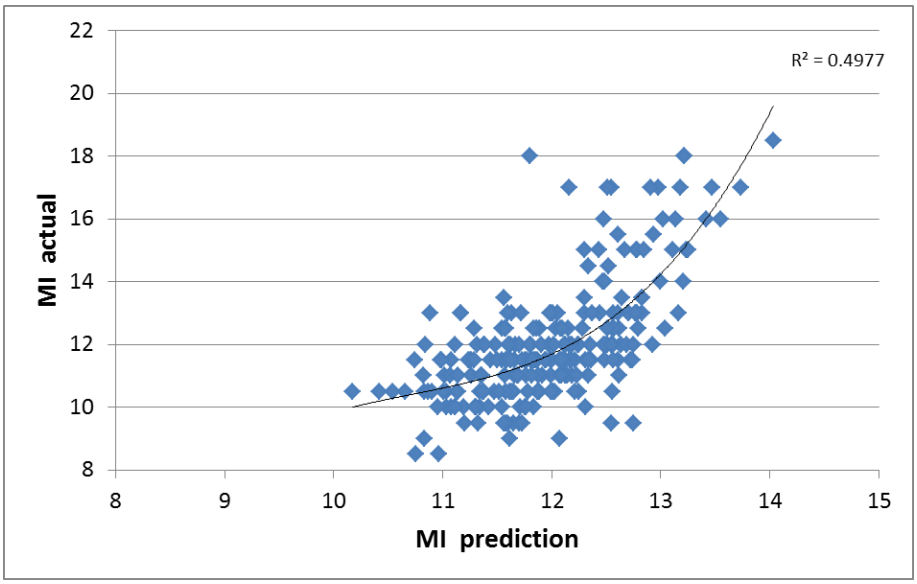
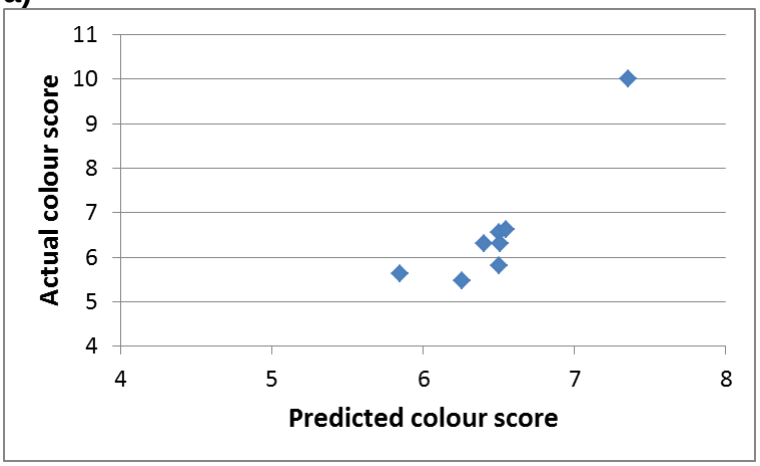


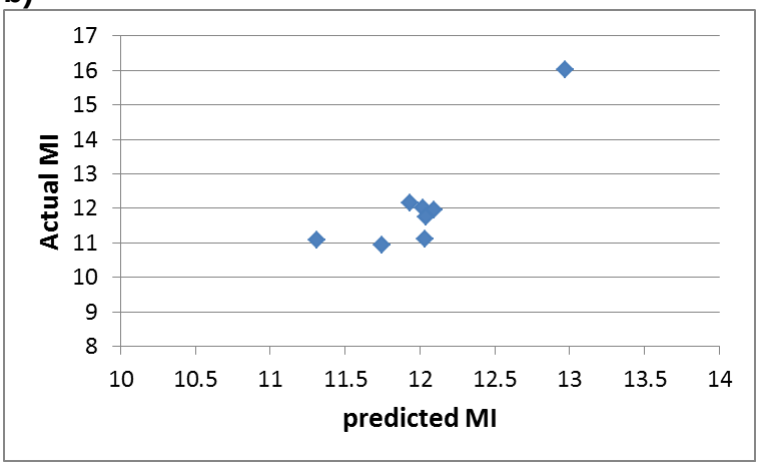
Figure 10. a) Actual colour score on day 18 and b) Actual MI on day 18 plotted against the value predicted from measurement of CF characteristics and head size at harvest. Prediction models are 0.43 size – 0.008 Fα +5 and 0.46 size-0.009Fα +10.6. The r value for line fit is 0.71 in both cases.

The same models were tested for their ability to predict the quality of whole consignments in terms of colour score on day 18 and MI on day 18 (Fig 11 a, b). The relationship between Fα at harvest and average shelf-life of each consignment was also tested (Fig 11 c).

a)



b)



c)

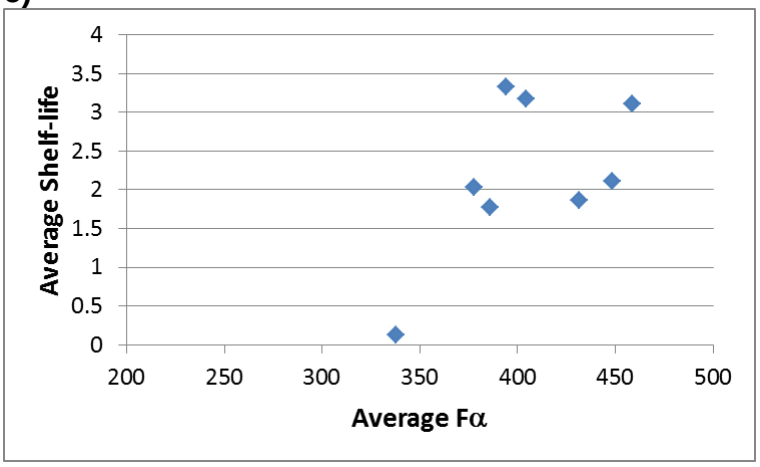


Figure 11. The relationship between actual and predicted colour score at 18 days, MI at 18 days for broccoli consignments. Each data point is the mean of 30 or 36 heads. C) shows the relationship between average shelf-life of each consignment and the average Fα measured.

Trial A. To optimise double pulse protocol

In July 2015, a series of measurements was carried out on 10 broccoli heads over 4 days at ambient in order to test aspects of the methodology. Three protocols for collecting CF data were compared; measurements on dark adapted heads, and double pulse protocols with 1, 3 or 5s delay between pulses.

This allowed two aspects of the methodology to be investigated.

Firstly a double pulse was used on the assumption that by using the fluorescence profile from the second pulse the state of the chloroplasts would be more uniform and less affected by differences in the ambient light conditions. To test this a simple comparison of data from the different protocols was carried out by calculating the correlation between specific characteristics for each head. Table 4 shows the correlation coefficients between characteristics measured using the dark adapted protocol and the other protocols for selected CF characteristics. Interestingly there was no evidence that the second pulse gave more reliable data (i.e. more strongly correlated with the data from the dark adapted protocol) than the first. The correlations are much stronger for Fv and Fv/Fm than for F0, F2 and F3.

Secondly a comparison of data from repeat measurements from the same heads gave an indication of the variability introduced each time the CF probe was repositioned on the broccoli head. Interestingly the data is much more stable for Fv and Fv/Fm than for F0, F2 and F3, which is probably one reason for the observation of weaker correlations observed (Table x) and may also contribute to the high level of noise observed in Figure 11 . Possible contributing factors to the variability are interference of background light and variability of chloroplast content across the broccoli head. Background light would cause an erroneous increase in detect fluorescence which would introduce errors that would be larger for F0, F1-F5 than for Fv (=Fm – F0).

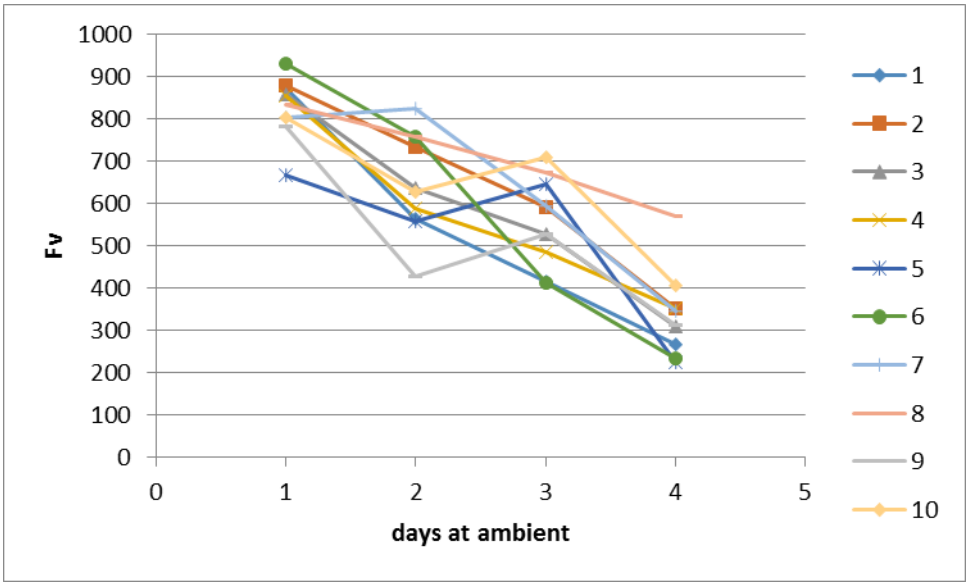


Figure 12. Fv measured on 10 individual heads (labelled 1-10) over 4 days storage at ambient.

Table 4. Correlations between CF characteristics measured by different protocols. Comparison of repeat measurements considered the first pulse for different pulse protocols.

	Correlation coefficient r						
	Dark adapted v 1s delay		Dark adapted v 3s delay		Dark adapted v 5s delay		Comparison of repeat measurements
	Pulse 1	Pulse 2	Pulse 1	Pulse 2	Pulse 1	Pulse 2	
Fo	0.593	0.528	0.642	0.574	0.619	0.623	0.700
F2	0.565	0.508	0.647	0.566	0.621	0.600	0.694
F3	0.544	0.499	0.644	0.565	0.634	0.590	0.701
Fv	0.843	0.844	0.861	0.844	0.883	0.889	0.933
Fv/Fm	0.824	0.820	0.818	0.769	0.878	0.874	0.899

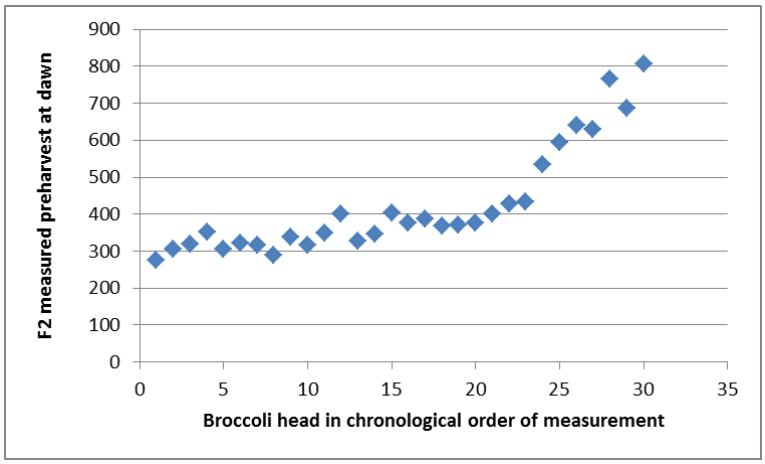
Trial B. To compare preharvest and postharvest CF measurement and to determine impact on shelf-life of length of low temperature storage period.

During Trial B CF characteristics were measured on 30 heads pre-harvest and postharvest on the same day to obtain information on the relative merits of field and packhouse measurements. The pre-harvest measurements were taken pre-dawn to avoid interference of sunlight.

Figure 12a shows the F2 yield measured on 30 heads starting at 04:45 am. The sun rose at 5:40 (Head 23) from which point the F2 measurement increased, indicating the interference of sunlight. As expected sunlight does not have such an impact on the Fv measurement (Figure 12b), as this is the difference between yields.

These observations are consistent with the observations of Trial A, indicating the importance of shielding the probe against external light during the measurement.

a)



b)

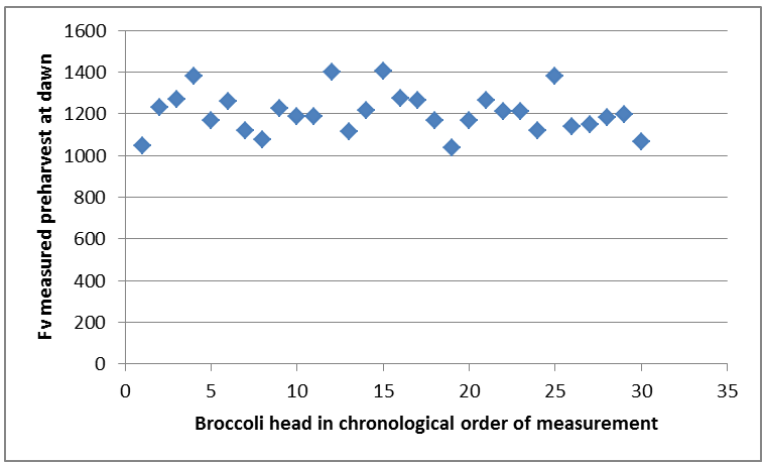
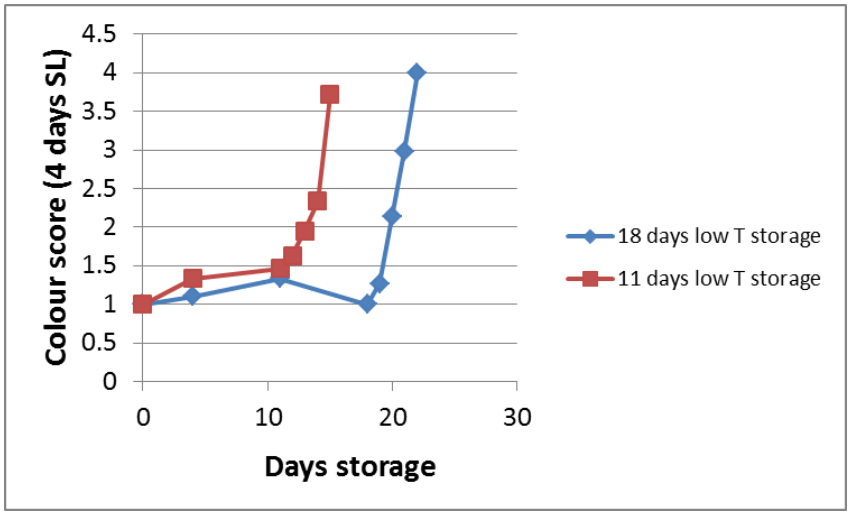


Figure 13. F2 and Fv measured for broccoli heads in the field starting pre-dawn.

Trial B was also carried out to determine the impact of different storage times. Contrary to expectations the effect of 1 week of low temperature storage (comparing 11 days and 18 days) had only a small effect on subsequent shelf-life as see by the change in colour score and maturity index (Figure 13 a and b)

a)



b)

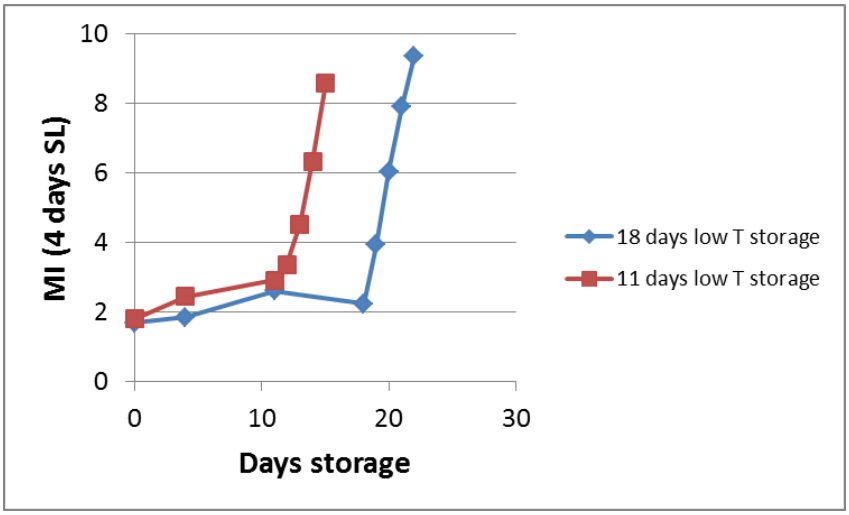


Figure 14. Colour score and maturity index assessed during high humidity storage at 1°C, followed by shelf-life conditions under high humidity at 18°C. 30 broccoli heads were stored at low temperature for 11 days and 30 heads for 18 days. Arrows indicate the point at which heads were moved to shelf-life conditions.

Discussion

During the first year of this project the objective was to identify a non-destructive method to assess head maturity during development in the field, at harvest and during storage, and to be able to predict subsequent storage/shelf-life behaviour. A CF characteristic Fβ measured at harvest was found to correlate with quality after 4 days of shelf-life. During this second year of trials a wider range of varieties, growing site and harvest time were used to obtain more varied consignments of broccoli in order to test the use of CF more rigorously. Unfortunately during this season the response of broccoli was very consistent through the season, apart from the last consignment of broccoli harvested in November, which was of poor quality. Nevertheless it was possible to develop a predictive model using CF for the rate of broccoli deterioration following removal from low temperature storage. This model is improved by the inclusion of broccoli head size.

A more detailed investigation of the protocols used suggest that there is no advantage of using a double pulse protocol. This finding will allow a much more rapid assessment of broccoli heads. For these trials a standard unadapted chlorophyll fluorimeter was used, and four individual measurements made across each broccoli head. The results obtained suggest that variability is introduced due to variability across each head. An adapted probe with several sensors and designed to reduce external light entering the probe may have a very significant effect on reducing errors. It is possible that this would strengthen the predictive power of CF considerably.

A trial was carried out to compare the use of CF measurements in the field before harvest with those within the laboratory after harvest. The conclusion from these trials was that it is impractical to make measurements in the field for two reasons; the sensitivity of the equipment to bright sunlight, and the variability in response of the broccoli heads when exposed to bright sunlight. Light levels within the laboratory, and in a packhouse, are much lower than in full sunlight, so that the interference with measurements is minimal. For these reasons the final recommendations will be to carry out measurements inside after harvest.

For the trials this year broccoli heads were covered with black plastic during transport to the laboratory. In the next season the effect of shading during transport will be tested in more detail so that a practical protocol for using the equipment developed can be established for recommendation to growers.

Conclusions

A predictive model for broccoli keeping quality has been developed using CF and head size measured at harvest. This model is only suitable for measurements made inside a packhouse where the effects of full sunlight on the broccoli head and on the measuring equipment is avoided.

In order to determine whether CF can therefore be a practical tool to help the broccoli growers the final phase of this project will need to concentrate on the following priorities

- Design and test a specialised sensor head, with multiple sensors and shaped to reduce external light interference.
- Validate the predictive model for heads harvested over a wider range of conditions
- Develop a protocol for use and test the ability of CF using this protocol in a commercial situation to distinguish storability of consignments at harvest and during storage in order to improve crop scheduling.
- Test the use of CF measurements during head development as a means to optimise growing practices for improved quality/storability.

Knowledge and Technology Transfer

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We would like to thank T.E. and S.W. Bradley who grew plots of broccoli for these trials at their Boundary Farm and allowed us to sample from their own crops. We would also like to thank Lincolnshire Field Products Ltd and T.H. Clements Ltd who allowed us to harvest broccoli heads from their fields in Lincolnshire. Thanks is also due to Hansatech Instruments Ltd for providing us with a chlorophyll fluorimeter and prototype chlorophyll meter and have technical advice.

Glossary

CF Chlorophyll fluorescence

Fv Variable component of the chlorophyll fluorescence transient rise

Fα Fβ, FΩ chlorophyll fluorescence characteristics identified as useful for predicting broccoli shelf-life. These have not been described precisely to maintain commercial confidentiality

MI Maturity index, calculated as = head colour score + floret loosening score+ Stem turgor Bud compactness score +Budd elongation score

SL Shelf-life

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