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Using induced chlorophyll production to monitor the physiological state of stored potatoes (*Solanum tuberosum* L.)

Jessica M.R. Garnett[a,b], Nikolaus Wellner[a], Andrew G. Mayes[b], Gerard Downey[c], E. Kate Kemsley[a,*]

[a] Quadram Institute Bioscience, Norwich Research Park, Colney, Norwich, NR4 7UA, UK
[b] School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK
[c] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

ABSTRACT

A Visible/Near-infrared (Vis/NIR) spectrometer equipped with a fibre-optic probe was used to stimulate and measure chlorophyll production in potato tubers, at low levels that produce no visible greening in the skin. Subtle responses to changes in the light stimulus were also tracked. When used with a static experimental setup, these measurements are precise. However, the technique is very sensitive to the exact geometry of the tuber-probe arrangement, and careful positioning of the probe is crucial. Complementary studies established that tissue under the apical buds ('eyes') has greater capacity to produce chlorophyll than other locations on the tuber surface. A long-term study of multiple tubers suggested that different cultivars behave differently in terms of the rate of chlorophyll production. These behavioural differences may be related to the batch dormancy status; validating this potential relationship is the focus of ongoing work.

1. Introduction

In many potato- (*Solanum tuberosum* L.) growing countries, a substantial proportion of the crop is stored under controlled conditions to provide a year-round supply. The stored tubers need regular inspection to monitor quality. Automated optical and/or spectroscopic methods, such as visible and near-infrared (Vis/NIR), are highly desirable for making post-harvest measurements in industrial environments. Visible and hyperspectral imaging can be used to detect blemishes and diseases such as scab, rot or blight, which manifest as skin defects and/or colour changes. It is also straightforward to monitor 'greening', the production of chlorophyll in the tubers, which is of particular concern with regard to food safety because increased levels of both chlorophyll and toxic glycoalkaloids are stimulated by light (Grunenfelder et al., 2006b).

Recent hyperspectral imaging has also clearly shown that sprouts have a different optical reflection to tubers (Wenhao et al., 2014). However, there are no externally visible effects in advance of sprouting. Therefore, visual inspection or imaging systems which are used for quality control can only flag up spoilage due to sprouting after it has occurred. The ability to predict the onset of dormancy break would be a major step forward in preventing or reducing losses.

There has been much progress in understanding developmental

changes and their regulation during the growth of potato tubers. Many compositional changes also occur post-harvest and during storage. These include changes in fatty acid and sugar concentrations, membrane permeability, electrolyte leakage and hormone levels (Knowles and Knowles, 1989; Spychalla and Desborough, 1990). A review by Suttle (Suttle, 2004) showed that the synthesis and action of hormones are important in the regulation of dormancy within a tuber. Several studies have looked specifically at the changes in hormone levels within tubers during sprouting (Aksenova et al., 2013; Friedman and McDonald, 1997; Sorce et al., 2000, 1996). Measuring these processes directly requires often lengthy laboratory analysis.

Visual inspection only registers light reflected off the tuber surface. Near infrared light does penetrate beneath the skin and can be used to monitor changes in the tissue, but the excitation of vibrational overtones is much weaker than electronic transitions, and the NIR spectra are dominated by the most abundant components (water, starch, sugars). Whilst there have been many reported applications of NIR for assessing potato quality during storage and processing, these have been focussed predominantly on compositional changes of the major components (i.e. water loss, and changes in starch and soluble sugars) and the concomitant processing properties (Lopez et al., 2013).

Minor metabolites and the developmental changes associated with

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* Corresponding author.
  E-mail address: kate.kemsley@quadram.ac.uk (E.K. Kemsley).

these are much harder to assay. One NIR study sought to predict the sprouting capacity of seed potato tubers to determine optimum planting date (Jeong et al., 2008). However, to date there have been no reports of this technique being used to assess the (undesired) sprouting potential of food potatoes during commercial storage.

Previous studies on dormant tubers have shown that hormonal development begins in the apical buds ('eyes') (Aksenova et al., 2013; Sorce et al., 2000, 1996). We hypothesise that Vis/NIR reflectance measurements made on the eyes of potato tubers may be able to detect early-stage tissue changes before any sprouts emerge. These changes may be compositional, i.e. the breakdown of starch granules, or there could be functional changes in the tissue metabolism, such as its potential for reacting to light stimuli. Potato tubers stored in the dark generally have negligible levels of chlorophyll (Dao and Friedman, 1994). However, once they are exposed to light, stimulation of chlorophyll synthesis occurs (Jadhav and Salunkhe, 1974). The chlorophyll is most concentrated in the outermost few millimetres of the tuber; eventually this causes surface greening (Gull and Isenberg, 1960). There have been indications that the photosynthetic apparatus can be influenced by storage (Bianchi et al., 2014). It is reasonable to surmise that it may also react to tissue changes associated with dormancy status.

In this paper, we describe a series of experiments investigating the use of Vis/NIR spectroscopy to monitor potatoes involving the stimulation and concurrent measurement of chlorophyll in and around tuber eyes. We discuss a variety of practical considerations relating to the sensitivity, specificity and reproducibility of the analysis, and present first evidence for a link between the dormancy status of tubers and the rate of stimulated chlorophyll production. The eventual aim is to provide a tool for better crop management by predicting the onset of dormancy break.

2. Material and methods

2.1. Tuber cultivars, harvests and suppliers

Potato tubers of a range of different cultivars and harvest seasons were obtained from various suppliers. The cultivars covered various phenotypes: 'floury' and 'waxy'; red and white skin; and with storage properties recognized as poor, average or good. Details of the tubers used in the experiments reported in the present paper are given in Table 1. All these experiments have been repeated at various times to confirm findings. These are listed in Supplementary Table 1.

Tubers were cleaned with a brush or air gun, unless otherwise stated, to remove excess dirt before analysis. Analysis was conducted at room temperature in an air-conditioned laboratory (nominal 21 °C, 50–60% humidity) under artificial ambient lighting with fluorescent light tubes (270 lx). Stored tubers were kept in dark, ventilated units at a temperature between 4–6 °C ('cold storage') and humidity of 80–90 %. For each measurement as required, the tubers were removed from storage in an insulated polystyrene box containing an ice block and brought into the laboratory for analysis.

2.2. Spectral acquisition

Vis/NIR reflectance spectra of potato tubers' skin surface were recorded using an EPP2000-NIR-200 spectrometer equipped with a VIS-NIR silica fibre-optic reflectance probe (StellarNet, Inc., Tampa, Florida, USA). The SL1 Vis/NIR light source was a Tungsten/Halogen lamp with 200 W/m² output, colour temperature 2800 K. The total light intensity (from the spectrometer light source plus stray laboratory light) was measured as 698, 694, 683 and 666 lx (J/m²) for distances of respectively 0, 1, 2, and 3 mm from the tip of the fibre-optic probe.

To collect a spectrum, the probe and tuber were each clamped into position, with the probe oriented perpendicular to the tuber surface. The distance between the tip of the probe and the tuber skin was arranged to be approximately 3 mm. Occasionally, the intensity of the

Table 1
Summary of the experiments described in detail in the present paper, giving information on the cultivars analysed, the supplier, the year of harvest, and the relevant figure.

Experiment Description	Conditions	Cultivar	Supplier	Year	Figure
Static monitoring over two days	During the analysis period, a tuber was constantly exposed to light (from the spectrometer source) and kept at room temperature. These tubers were freshly harvested and had not been kept in storage.	Cultra	Country Crest Farm, Rathmooney, Dublin, Ireland.	2015	Fig. 1
Static monitoring with intermittent light exposure	During three analysis periods (5-6 hours each, once a day) the tuber was exposed to light. Between analysis periods, all lights were turned off. These tubers had been in cold storage for several months before analysis.	Orchestra	Produce World, Sutton Bridge, Lincolnshire, UK	2017	Fig. 2
Repositioning repeatability	The tuber was exposed to room temperature and laboratory lights for 48 hours before analysis, following several months of prior cold storage.	Royal	Sutton Bridge Crop Storage Research, Spalding, Lincolnshire, UK	2016	Fig. 3
"Line" Experiment	During the analysis period, the tubers were constantly exposed to light and kept at room temperature. The tubers had been in cold storage for several months before analysis.	King Edward	Produce World, Sutton Bridge, Lincolnshire, UK	2017	Fig. 4
Long-Term Analysis	Four samples of tubers were collected from suppliers shortly after harvest. Six tubers from each batch were wash gently with cold water and put into cold stored at QIB institute. The tubers were only removed from cold dark storage for the period of analysis (~10 minutes on each occasion)	Mozart, King Edward, Maris Piper	Produce World, Sutton Bridge, Lincolnshire, UK. B & C Farming, Marsham, Norfolk, UK G's Fresh, Barway, Cambridgeshire, UK	2014	Fig. 5

reflectance signal required this to be adjusted slightly to optimise the detected signal intensity (to enhance a weak signal, or to avoid saturating the detector). Depending on the experiment, typically between 50–100 scans were collected with an integration time of 20–30 ms, which were averaged to produce each spectrum. After each positioning of the probe and tuber, a 'dark' signal was recorded with the source switched off, to compensate for stray light background.

In general, one of two sampling modes was employed. Either the probe and tuber were kept clamped in place throughout, with the probe positioned to interrogate the same spot on the tuber's surface; or alternatively, tubers were newly clamped, and the probe aligned for each individual Vis/NIR recording.
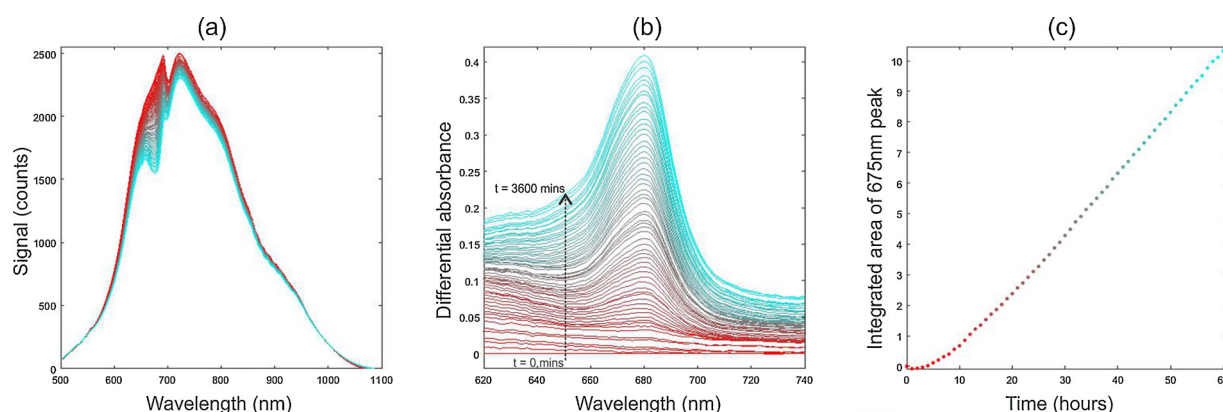
2.3. Data handling

Single time-point spectra and spectral time series were collected using the StellarNet SpectraWiz software and saved as ASCII files. All data processing and visualisation was carried out in Matlab (The MathWorks, Inc., Natick, MA, U.S.A.) with the Statistics and Machine Learning Toolbox installed. A combination of Matlab functions and in-house written scripts were used to carry out a range of pre-processing and statistical methods.

All single-beam spectra were baseline-corrected to remove the linear detector noise offset, and standard normal variate (SNV) normalised (Barnes et al., 1989). Kinetic spectra were calculated by referencing to the first spectrum in each time-series. This allowed changes at each location to be monitored and compared across tubers, by factoring out initial cultivar- and condition-dependent differences in the skin.

3. Results

3.1. Static monitoring of an eye with continuous light exposure

To conduct a kinetic analysis which reproduces classic light exposure measurements (Anstis and Northcote, 1973), Vis/NIR spectra were recorded at regular time intervals over an extended period while the tuber was exposed continuously to light from the spectrometer source as well as from the standard laboratory conditions. During this experiment, probe and tuber were clamped in place throughout (hence 'static' monitoring), with the probe positioned to interrogate a single eye on the tuber surface. This experiment has been repeated multiple times with various tubers from different harvests (see Supplementary Table 1). The trends observed, discussed below, have held true for all the confirmatory experiments conducted, irrespective of tuber species, skin colour, and whether the tuber had been chemically treated.
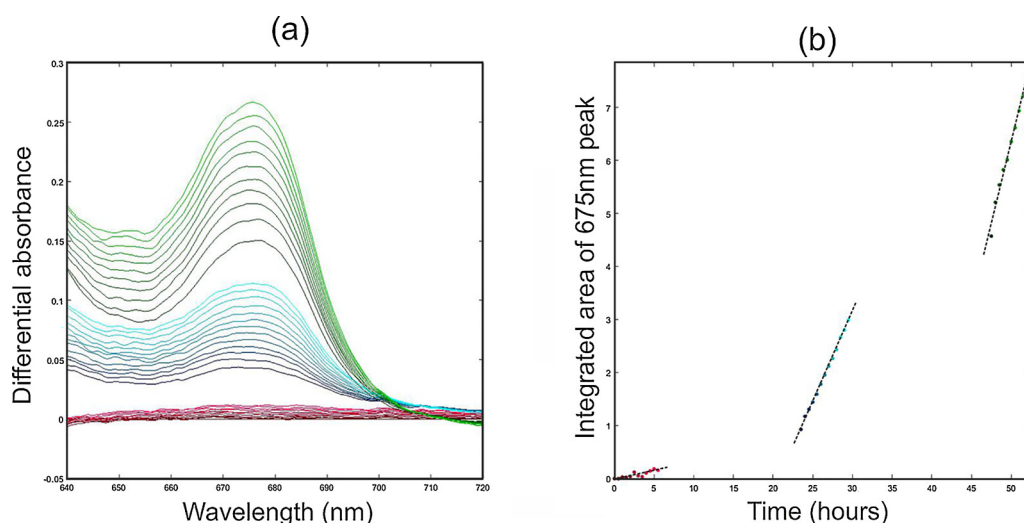
Fig. 1(a) shows the results from a cv Cultra tuber measured hourly for 66 h. The key changes in the spectra are seen between 660 nm and 690 nm. These can be explained by the production of predominantly chlorophyll *a* in the potato tuber during the analysis, which exhibits absorbance maxima at ∼475 and ∼675 nm (Petermann and Morris, 1985; Friedman and McDonald, 1997). The 675 nm band of chlorophyll *a* falls within the region of peak sensitivity for the combination of optical components (light source, monochromator, fibres, detector) used in our work. In contrast, the band at 475 nm is beyond the usable range. This is not a disadvantage for measuring chlorophyll, however, since the 475 nm band overlaps considerably with confounding absorptions from skin pigments, whereas the longer wavelength band does not. Note that whilst we have used a spectrometer with visible and near-infrared capability, this measurement could be conducted using an alternative system, for example UV/Vis, provided its operating wavelength covers the region of interest.

Fig. 1(b) shows the kinetic spectra in the wavelength range 620–740 nm, highlighting the main region of interest around the 675 nm chlorophyll *a* band. After an initial lag phase of about 8–10 hours, the rate of change in the signal gradually increases until it reaches a steady state, which continues from about 24 h until the end of the 3 day measurement. This is visualised clearly in panel (c), which shows the integrated area of the 675 nm peak (calculated with respect to a local baseline from 655 to 700 nm) as a function of time.

3.2. Static monitoring of an eye with intermittent light exposure

A static probe arrangement was also used to investigate the responsiveness of a tuber in terms of its chlorophyll production when it is exposed to cycles of light and dark. A tuber was clamped into position with the probe aligned with one of the tuber's eyes. Kinetic spectra were recorded (one every 30 min) during periods of 5–6 hours illumination, each day for 3 consecutive days. In between these periods, all light sources in the laboratory were turned off.

Fig. 2(a) shows the chlorophyll band in the kinetic spectra collected from a cv Orchestra tuber (see Table 1). Fig. 2(b) shows the areas of the 675 band as a function of time. The results demonstrate clearly two key points. First, the high sensitivity of the chlorophyll response to the presence/absence of light: after the initial lag phase, chlorophyll is produced within the tuber whilst it is exposed to light, and as soon as the stimulus is removed, production ceases. In a related experiment (data not shown), the laboratory lights were turned off periodically whereas the spectrometer source was kept on continuously; the chlorophyll band was found to increase at a nearly constant rate. This confirmed that the spectrometer light source is the main stimulus even when experiments are conducted under ambient lighting conditions,

Fig. 1. Static monitoring experiment: kinetic spectra recorded hourly for 66 h from a single eye; the tuber was exposed to light (spectrometer source and ambient) and at room temperature throughout. Panel (a) shows the single beam spectra from each time point. Panel (b) shows the kinetic spectra obtained by ratioing each subsequent spectrum to the first, focusing on the region around the 675 nm peak. Panel (c) shows the summed area of the 675 nm peak as a function of time. In all panels, data for the initial time point is plotted in red, with a gradual colour change until the last time point, which is plotted in blue.

Fig. 2. Spectral data of a tuber's eye, recorded every 30 min for 5–6 hours over 3 consecutive days. The tuber was only exposed to light during the analysis periods. Panel (a) shows the kinetic spectra and (b) the summed areas of the 675 band against time. The colour gradient of dark to light represents the first to last spectra recorded in a single day, whilst the red, blue and green represent days 1, 2 and 3 respectively.

consistent with the measured ratio of 3:1 for the spectrometer:laboratory light source intensities. Second, the rate of chlorophyll production is not constant. This is seen most evidently in panel (b): the gradient of the results within each analysis period increases each day.

3.3. Repositioning repeatability, assessed by measurements from two eyes on the same tuber

The static monitoring experiments clearly show that Vis/NIR spectroscopy has the required sensitivity to detect small changes in the chlorophyll content of tuber skin. The spectra demonstrate excellent signal-to-noise ratios. The measurements can track changes in real-time, even at low levels of chlorophyll. Note that none of the tubers were visibly green at the end of these experiments.

However, a static experimental setup that examines only a single tuber is not practical for industrial applications. Measurements need to be made fast and, crucially, on multiple tubers to give a reliable batch average. This is especially important because there is large inter-tuber variability (to be discussed in 3.5). Making multiple high-throughput measurements involves repositioning the probe to analyse different eyes with different shapes on many tubers. Some variance arising from irreproducible optical alignment is inevitable, whether using a fibre-optic probe or indeed any 'point-and-collect' instrumentation.

An experiment was devised to investigate the error associated with the alignment step for different eye shapes. A tuber was taken from dark storage and exposed to laboratory lights for 48 h to stimulate the presence of chlorophyll under the skin, as tubers stored in the dark contain only negligible amounts of chlorophyll (Dao and Friedman, 1994; Edwards and Cobb, 1999; Kozukue et al., 2001). Ten Vis/NIR spectra were recorded from two of the tuber's eyes, one shallow (close to the skin surface) and one deep-set (sunken into the skin surface). The tuber was unclamped after each Vis/NIR recording, then realigned with the probe and re-clamped before the next measurement.

Fig. 3 shows the data obtained from a cv Royal tuber (see Table 1). The ten raw spectra as collected are shown in panels (a) for the deep-set eye and (d) for the shallow eye, demonstrating the variability of the overall signal intensity arising from small differences in probe positioning. Some of the overall variation in these raw spectra is removed by the SNV correction, as shown in panels (b) and (e). However, closer inspection of the 620 to 700 nm wavelength range shows that considerable variation persists, particularly for the deep-set eye (panel (c)). This is comparatively less for the shallow eye (panel (f)). Shallow eyes were, therefore, used where available for all subsequent experiments.

It is clear, then, that moving from static probe experiments (as described in 3.1 and 3.2) to studies in which the tuber and probe are aligned anew before each measurement results in considerable irreproducibility in the spectra overall as well as in the calculated band areas (see the boxplots in Fig. 3(g)). It may also suggest locally variable chlorophyll content, which is explored in the next section.

3.4. Comparison of the responses from the eye and the surrounding tissue in a tuber

An experiment was designed to investigate the differences between the chlorophyll production of an eye and the surrounding surface area. Measurements were made at five positions (labelled a–e) on a tuber's surface, with two positions to the left (a and b) and two to the right (d and e) of the eye. The central measurement position (labelled c) corresponded to the tuber eye. Thus, adjacent areas arranged in a line traversing the eye were interrogated, with no overlap. On three successive days, Vis/NIR spectra were recorded at three timepoints each day, approximately 3 h apart.

At each timepoint, the measurements from each position were performed in triplicate, with repositioning of the probe before collection of each spectrum. This is in recognition of the results from the previous study which implicated repositioning of the probe as a major source of measurement variance. Between the measurements, the tuber was left at room temperature and exposed to constant ambient laboratory lighting.

The data in Fig. 4 were obtained from a cv King Edward tuber (see Table 1). The image in the upper centre of Fig. 4 shows the experimental arrangement. The five panels (a) to (e) relate to the five positions on the tuber. In each panel, the average area of the 675 nm peak is shown for every time point. All five panels clearly show a relationship between the magnitude of the chlorophyll band and time. However, the greatest rate of change, and largest chlorophyll band at the end of the experiment, is obtained from the central eye measurement position (panel (c)).

These findings strongly imply that enhanced chlorophyll production is highly localised at the area directly underneath the eyes. Analogues of this line experiment were repeated with other cultivars and harvest seasons (see Supplementary Table 1) and comparable outcomes were obtained throughout.

3.5. Long term measurement of eye and skin chlorophyll content in cold-stored tubers

In experiments 3.1 through 3.4, we have exploited the use of temperature to accelerate biochemical processes, removing the tubers from
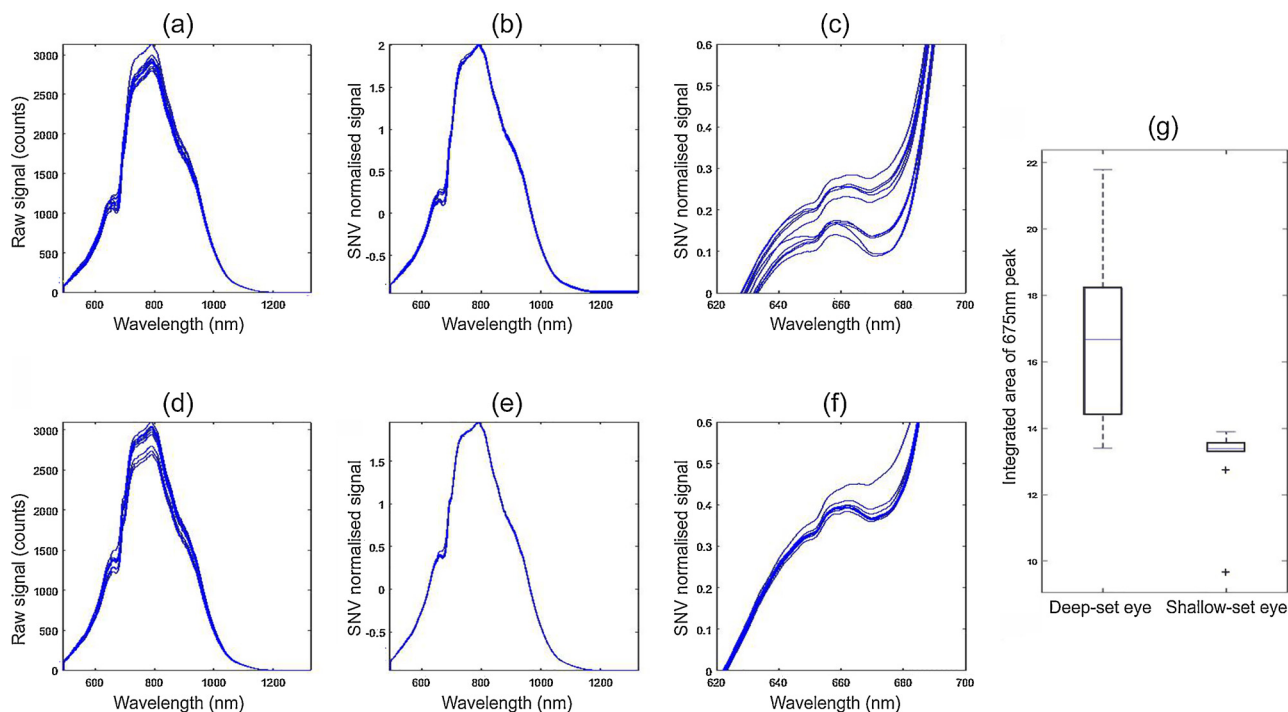
Fig. 3. Illustrating the spectral variation caused by tuber repositioning. Panels (a) to (c) show results from a deep-set eye, and (d) to (f) from a shallow eye. In each case, the panels from left to right show: 10 spectra as recorded; the same 10 spectra after SNV correction; the SNV-corrected data focussing on the wavelength range 620–700 nm. The boxplot in (g) compares the integrated area measurements of the chlorophyll band from the two eye types.

cold storage and conducting experiments in ambient laboratory conditions. This is a recognized way of speeding up measurements related to dormancy status (Vanittersum and Scholte, 1992). In commercial practice, tubers are invariably stored at temperatures below 7 °C, as this is the primary means of suppressing dormancy break. Therefore, the

aim of experiment 3.5 was to determine whether similar observations of stimulated chlorophyll production could be obtained from tubers that were kept in conditions resembling commercial cold storage over a period of several months.

Six tubers from each of cvs Mozart, King Edward and Maris Piper
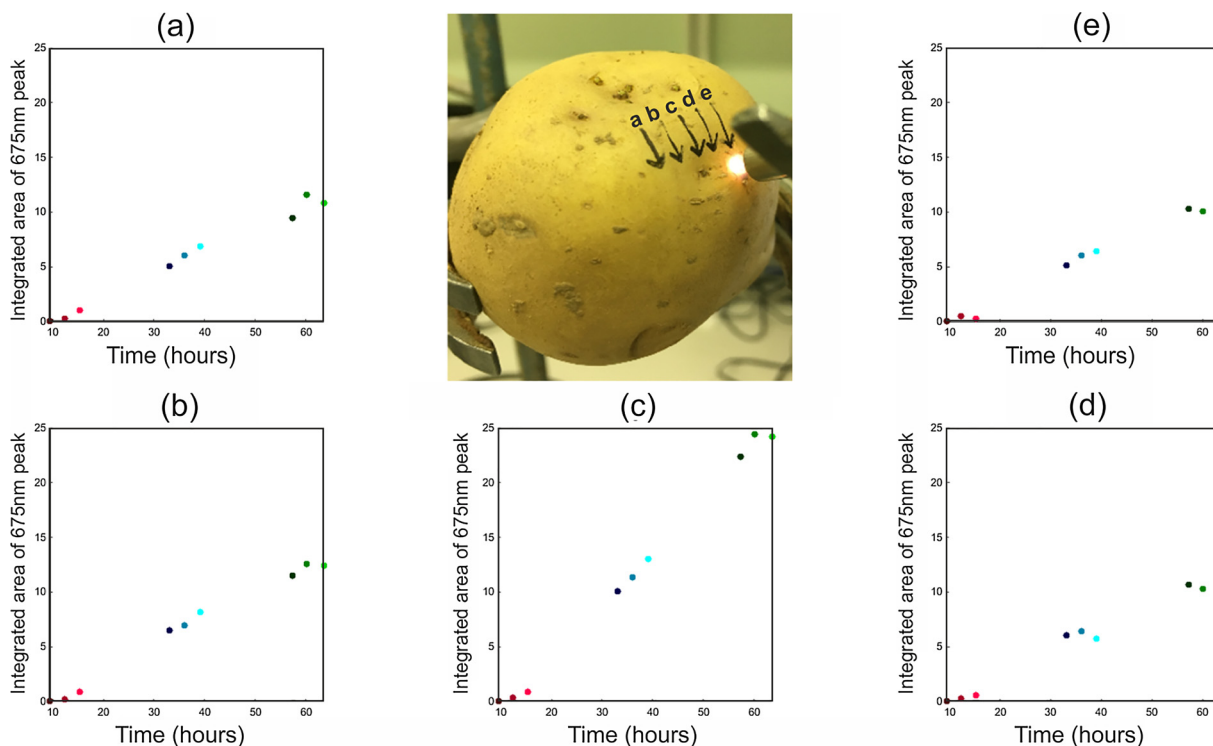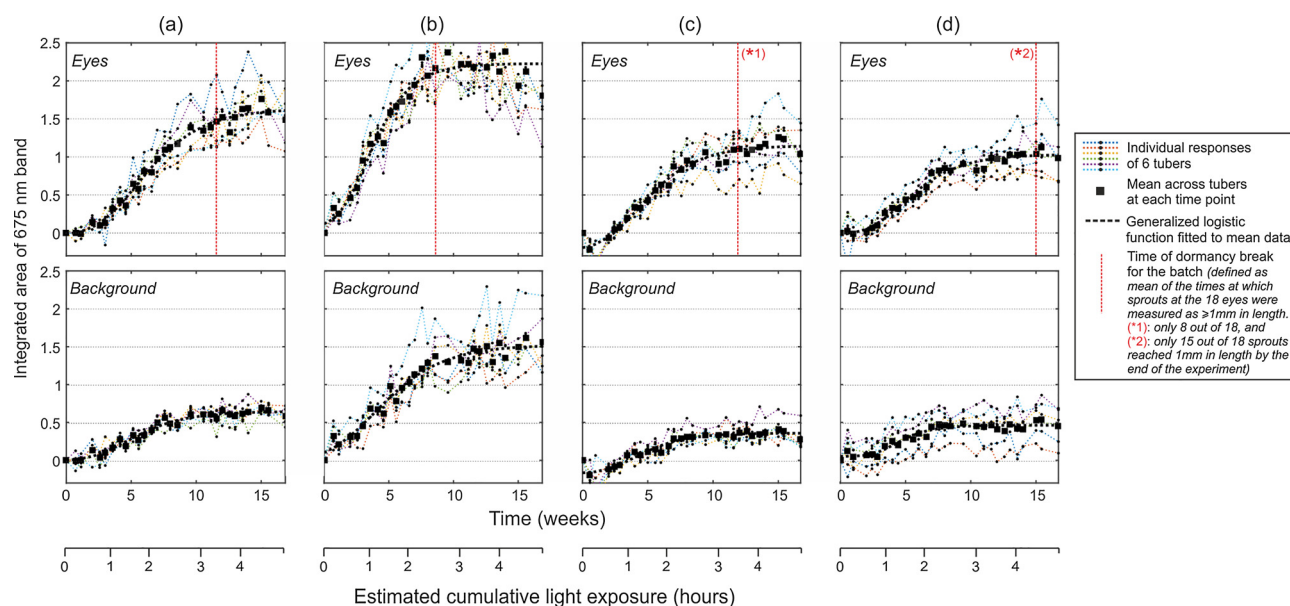

Fig. 4. "Line" experiment conducted across the eye of a cv King Edward tuber. The upper central image shows the experimental arrangement, with the different positions used to take measurements indicated. The probe is shown here taking a measurement from position e. The central position c corresponds to the tuber eye. Panels (a) through (e) show the average integrated 675 nm band from triplicate measurements, taken at three timepoints each day, for three days, as a function of time. The central eye position clearly shows the greatest chlorophyll production over the analysis period (panel (c)).

tubers were used in this experiment (see Table 1). The tubers were retrieved from cold storage and analysed twice a week for 16 weeks. Removing one tuber from the insulated box at a time, each tuber was clamped into position such that the probe was aligned with a demarcated area of the skin with no eyes (referred to as the 'background'). The spectrum was recorded, and the tuber and probe repositioned for two additional measurements of nominally the same spot. Similarly, three spectra were recorded from each of three selected shallow eyes on the tuber, before returning it to the insulated box, and subsequently to cold storage. This protocol resulted in 12 spectra being collected from each tuber at each measurement session.

This experiment can be considered as an approximate analogue of 3.2, as each tuber was again intermittently exposed to light while measurements were taken. Key differences in 3.5 are that: the periodic light exposure (as a combination of spectrometer source and ambient room lighting) amounted to approximately 10 min at each measurement session; measurement time points were separated by days rather than hours; and the tubers were returned to dark and cold storage in between measurements. Precluding the use of a static experimental arrangement, this protocol is intended to demonstrate how the method could be implemented in an industrial situation.

Fig. 5 shows the integrated areas of the 675 nm band plotted against time. In each of the pairs of panels (a)–(d), the upper panel shows the data collected from the eyes and the lower from the background. Each 'Eye' panel shows the mean (of nine eye measurements) from each of the six tubers (coloured lines), along with the batch mean (filled black squares). Each 'Background' plot shows the mean of three measurements from the individual tubers, and the batch mean. All plots are shown on the same horizontal and vertical axes to aid comparison. In addition to the elapsed storage time in weeks, the horizontal axes are also labelled with an alternative scale which shows the estimated cumulative light exposure experienced by the tubers.

Both the eye and background data strongly suggest a sigmoidal response to describe the change in the chlorophyll as a function of time. A generalized logistic function (Richards curve) was fitted to the mean data in each case, and these are indicated by the dashed black lines. The goodness-of-fit as expressed by the R^2 statistic is > 0.9 in all instances.

We note that the lag phase at the beginning of analysis has been observed previously (see for example Fig. 1(c)), whereas the asymptotic phase has not. It is possible that the latter is an indication of damage to the photosynthetic apparatus caused by long term cold storage (Bianchi et al., 2014), which did not occur in the experiments conducted at room temperature throughout.

This experiment confirms that chlorophyll is produced in greater amounts at the eyes than elsewhere on the tuber skin. This finding holds true for all the cultivars studied. Compare the upper and lower panels of Fig. 5 in each case: the absorbance of the eyes is typically 1.5–2 times that of the corresponding background.

Marked on the upper panels (red vertical dashed lines) are the times at which dormancy was broken for each batch. Using industry viewpoints as a guide, we define this as occurring at the mean of the times at which the 18 eyes had produced ≥ 1 mm sprouts. The calculated values are consistent with growers' knowledge about the various cultivars: cv King Edward, for instance, is known for having a short dormancy period (and consequently being difficult to store), whereas Mozart and Maris Piper are considered to have medium dormancy periods. Note that the calculated dormancy break times for the cv Maris Piper batches are underestimates: in both cases, not all the eyes produced 1 mm length sprouts even at the end of the study.

Remarkably, differences between the chlorophyll response curves hint at a potential relationship with the batch dormancy behaviour. For instance, cv King Edward (Fig. 5(b)) shows a near-absence of a lag phase and a relatively early onset of the asymptotic phase; the rate of chlorophyll production is also highest for this cultivar. In contrast, the cv Maris Piper batches, which were the last to break dormancy, show a much lower rate of chlorophyll production. Finally, we note that even though the two Maris Piper batches were supplied by different growers, their behaviour is notably similar for both the eye and background data.

4. Discussion

The results presented in this paper show that chlorophyll can be readily detected using Vis/NIR spectroscopy. In particular, the 675 nm band of chlorophyll a provides a clear measurable signal within the

Fig. 5. Chlorophyll production in a long-term storage experiment, showing the integrated areas of the 675 nm band in tubers of the following cultivars: (a) Mozart, (b) King Edward, (c) and (d) Maris Piper (from different growers). In each case, the upper panel shows data from the eyes, and the lower panel from the background. Within each plot, mean data from 6 tubers are indicated by different coloured broken lines, as per legend. The mean value across all six tubers is indicated by the filled black squares. Richards curves fitted to the mean data are indicated by the dashed black lines. Finally, the mean time at which the batch was assessed to have broken dormancy (mean times at which sprouts from the 18 eyes were measured as ≥ 1 mm in length) is indicated by the vertical red dashed lines. For the cv Maris Piper, not all sprouts reached 1 mm in length before the end of the study; these eyes were recorded as having a sprouting time equal to the study date.

optimal operating wavelength range of a standard Vis/NIR device. The Vis/NIR technique involves illumination of the sample with a suitable light source. Thus, the measurement process inherently acts to stimulate the production of chlorophyll in potato tubers, along with any other (e.g. ambient) light sources that are present.

This is evident from the results of the static experiment 3.1, which tracks the stimulated production of chlorophyll under tuber eyes as represented by the integrated area of the 675 nm band. The response curve here is also consistent with the concept of a lag phase in the chlorophyll production and is credible from a biological perspective. Immediately after removal from dark storage, tubers contain neither chlorophyll nor proto-chlorophyll, so there is lag phase of several hours before measurable quantities of chlorophyll begin to be produced (Anstis and Northcote, 1973; Virgin, 1993). Thereafter, there is a steady production that depends on the intensity of the light stimulus and on temperature (Grunenfelder et al., 2006a). Chlorophyll production is cumulative but requires continuous exposure to light; it ceases if the tuber is returned to the dark. However, once the chloroplasts have been primed, there is no further lag phase, and a tuber returned once more into the light resumes chlorophyll production at the previous rate. Vis/NIR spectroscopy is readily able to track this behaviour (experiment 3.2; Fig. 2).

The uncertainty (noise) arising directly from the Vis/NIR measurement is very low. This is demonstrated well by the static experiments: in a fixed sampling arrangement, the increase in the band area as a function of time is exceptionally smooth (Fig. 1 (c)). However, such an arrangement is not practical for high-throughput screening, which requires analysis of many tubers. Considerable measurement uncertainty arises from the sensitivity to the precise optical geometry of the tuber-probe arrangement. This is evident from repositioning the probe even only slightly between readings. The uncertainty can be sub-divided into two primary sources. First, the variation associated directly with the amount of reflected light collected by the probe at a tuber surface of non-uniform shape (exemplified by the deep-set vs shallow eye experiment, 3.3). Second, intrinsic localised variation in the chlorophyll content on the tubers (as shown in the line experiment, 3.4). The only way to mitigate these measurement uncertainties and obtain a reliable batch average is to sample many tubers.

The results of experiments 3.4 and 3.5 clearly show that given the same conditions of stimulation, considerably more chlorophyll is produced at the eye compared with the surrounding tissue. This observation fits with the notion that the apical bud is the most biologically active tissue in the tuber (Aksenova et al., 2013), presumably producing more chloroplasts than the starch-filled storage cells (Anstis and Northcote, 1973). It is thus credible that the eyes are more sensitive to a light stimulus than the surrounding skin tissue.

Over several months storage, the stimulated chlorophyll production changed markedly over time in both tuber eyes and skin. The magnitude of the chlorophyll *a* band as a function of time could be modelled effectively by a Richards curve in all cases. It could be argued that this behaviour is due to general ageing effects, such as water loss, or possibly damage to the photosynthetic apparatus in prolonged cold storage. However, these processes should affect all tubers in the same way. Our observations indicate that different cultivars have different, potentially characteristic chlorophyll production rates, suggestive of an underlying biological variation related to their known dormancy periods. Bianchi et al (Bianchi et al., 2014) have shown that the fluorescence from the photosynthetic apparatus decreases during storage and has a minimum before sprouting. In a comparable way, our results suggest that the ability of tubers to produce chlorophyll under controlled stimulation may carry information indicative of their physiological state.

5. Conclusions

Using a fixed experimental arrangement, Vis/NIR spectroscopy with a fibre-optic probe can readily track stimulated chlorophyll production in potato tubers, at low levels that produce no visible greening. However, the Vis/NIR reflectance mode of measurement is highly sensitive to the exact geometry of the tuber-probe positioning, and best efforts should be made to make this as repeatable as possible to minimize unwanted variance. Since in practice it is impossible to achieve complete reproducibility, this will inevitably be a source of error that any practical application will need to contend with

From experiments 3.4 and 3.5, we conclude that the tissue under the eye is more biochemically active, in that it appears to have greater capacity to react to the light stimulus than elsewhere on the tuber. In terms of measurement protocols, an imperative then is the careful positioning of the probe to focus on the eye region, to maximize the response of interest. Experiment 3.5 also suggests that different cultivars behave differently with regards to the rate of chlorophyll production, and further, that these behavioural differences may be related to the batch dormancy status. Substantiating this putative relationship is the focus of ongoing studies, to be reported in a subsequent publication.

Vis/NIR is a low-cost, robust and industry-friendly technology, and this work has the potential for translation into commercial settings. The eventual aim is to provide a tool that can accurately predict the onset of dormancy break. From a crop management perspective, such advanced warning would allow corrective action to be taken. This would be a commercially valuable proposition, reducing waste due to spoilage during storage.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2018.07.014.

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