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

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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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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/m^2 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/m^2) 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