

Project title: Application of chlorophyll fluorescence for prediction of harvest maturity in broccoli

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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 has been identified that 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, due 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 used to map changes in the health of broccoli during storage and shelf-life (FV395) 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 determine the optimum harvest window to extend the storage/shelf-life of broccoli
- 5) To model broccoli head maturity, including biochemical and morphological changes in terms of chlorophyll fluorescence profile.

The specific objective for the first year of the project was to identify a measurement using chlorophyll fluorescence at harvest that could predict the subsequent keeping quality of broccoli heads.

Broccoli heads with a range of maturity were harvested from five field trials (three with cv. Steel, and two with cv. Iron Man) between July and October 2014. The quality of the heads was followed over two weeks of low temperature storage, followed by shelf-life determination at 18°C. A full range of quality assessments were measured at each stage including head diameter, weight, colour by machine measurement, visual scoring of colour, stem turgor, head colour, bud compactness, bud elongation, floret loosening.

On the basis that the main changes associated with quality that we were observing were colour and floret loosening, an overall Maturity Index was developed by combining these observations (Maturity Index score = Head colour score + floret loosening score). An increase in Maturity Index indicated loss of quality.

Figure A shows the data for a trial on cv. Steel carried out in July, with the Maturity Index for individual heads. It is notable that at harvest the heads were indistinguishable in terms of Maturity Index, but as storage progressed they exhibited a range of keeping qualities,

indicated by an increasingly wide range in Maturity Index.

In order to achieve the objectives of the overall project, the specific objective of these trials was to identify a measurement at harvest that could predict the subsequent keeping qualities of broccoli heads. For practical reasons we chose to relate characteristics measured at harvest to the Maturity Index after four days of shelf-life (indicated by an arrow in Figure A)

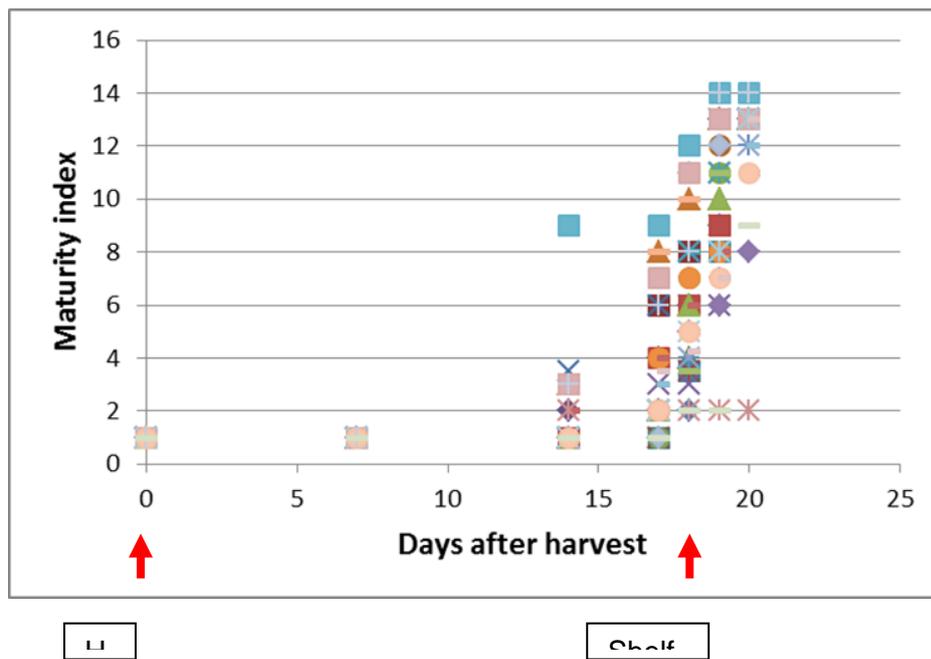


Figure A: The maturity index of individual heads of broccoli during 14 days storage at 1°C followed by shelf-life determination at 18°C, showing how heads that appear to have identical maturity at harvest deteriorate at very different rates. An increase in Maturity Index indicates loss of quality.

A wide range of characteristics measured at harvest were tested for their ability to predict deterioration of heads, including head diameter, colour by machine measurement and a range of chlorophyll fluorescence characteristics. As expected, head diameter was quite a good predictor of maturity index after four days of shelf-life – it is well known that larger, more mature heads have a shorter storage life, a rule of thumb that growers use to some extent to control stocks.

We found no useful relationship between colour measurement at harvest and subsequent keeping quality but we identified one particular chlorophyll fluorescence characteristic that we found correlates consistently with Maturity Index after four days of shelf-life and we used this to produce a simple predictive model. The data indicates that the two cultivars we have tested follow the same or very similar models, but the model can be improved by including head size to make more accurate predictions of storage and shelf-life behaviour.

The indications so far are that measurement of this particular chlorophyll fluorescence characteristic may prove to be a practical method to assess broccoli storage potential. The evidence is 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 B shows a plot between the predicted and actual Maturity Index for four consignments of broccoli.

There may also be potential to use this technology as a tool to help growers make decisions on changes to crop management in the field which will improve a crop's keeping qualities.

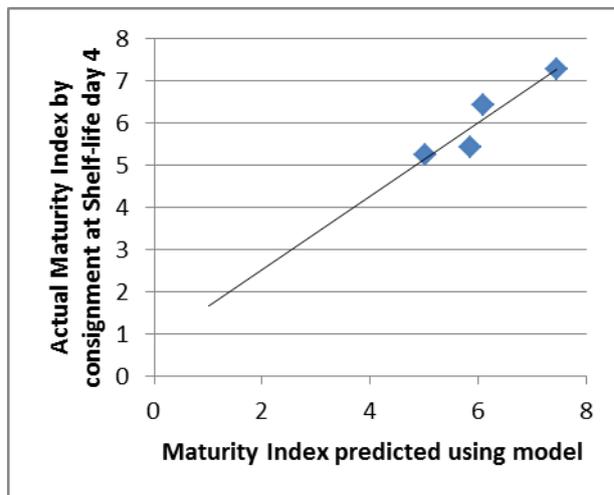


Figure B. Actual v predicted Maturity Index after 4 days shelf-life using the model developed using head diameter and chlorophyll fluorescence

Priorities for the next phase of this project will be:

- Design a specialised sensor head, increase accuracy and speed up measurement
- Test the ability of chlorophyll fluorescence in a commercial situation to distinguish storability of consignments at harvest and during storage in order to improve crop scheduling. This will include refining the measurement practices, such as stage of measurement (before/after harvest), time of day, number of heads.
- Test the use of chlorophyll fluorescence measurements during head development as a means to optimise growing practices for improved quality/storability.

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.

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 (FV395) 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. The use of models, such as the “Wellesbourne Cauliflower Model” to predict the time taken to reach the required head size (7-20 cm) incorporate the effect of solar radiation and temperature to estimate the effective day-degrees during the growing season (Wurr *et al* 1991, 1992, FV57a). 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. 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 antioxidants, made up of ascorbic acid, phenolic acids, flavonoids (quercetin and kaempferol). Quercetin 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, Staack and Jeffery 2002). 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 isothiocyanate and indole-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)
- 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 determine the optimum harvest window for extending the storage and shelf-life of broccoli
- 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.

Materials and methods

Field trials

Field trials were grown in clay soils on land belonging to Boundary Farm, owned by T.E. and S.W. Bradley, near Preston, Kent CT3. For most trials, plants of Iron Man and Steel were provided by the Allium and Brassica Centre at planting, and subsequent trial management was undertaken by T.E. and S.W. Bradley. For the last trials of the season Steel plants were provided by T.E. and S.W. Bradley. The broccoli heads were harvested in July, September and October 2014.

Chlorophyll fluorescence (CF) measurement

Chlorophyll fluorescence (CF) measurements were made using a Handy Pea Chlorophyll Fluorimeter (Hansatech Instruments Ltd, King's Lynn, UK). The measuring head was used with an adapted leaf clip, which is necessary 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.



Figure 1. Handy Pea Chlorophyll fluorimeter

The fluorescence transient was measured immediately following a double pulse sequence (2s pulse $2000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 3s delay, 2s pulse $2000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). 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.

Figure 2 shows a typical fluorescence transient obtained from photosynthetic tissue. The fluorescence yield at several points on the trace are measured: F_0 (minimum fluorescence yield), F_m (maximum fluorescence yield), F_v (variable fluorescence = $F_m - F_0$), F_1 , F_2 , F_3 , F_4 , F_5 (fluorescence yield after 10, 30, 100 μ s, 1, 3, ms respectively). In addition T_{fm} (time to reach f_m) 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.

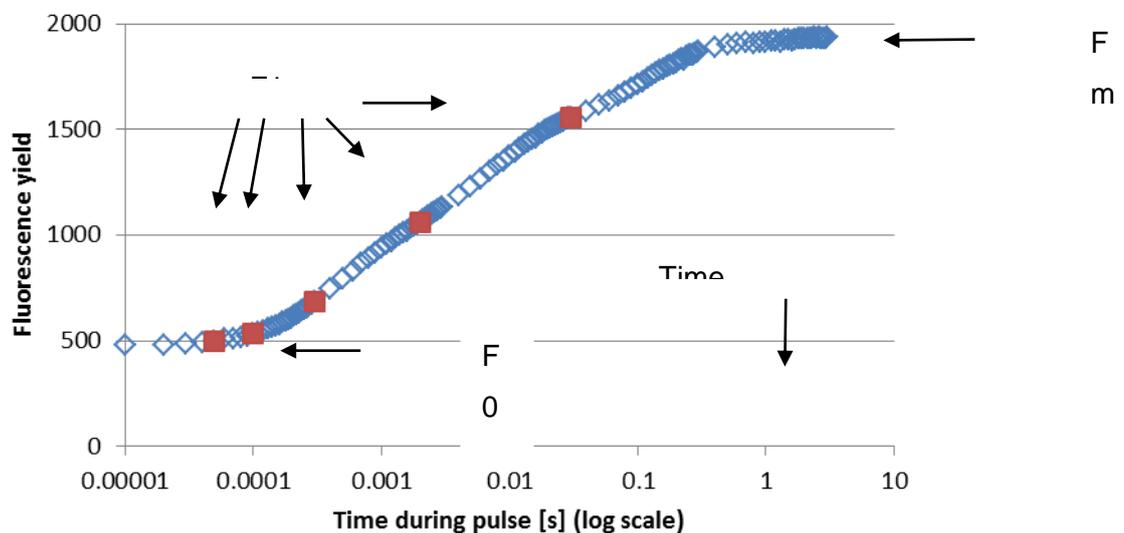


Figure 2. A typical trace of fluorescence yield from a broccoli head exposed to a 3 s light pulse obtained using a non-modulated fluorimeter such as the Handy PEA (Hansatech Instruments Ltd, UK). Some of the parameters used to calculate the fluorescence characteristics are indicated on the figure including F_0 (initial fluorescence yield), $F_1 - F_5$ (Fluorescence yield at 50 μ s, 100 μ s, 300 μ s, 2 ms and 30 ms respectively), F_m (maximum fluorescence yield), time to reach F_m .

Season 1 field trial. To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.

The broccoli heads used in the main trials reported here were harvested from field trials as follows:

Variety	Date harvested	No. heads and head size range
Iron Man	11 th July	29 heads 9 -13 cm
	16 th July	20 heads >9 cm
Steel	11 th July	19 heads 9 – 13 cm
	16 th July	26 heads >9 cm
Iron Man	22 nd September	13 heads 9 – 13 cm
Steel	30 th September	26 heads 10 – 18 cm
Steel	23 rd October	52 heads 8-15 cm

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 the heads were 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 at more frequent intervals during the shelf-life period.

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).

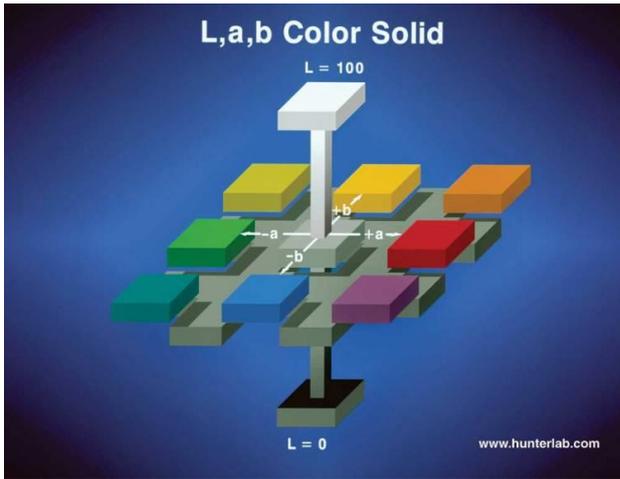


Figure 3. the $L^* a^* b^*$, colour space and Minolta colour meter used to measure machine colour values.

Chlorophyll Fluorescence measurement protocol

CF characteristics were measured using the Hansatech Handy Pea, using a double pulse protocol in four positions in a line across the head (outer, inner, inner, outer whorl).

Maturity assessment

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

- Stem turgor (Turgid – flaccid) 0, 0.5, 1
- Head colour (Green-purple, green, yellowing) 1, 2, 3, 4, 5, 6, 7, 8,9,10
- Bud compactness (compact – loose) 0, 0.5, 1
- Bud elongation (Flat head with no elongation – increasing unevenness as buds elongate) 0, 0.5, 1, 1.5, 2
- Floret loosening (tight – loose) 0, 1, 2

On the basis that the main changes observed were colour and floret loosening, an overall maturity index (MI) was developed as $MI = \text{Head colour score} + \text{floret loosening score}$.

Results

Season 1 field trial. To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.

Broccoli heads with a range of maturity were harvested from five field trials (three with variety Steel, and two with variety Iron Man) between July and October 2014. The quality of the heads, followed over two weeks low temperature storage, followed by shelf-life determination at 18°C are illustrated in Figures 4-8

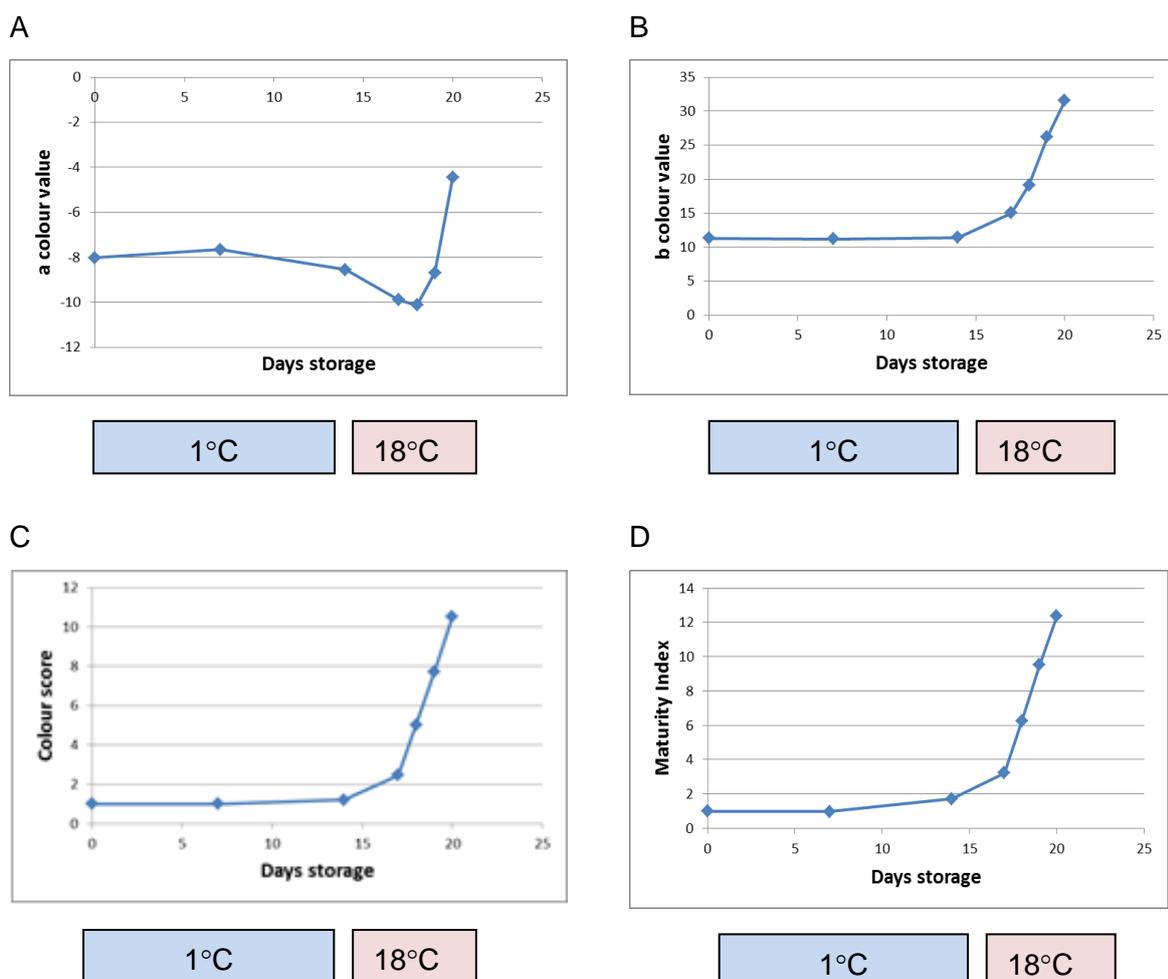


Figure 4. Characteristics of broccoli heads (variety Steel) harvested in July 2014, and stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A). L*a*b* colour score a, B). L*a*b* colour score b, C) visual colour score, D). Maturity index (colour score + floret loosening score). Each data point is the mean of measurements on 45 heads.

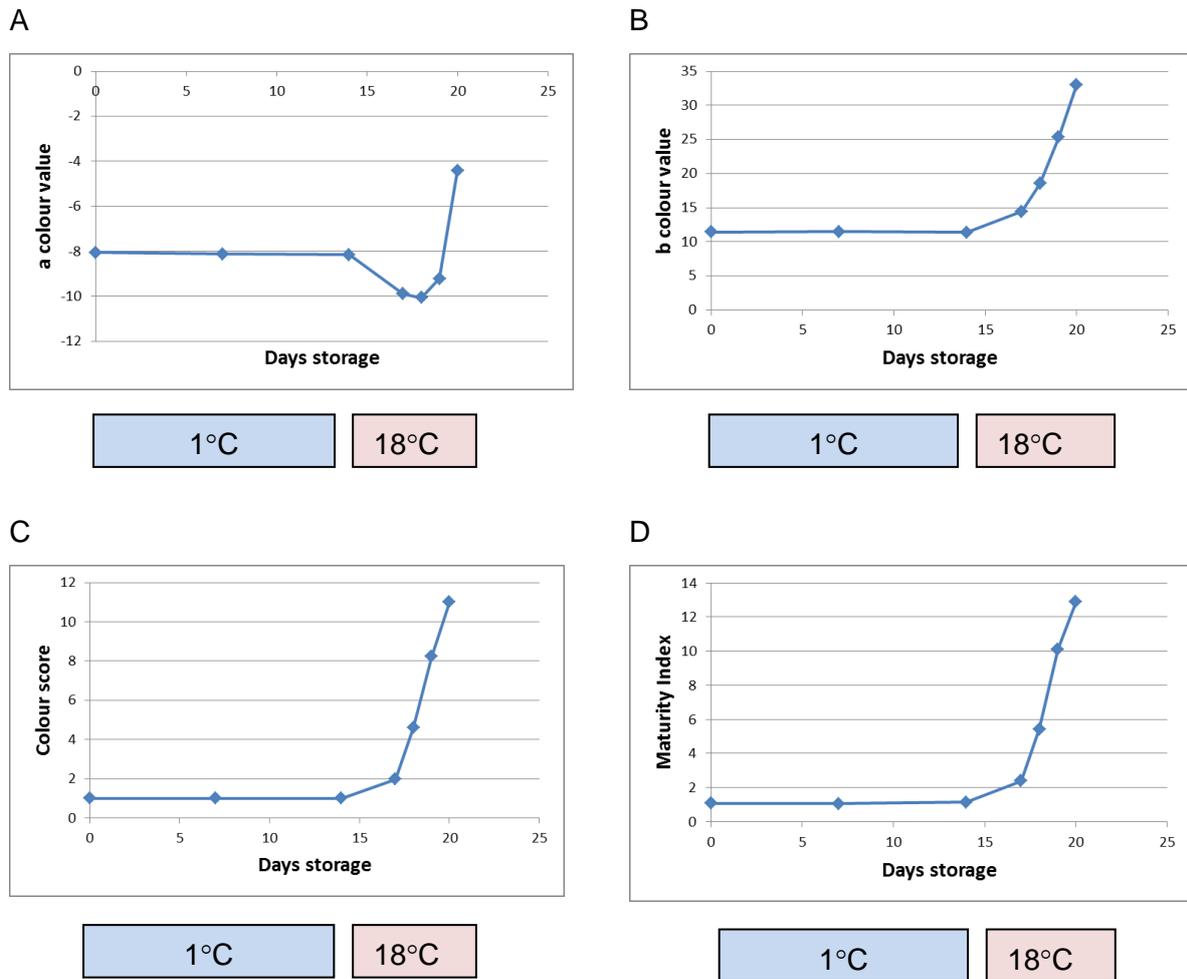
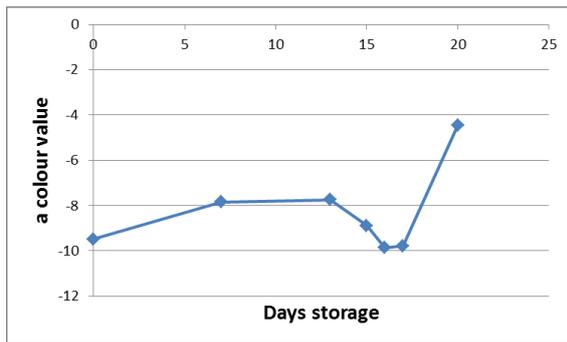


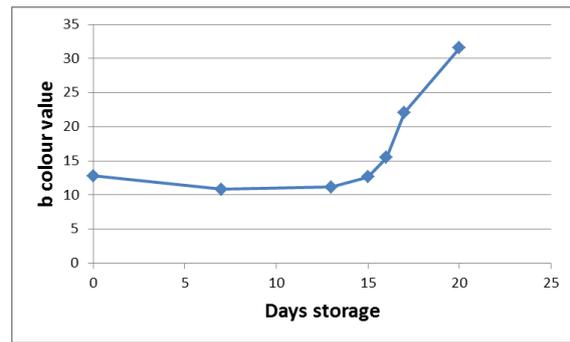
Figure 5. Characteristics of broccoli heads (variety Iron Man) harvested in July 2014, and stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A). A). L*a*b* colour score a, B). L*a*b* colour score b, C) visual colour score, D). Maturity index (colour score + floret loosening score). Each data point is the mean of measurements on 49 heads.

As observed in previous trials the a value of the Lab score changes in a complex way during head maturation; the value decreases as purple colouration is lost and the greenness increases, and then the value increases as greenness is lost during senescence. The behaviour of the b value is more straightforward, increasing as senescence leads to an increase in yellowness. The visual colour score was considered a good measure of maturation, especially when refined with a score for bud loosening to form the Maturity index (MI). Comparing heads from different trials, the rate of maturation (deterioration) varied with trial and was notably slower for Steel harvested in October.

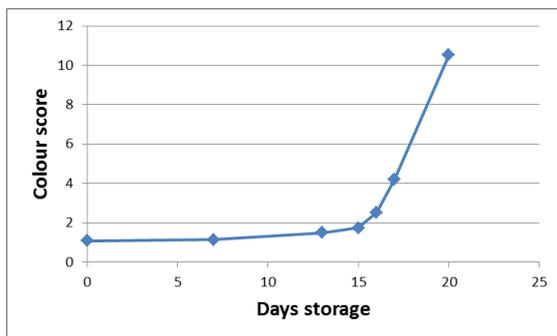
A



B



C



D

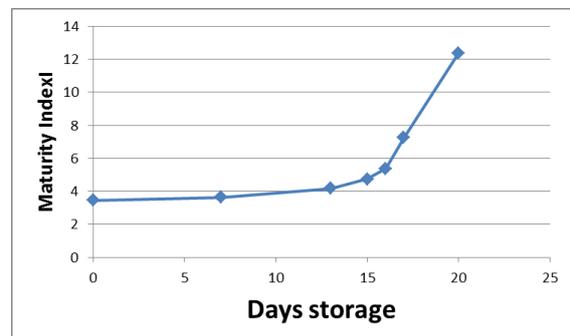
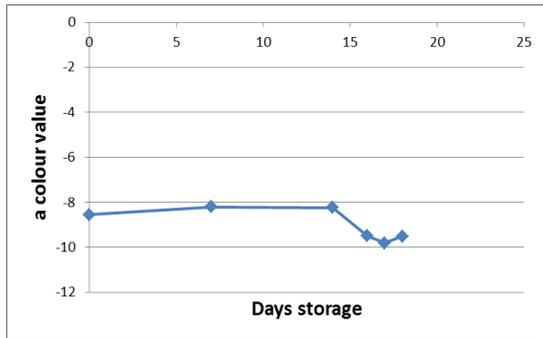
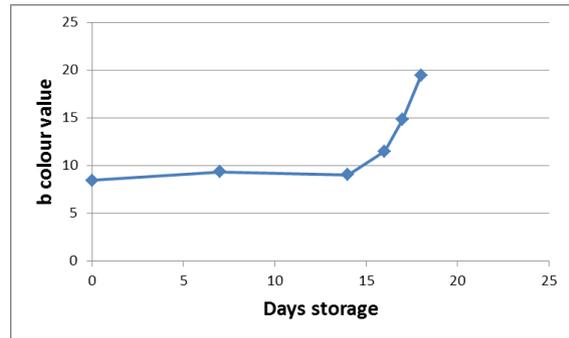


Figure 6. Characteristics of broccoli heads (variety Steel) harvested in Sept 2014, and stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A). L*a*b* colour score a, B). L*a*b* colour score b, C) visual colour score, D). Maturity index (colour score + floret loosening score). Each data point is the mean of measurements on 26 heads.

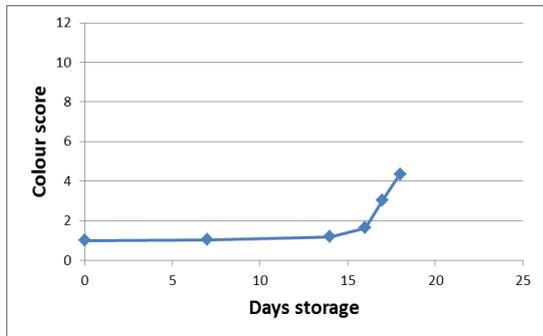
A



B



C



D

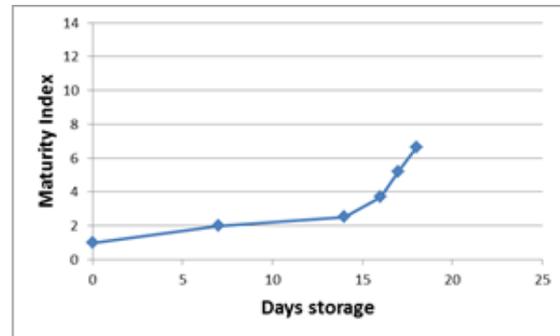


Figure 7. Characteristics of broccoli heads (variety Iron Man) harvested in September 2014, and stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A). L*a*b* colour score a, B). L*a*b* colour score b, C) visual colour score, D). Maturity index (colour score + floret loosening score). Each data point is the mean of measurements on 13 heads.

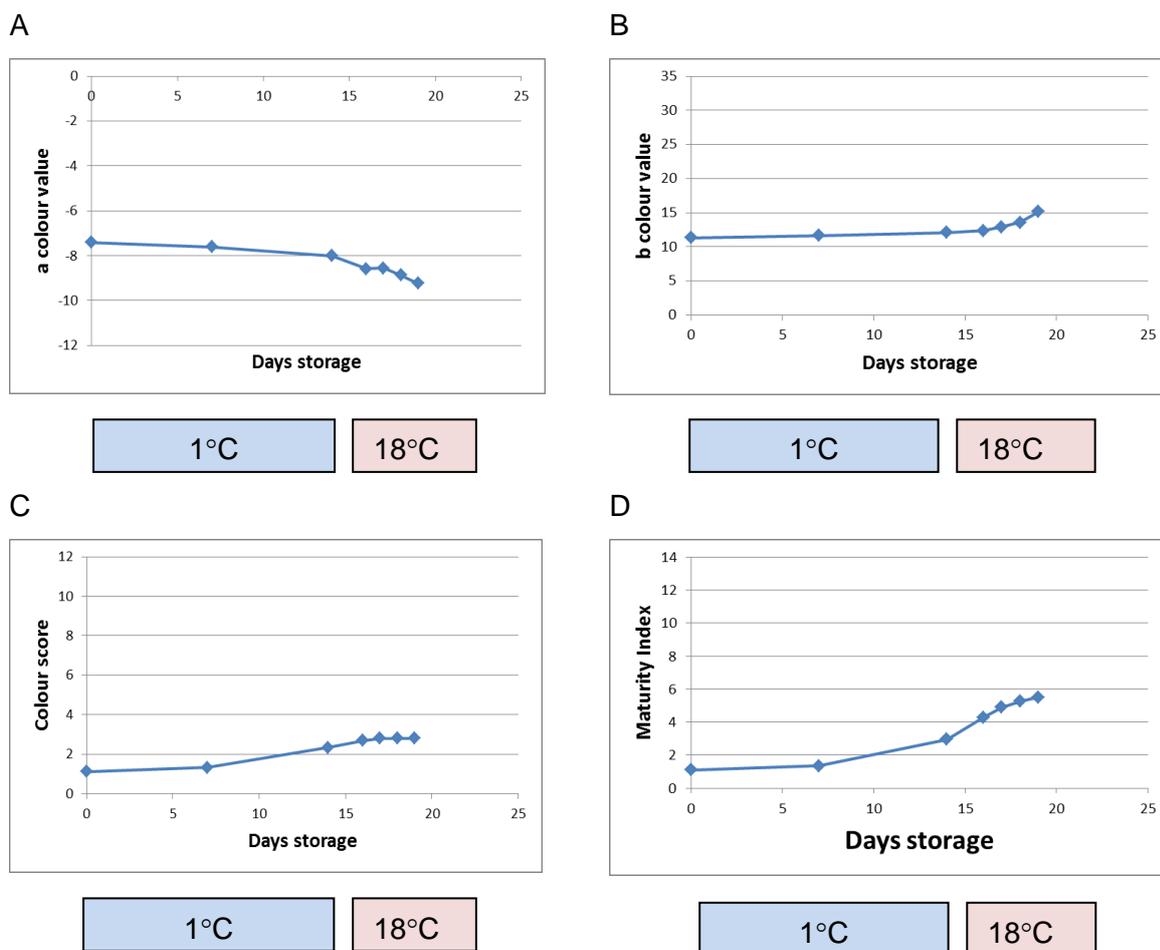


Figure 8. Characteristics of broccoli heads (variety Steel) harvested in October 2014, and stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A). L*a*b* colour score a, B). L*a*b* colour score b, C) visual colour score, D). Maturity index (colour score + floret loosening score). Each data point is the mean of measurements on 52 heads.

Figures 4-8 show data averaged over all the heads for each trial. In practice the heads harvested had a variable response to storage. Figure 9 shows the data for Steel in July, with the MI for individual heads. It is notable that at harvest the heads were indistinguishable in terms of MI, but as storage progressed they exhibited a range of keeping qualities, indicated by an increasingly wide range in MI.

In order to achieve the objectives of the overall project, the specific objective of these trials was to identify a measurement at harvest that could predict the subsequent keeping qualities of broccoli heads. For practical reasons we chose to relate characteristics measured at harvest to the MI after four days of shelf-life (MI-SL4) (indicated by an arrow in Figure 9).

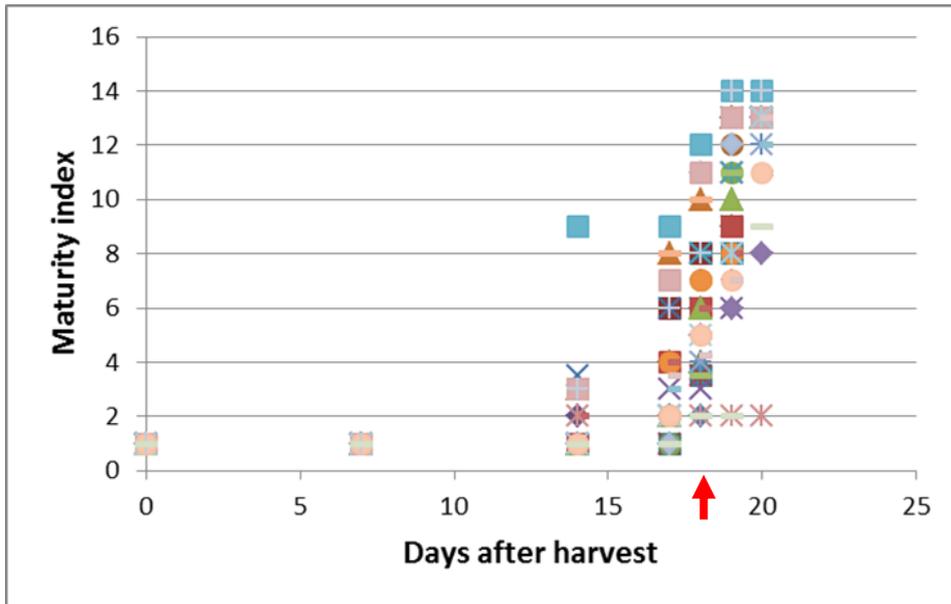


Figure 9.

MI of individual var. Steel broccoli heads following harvest in July 2014, and storage for two weeks at 1°C followed by shelf-life determination at 18°C. Each data point refers to a single broccoli head. On days 0 and 7 all heads had the same MI. The arrow indicates 4 days under shelf-life conditions.

A wide range of characteristics measured at harvest were tested for their correlation with MI-SL4, including head diameter, L*a*b* colour values and all CF characteristics measured (direct CF yields, and calculated physiological characteristics). A selection of these are shown in Table 1 for the individual trials. As expected, head diameter correlates positively with MI-SL4, consistent with the well-known observation that larger more mature heads have a shorter storage life. This is well known by growers, and may be used to some extent to control stocks, but of course any management strategy must take into account that small heads have a lower weight and therefore price. Increasing colour value b indicates increasing yellowness of heads. There is a negative correlation with b value and MI-SL4, indicating that high b value (indicating high yellowness) relates to low MI-SL4 and therefore longer shelf-life, which is unexpected. The most consistent correlation was a CF characteristic which we have termed $F\beta^1$, which has a negative relationship with MI-SL4, i.e. high $F\beta$ indicates low MI-SL4 and therefore longer shelf life. $F\beta$ is an indication of active (functional) chlorophylls, so that high $F\beta$ would be expected to indicate lower maturity.

¹ The details of the calculation of $F\beta$ are not given in this report in order to preserve commercial confidentiality.

Table 1. Correlation coefficients (r) for individual field trials between head characteristics measured at harvest and the MI after four days of shelf-life (MI-SL4). For the Iron Man trial harvested in September CF measurements were not collected immediately after harvest. *, **, *** significant to 5, 1, 0.1%

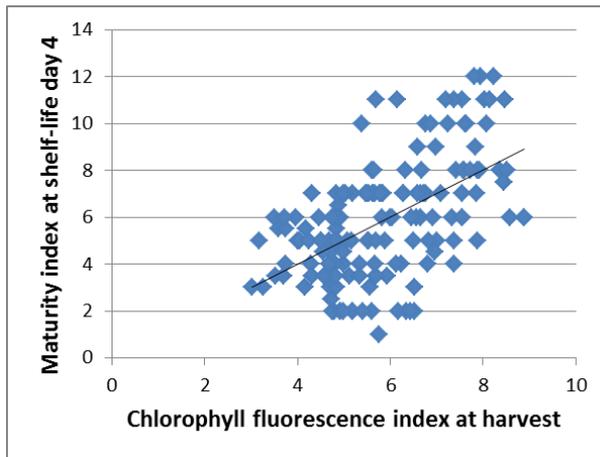
Trial date/variety	d.f.	Head diameter	(L*a*b* colour b value	Fβ	RC/CS
July/Steel	34	0.252	-0.283	-0.368*	-0.417*
Sept/Steel	24	0.413*	-0.570**	-0.391*	-0.425*
Oct/Steel	47	0.682***	-0.037	-0.436**	-0.156
July/Iron Man	47	0.717***	-0.307*	-0.780***	-0.704***
Sept/Iron Man	48	0.456**	-0.611***	--	--

The correlation coefficients were then calculated between harvest characteristics and MI-SL4 for all heads across all trials. (Iron Man in September was omitted as the CF characteristics were not measured at harvest). For colour b value the correlation was not significant ($r = 0.03$), but for Fβ value it was very significant $r = -0.54$

An algorithm to predict MI-SL4 from Fβ at harvest was calculated as $MI-SL4 = -0.0121F\beta + 17.131$. This can be improved by including head size, in which case the algorithm becomes $MI-SL4 = 0.286 * diameter - 0.00605F\beta + 8.301$

Figure 10 shows these algorithms fitted to each individual head in four trials and the average for each consignment. There was no indication from the data that the two varieties should have different algorithms.

a)



b)

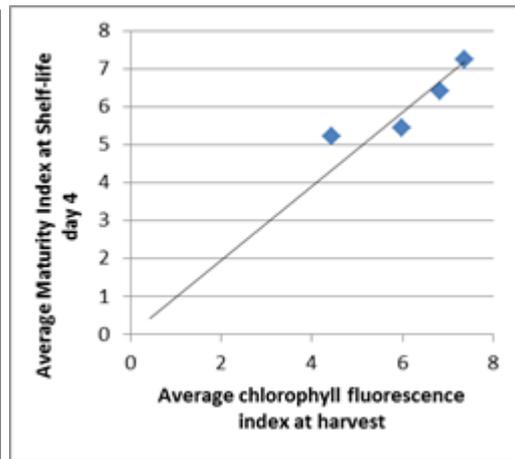
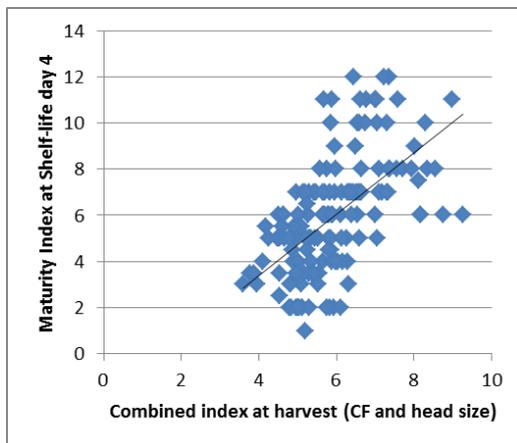


Figure 10.

Actual v predicted Maturity Index after 4 days shelf-life using the algorithm developed using $F\beta$. a) Each point relates to a single broccoli head. $MI-SL4 = -0.0121F\beta + 17.131$ $R = 0.54$
 b) Each point is an average over the whole broccoli harvest.

a)



b)

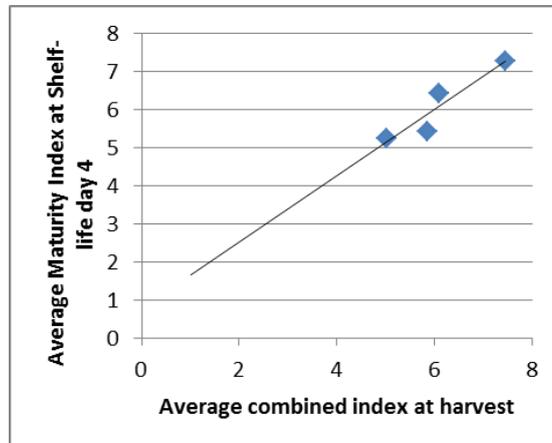


Figure 11. . Actual v predicted Maturity Index after 4 days shelf-life using the algorithm developed using head diameter and $F\beta$. a) Each point relates to a single broccoli head. $MI-SL4 = 0.286 \cdot \text{diameter} - 0.00605F\beta + 8.301$ $R = 0.59$ b) Each point is an average over the whole broccoli harvest.

Practical issues relating to the measurement of chlorophyll fluorescence characteristics in the field and packhouse

The results presented above indicate that the CF characteristic $F\beta$ may provide a tool to predict shelf-life of harvested broccoli heads. For CF to provide a feasible method for assessing broccoli heads in the field there are some practical issues that need to be addressed;

- The calculation of CF characteristics using non-modulated chlorophyll fluorimeters, as used in this study assumes that plant material is dark adapted, but dark adaptation (15 – 30 minutes) would make the measurement impractical
- External light can cause errors in measurements made by non-modulated fluorimeters
- It is important to design a measuring head taking into account the characteristics of a broccoli head, for example understanding changes in CF characteristics across a head.

Strategies to avoid the need for dark adaptation

In this study, instead of dark adapting the broccoli heads, a double pulse strategy was used. This strategy has been developed in earlier AHDB Horticulture projects for pome fruit (TF142) and for broccoli (FV395). Given that strong correlations were found, and robust algorithms were developed using this approach, this indicates that, in this case, dark adaptation is not necessary. In the next phase of the project it will be useful to find out whether a single pulse strategy, which allows a more rapid measurement, is feasible.

Errors due to external light

The measurements presented so far in this report were taken in a laboratory. External light in the field, especially on a clear sunny day, is higher intensity, so that it is harder to shield the measuring head. It is possible that where field measurements are necessary, that they will need to be taken at dawn to avoid bright sunlight.

Measurements with and without dark adaptation were tested in the field in July 2014. On a sunny day very low F_v/F_m was recorded and there was frequent detector overload. The main cause appeared to be external light entering the detector rather than light stress to the broccoli head. This is illustrated by Figure 12 which shows the F_v and F_o values measured over time in relation to sunshine levels. While F_v remained relatively constant, it was observed that high F_o corresponded to sunny periods. It is perhaps fortuitous that $F\beta$ which is most closely related to F_v has been identified as the most useful CF characteristic to be

measured. Tests indicated that even in the presence of strong background sunshine individual broccoli heads gave consistent Fv readings, regardless of sun levels and even though Fo was very variable.

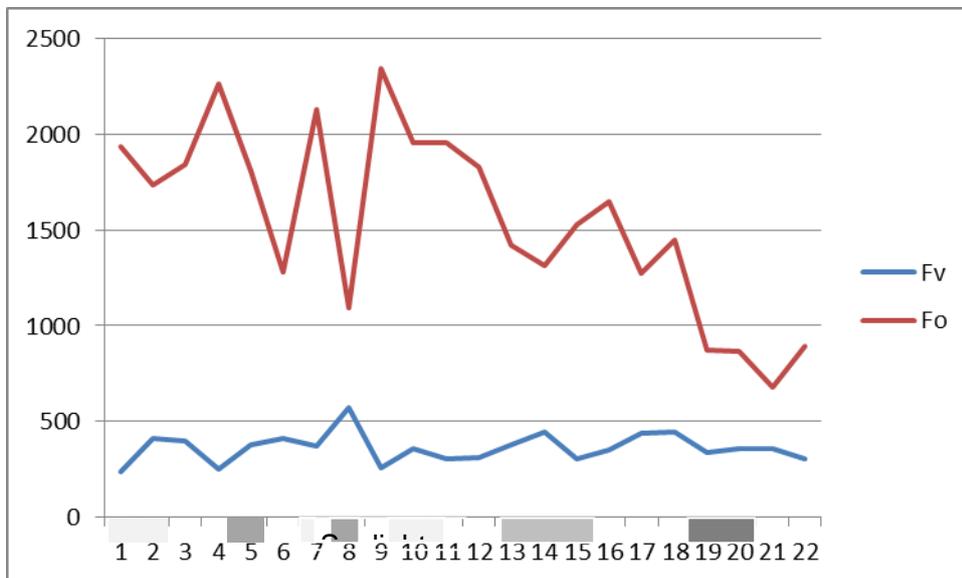


Figure 12. Fo and Fv measured from broccoli heads in the field during early afternoon in July 2014. The shading along the x axis gives an indication of sunshine levels during the measurements. This was estimated by observation. No direct measurement of light intensity were made.

Optimising head design

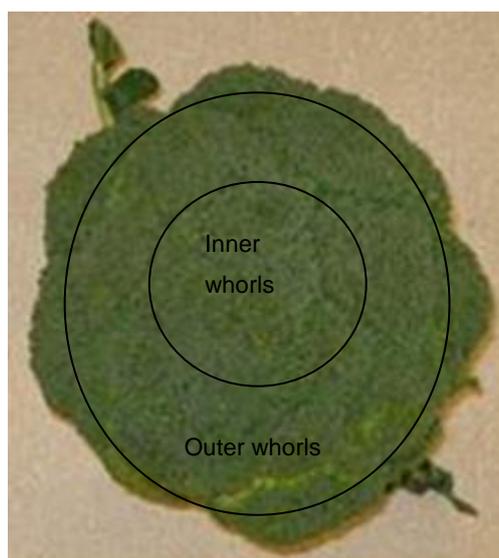


Figure 13. measurements of CF characteristics were taken from both inner and outer whorls of each broccoli head

For all the trials presented in this report either eight or six CF measurements were made from each head, with half from the inner whorls and half from the outer whorls. The values of CF for each head were calculated as the mean of the six or eight measurements. Significant differences in measurements between inner and outer whorl values were recorded in all cases tested (data not shown). Interestingly, and unexpectedly Fv was greater for the outer than inner whorls, even though the outer whorls are more mature. For a robust head measurement it is suggested that a measuring head should cover both inner and outer whorls.

Discussion

During Phase 1 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. The observation that there is a correlation between F β measured at harvest and quality after 4 days of shelf-life suggests that measurement of F β may prove to be a practical method. The evidence is not yet strong enough to suggest that F β could be used to grade individual heads, but to predict the behaviour of consignments. In addition there may be potential to use this measurement to adapt field management for broccoli heads with optimum keeping qualities.

Conclusions

F β has been identified as a CF characteristic that relates to the maturity of broccoli heads and can be used to predict storability.

Priorities for the next phase of this project will be

- Design a specialised sensor head, increase accuracy and speed up measurement
- Test the ability of CF in a commercial situation to distinguish storability of consignments at harvest and during storage in order to improve crop scheduling. This will include refining the measurement practices, such as stage of measurement (before/after harvest), time of day, number of heads.
- Test the use of CF measurements during head development as a means to optimise growing practices for improved quality/storability.

Knowledge and Technology Transfer

“The potential of chlorophyll fluorescence to assess maturity and storage life of broccoli heads”. Debbie Rees, Richard Colgan, Karen Thurston, Lisa Wray-French and Emma Skipper. Talk presented at the Brassica and Leafy Salads Conference 28th January 2015

The project was featured in the Vegetable Farmer, “Freshness Sells”, Professor Geoff Dixon

Glossary

CF Chlorophyll fluorescence

Fv Variable component of the chlorophyll fluorescence transient rise

F β chlorophyll fluorescence characteristic identified as most useful for predicting broccoli shelf-life

MI Maturity index, calculated as = head colour score + floret loosening score.

SL Shelf-life

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