# Final Report

# Sustaining Expertise in Potato Post-harvest Physiology

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# 1. SUMMARY

## 1.1 Aim

To understand the relationship between calcium and phosphate nutrition in potato tubers and how nutritional profile of tubers at harvest can influence the storage potential and in particular their propensity to undergo senescent sweetening and low-temperature sweetening during storage. In addition, the use of real-time respiration rates of tubers was evaluated to monitor tuber health during storage as a way of providing store managers an additional tool for store monitoring.

## 1.2 Methodology

Field trials investigating the incorporation of calcium and phosphate based fertilisers into the seed bed before planting, and topical application of liquid based calcium and phosphate products during the growing season were studied to assess the capacity for raising calcium and phosphate in tubers as a way to improve their storage potential. In particular storage trials were conducted to see if raising calcium content of tubers reduced their propensity to develop senescent sweetening when stored at 10°C. While increasing the phosphate content of tubers was investigated as a means to increase tuber resistance to low-temperature stress and sweetening.

Calcium plays an important role in maintaining cell health and tissue integrity and helps to delay the onset of senescence-related processes such as softening and tissue breakdown. In other long-stored commodities such as apple, susceptibility to low-temperature stress is associated with consignments with low-phosphate content. In these trials, tubers grown under a range of phosphate fertiliser rates were stored at low-temperature (3.5˚C or 5˚C) or at 10˚C and the concentration of sugars present in the tubers was measured alongside fry colours.

Respiration rates of tubers change during storage and increase around the time of sprouting. The use of real-time respiration measurements from inside the store affords the opportunity to provide additional tools to the store manager of the physiological status of the crop. In the first instance, the use of a real-time respiration chamber 'Mini-Pod' was tested at the Produce Quality Centre (PQC) using tubers of Markies and Pentland Dell collected at monthly intervals from crop stored at SBCSR. Respiration rates were compared to dormancy, sugar content and textural properties during the storage season.

## 1.3 Key findings

Application of phosphate fertilisers (triple super phosphate) at field rates of 150-300 kg $ha^{-1}$ increased phosphate content of Markies and Pentland Dell at harvest. In the first year (2015) higher tuber phosphate was linked to lower glucose and sucrose content of Markies at harvest.

Treatment effects were lost during storage at 3.5˚C or 10˚C and no effect of increased phosphate were observed on sugar profiles in Pentland Dell or Markies during storage.

Repeat application of phosphate the following year (2016) led to raised phosphate content in Markies and Pentland Dell tubers grown under phosphate supplementation of 150-300 kg $ha^{-1}$. Raising tuber phosphate content did not alter the sugar profile of Markies or Pentland Dell at harvest, however tubers grown under 300 kg $ha^{-1}$ P were lower in sugar content during storage at 3.5˚C: Fructose, glucose and sucrose content was lower in Pentland Dell tubers early (2-5 months) in the storage season, while Markies tubers were lower in glucose and sucrose. By the final inspection at 7 months treatment differences were lost.

Application of calcium based fertilisers (Gypsum, Tropicote or InCa) to Lady Rosetta increased calcium content of tubers at harvest. Generally, calcium application to Lady Rosetta and Pentland Dell led to a reduction in the glucose content of tubers at harvest, while tubers from plants grown under InCa and Tropicote application regimes resulted in a lower sucrose content. During storage at 10˚C, no decrease in sugars was observed in tubers harvested from plots receiving calcium. Repeat trials, in the second year found calcium pre-treatments marginally increased calcium content of Pentland Dell tubers at harvest. However, pre-treatment Markies with calcium failed to alter tubers sugar profile. In general, tubers' propensity to sweeten during storage was not affected by calcium pre-treatments with the exception that Pentland Dell tubers removed from store after 5 months storage at 10˚C subject to calcium treatments (50-200 kg $ha^{-1}$) were transiently lower in sucrose. After 7 months storage, sucrose content of tubers had increased to a similar levels across treatments. No treatment differences in fry colour analysis of tubers subject to calcium pre-treatments was observed.

Tuber respiration rates of Pentland Dell stored at 3.5˚C and 10˚C were constant and similar between storage temperatures for the first 5 months of storage and before rates increased between 6-7 months of storage. For tubers stored at 10˚C, the rise in respiration occurred alongside a rise in sucrose content and was in line with an increase in fry colour. Changes in reducing sugar accumulation of in tubers stored at 10˚C were not related to changes in respiration or the pool of reducing sugars. In contrast, for tubers stored at 3.5˚C, sucrose and reducing sugar content increased (LTS) within the first month of storage alongside an increase in fry colour but this occurred 4 months before a rise in respiration was observed. Changes in reducing sugars were not well correlated with respiration rate. The rate of respiration in Markies stored at 3.5˚C or 10˚C, were similar and stable for the first 5 months of storage before $CO_2$ production increased; the relationship between fry colour, respiration rate and reducing sugar content was poor. Dormancy break occurred within the first month of storage irrespective of storage temperature; with regular CIPC application the presence of regrowth was similar across

storage temperatures. The rise in respiration in tubers stored at 3.5˚C was not correlated with changes in sucrose, reducing sugars.

## 1.4 Practical recommendations

Application of phosphate (300 kg $ha^{-1}$) increased phosphate content of Markies and Pentland Dell tubers at harvest in one year out of two in grown in a field site low in Phosphate (P index 1). High phosphate application reduced the sucrose concentration of tubers at harvest.

Real-time respiration of tubers in store using SafePod technology may have utility in identifying stores where a rise in respiration is linked to sweetening, this will need further investigation across a wider range of varieties and temperatures.

# 2. INTRODUCTION

## 2.1 Background

### 2.1.1 Calcium - role in reducing senescent sweetening

Calcium plays an important role in maintaining cellular function, retaining membrane integrity through enhancing cell to cell cohesion, facilitating sugar loading from the apoplast into cells and resisting the spread of disease. Calcium distribution in potato is not uniform, with higher concentrations at the stolon end decreasing towards the apical end. The extent to which this impacts on tuber physiology is not well documented. In fruit, low calcium increases the propensity for senescent breakdown. In potato, low calcium is associated with russet skin spot. While few soils are considered low in calcium, its movement within the plant is restricted to the xylem, making uptake into tubers hard to manage.

Granular application of calcium is known to reduce russet spot, however, there is little calcium movement from the above ground parts into the tuber. The bioavailability of calcium and the extent of its inactivation through binding to oxalates and phosphates has an important bearing on calcium activity. Moreover, calcium can be displaced from binding sites within the cell wall matrix by $K^+$ and $Mg^{2+}$ and so the balance of these minerals influences the ability to maintain tissue integrity.

Calcium's role in stimulating sprout vigour (Dixon 1983) and reattachment of the vasculature may also influence plant establishment from seed tubers. Quantifying calcium, and the ratio of calcium : potassium and calcium : magnesium at harvest may provide an indication of the tubers' propensity to develop senescent sweetening.

### 2.1.2 Phosphate - role in reducing sensitivity to low-temperatures

The propensity to develop storage disorders such to sensitivity to low-temperature sweetening (LTS) and senescent sweetening (SS) are not well characterised in potato tubers, but lessons may be learnt by comparison with top fruit. In apple, sensitivity to low temperature breakdown is linked with low phosphate content (<9 mg $100^{-1}$) of fruit at harvest. In potato, low phosphate content during tuber development is associated with low dry matter (DM) content and reduced yield. While work has focussed on improving uptake of phosphate from the rhizosphere, less is known on the impact of phosphate on storage quality or sensitivity to low temperature sweetening (LTS). Development of LTS has a number of phases; from starch breakdown (quality and quantity of starch, size of starch granules), through to downstream activities of acid invertases converting sucrose to glucose and fructose, and the ability of tissues to respire sugars. These all impact on accumulation of sugars under low-temperature conditions. Cultivars resistant to LTS tend to have high amylose content, a high ratio of amylose: amylopectin and large starch granule size. Raising phosphate availability increases DM content of tubers through

an increase in starch synthesis (70%) primarily through induction of starch branching isoenzymes (SBE I, II) yielding high quality amylopectins. In addition, phosphates are associated with phospholipids in the amyloplast membranes protecting starch granules from hydrolytic breakdown. Moreover, phosphate is incorporated into phosphate mono-ester linked to glucose residues within the starch molecules. The activities of acid invertases and associated inhibitor molecules are a main regulator of LTS activity. Quantifying the content of phosphate in tubers as a proportion of DM content at harvest and during storage alongside the ratio of amylose/amylopectin and invertase activity will characterise the impact of phosphate content at harvest on tuber sensitivity to LTS.

## 3. MATERIALS AND METHODS

### 3.1 1st year field trials: Field application of Phosphate and Calcium treatments at planting to reduce low-temperature and senescent sweetening in stored crop: 2015-2016.

In the first year (2015) a field trial was set up and managed with the kind assistance of Mr Matt Smallwood (McCains Food GB Ltd). The trial site was kindly supplied by Mr James Daw (W.B. Daw and son) in Thorpe Constantine, Staffordshire. The site was a classified with a P index of 0. Four randomised plots per treatment per variety were planted. Each plot consisted of 4 rows of 12 tubers with the outer rows used as guard rows. For the calcium/senescent sweetening trial the varieties: Lady Rosetta, Markies and Pentland Dell were used, and for the phosphate trial: Markies, Pentland Dell and Innovator were trialled. Potatoes were planted on the 24th April 2015 and were trickle irrigated. Soil analysis before planting showed the plot identified for the calcium trial had a calcium content of 980 mg/kg.

The calcium trial consisted of five products used at recommended field rates; gypsum ($CaSO_4$. $2H_20$) containing 23% calcium and 19% sulphur was applied at a field rate of 2500 kg $ha^{-1}$, Tropicote (Yara) a granular calcium nitrate compound containing 26.3% CaO, 15.5% N was applied as a base dressing of (380 kg $ha^{-1}$), Calcifert-lime (39% Ca) was applied at 300 kg $ha^{-1}$, Tropicote and Calcifert treatments supplied 100 kg $ha^{-1}$ of available calcium, and gypsum supplied 500 kg $ha^{-1}$ available calcium. InCa (Plant Impact) a liquid formulation (6% Ca w/v, 5.4% N) was applied as a foliar spray at 1.5 L $ha^{-1}$ in a volume of 200 L $ha^{-1}$ during canopy development at recommended growth stages: tuber initiation stage, initiation and bulking and a third application at first flowering; 27/6/2015, 10/07/2015 and the 22/7/2015. Untreated controls were also included. In order to readdress the excess of sulphur applied as part of the gypsum treatment ($CaSO_4.H_2O$) other treatment plots received an additional Ammonium Sulphate base dressing (275 kg $ha^{-1}$) providing 165 kg $SO_3$ $ha^{-1}$. Before treatment, soil sulphur content was 15 mg $kg^{-1}$.

Nitrogen was applied according to recommended rates for each variety: Lady Rosetta received 230 kg N $ha^{-1}$, Markies 180 kg N $ha^{-1}$, Pentland Dell 220 kg N $ha^{-1}$. All but 30 kg was applied as pre-planting base dressings, while the remaining 30 kg of Nitrogen was applied via the trickle-fertigation-system.

The phosphate trial consisted of one product; Triple Super Phosphate containing 46% phosphate that was applied at 3 rates 217.4, 434.8 and 653.2 kg $ha^{-1}$ producing 100, 200 and 300 kg $ha^{-1}$ of available phosphate. An additional treatment was 200 L $ha^{-1}$ foliar applied phosphate treatment that was applied on three occasions (11/6/2016, 19/6/2016, 27/6/2016) and untreated control plots.

After full canopy emergence, (July 2015) chlorophyll fluorescence measurements were taken using the Handy Pea handheld fluorimeter (Hansatech ltd) on fully expanded, leaf samples, to measure effects of treatments on chloroplast activity as a proxy measurement of plant health.

After desiccation of the canopy on 30th September 2015, a sample of tubers from each treatment was harvested and transported to the Produce Quality Centre. A second harvest at commercial lifting (21-22 October 2015) of 120 tubers from each plot was lifted by hand. Tubers from the commercial harvest were transported to Sutton Bridge Crop Storage Research.

### 3.2 1st year - Storage Treatments 2015-2016

For the calcium trial 100 tubers of Markies, Pentland Dell and Lady Rosetta from each plot (4 replicate plots giving 400 per treatment) were stored at 10°C. Individual treatment-plots were stored separately in trays which were distributed within the store within a randomly distributed design, which was maintained at each sampling occasion. Twenty tubers per plot were sampled immediately as a harvest sample. The remaining 80 tubers were sequentially sampled during storage.

For the phosphate trial, 120 tubers per plot were harvested into nets (480 per treatment). 20 tubers per net were sampled at harvest, the remaining 100 tubers per plot were split into two trays, one tray was stored at 10°C and the other at 5°C.

Trays were randomised within the store and plot positions were maintained at each sampling occasion. Potatoes were treated with CIPC after curing and repeat treatments applied in January 2016.Tubers were sampled in November (Harvest), January, end of February, April with a final assessment at the beginning of June.

For the calcium trial 20 tubers per tray were sampled, while for the phosphate trial 15 tubers per tray were removed on each occasion. Tubers were placed in paper bags and transported in bulk ½ tonne potato bins to the Produce Quality Centre, where tubers were temporarily placed in 10°C or 5°C stores prior to processing for analysis. Tubers were scored for dormancy break and bud/sprout movement, before tissue samples from opposite eighths of the tuber were taken and frozen at -20°C. Samples were then freeze dried for 48 hours. Freeze dried samplers were stored in screw-tight pots within sealed boxes containing silica gel. Dry weights of samples were calculated from the fresh and freeze-dried samples. Samples were ground to a fine powder, using a pestle and mortar. Sub-samples were sent to Yara Analytical for mineral analysis. The remaining samples were used for sugar analysis.

## 3.3 2nd year field trials: Phosphate and Calcium application to reduce low-temperature and senescent sweetening 2016-2017

The second year field trials (2016) were set up by Matt Smallwood (McCain) to explore the supplementation of phosphate and calcium in potato cultivation in order to influence tubers susceptibility to low-temperature sweetening and senescent sweetening respectively. The trials were planted at J Daw & Son at Rugeley Staffordshire on the 30th April 2016. Soil type was Sandy Loam and indices were representative for the area (P 23ppm, K 130ppm, Mg +2 and Ca 1180ppm).

The trial was set up as a two split plot design, with subplots of Markies and Pentland Dell and main plot treatments for Phosphate and Calcium. Each plot consisted of 100 tubers planted over 4 rows with a 3 m gap between plots. All phosphate was applied using Triple Super Phosphate (46% P) and Calcium using Yara Tropicote (Calcium Nitrate 15.5% N 19% Ca). Fertiliser was applied to the destoned bed at planting and incorporated into the bed using a nematicide bedtiller prior to planting. The treatments for phosphate were; no additional phosphate (P0), 75, 150 & 300 kg $ha^{-1}$.

The calcium treatments consisted of no additional calcium 50, 100 and 200 kg $ha^{-1}$, of Tropicote. Four plots per treatment were used. During the growing cycle canopy development was measured as a proportion of canopy cover using a grid every 7-10 days. Tubers were harvested 10th October 2016, 145 days after planting from 6m test digs from each plot and tubers were graded in 10mm size bands and the weight recorded as total yield and marketable yield (t/Ha >45mm).

### 3.2.1 2nd year – Storage treatments

After harvest tubers were transported to Sutton Bridge Crop Storage Research. On arrival, the tubers from individual plots were placed into trays- with the field plot replicates maintained during storage. After storage temperature pull down and curing, tubers treated with phosphate were split between two storage temperatures (10 and 3.5°C) while calcium-treated tubers were maintained at 10°C. All tubers were treated with CIPC at the start of storage and on a second occasion at the beginning of April.

Tubers were transported to the Produce Quality Centre where a series of measurements were made and samples were taken for subsequent analysis. Samples for sugar analysis and fry colour were taken at harvest (November 2016) and again after 2 months storage (January 2017); 5 months (March/early April 2017) and the final assessment was after 7 months (June 2017). Tubers were sampled for respiration and tissues then sampled for texture and for sugars from opposite eighths and the middle of the tuber (Peri-medulla). Tissues were also frozen in

liquid nitrogen and stored at -80ºC for ascorbic acid content and for mineral analysis (see below for details).

### 3.4 3rd year – Storage treatments

In the third year, Markies and Pentland Dell were sourced from commercial stocks and transported to SBCSR, tubers were sub-sampled into storage trays. Tubers were subject to a controlled temperature pull down to a final storage temperature of 3.5°C or 10°C. At intake, and subsequently at monthly intervals. 4 replicates of 20 tubers per replicate tubers were sampled and transported to the Produce Quality Centre. Tubers were placed into stores at 3.5°C or 10°C and allow to reacclimatise to temperatures before being placed inside respiration chambers (mini-pods). Sprout assessments and samples of tissue from opposite eighths and from the central medulla region were taken for sugar analysis; additional tissue samples were frozen at -80°C for ascorbic acid analysis and longitudinal slices were taken from the central region of tubers for ROS activity.

### 3.5 Sugar Analysis

Samples of tissue were taken from opposite eighths of the tuber and from the central cortex (peri-medulla) at harvest (November), and subsequently during storage. In years 1 and 2 after 2 months (January), 5 months (April) and 7 months (June). In the third year tubers were placed for 7 months in air storage at 10°C, for tubers varieties Pentland Dell and Markies that were pre-treated with calcium products, and 10 and 5°C for Markies pre-treated with phosphate products. Tissue samples were stored briefly at -20°C before being freeze dried. Sugar analysis was performed using 0.2 g with 1.6 ml of 80:20 (ethanol : water) for 2 hours at 70°C in a shaking water bath. Samples were centrifuged at 10,000 g for 5 minutes, the supernatant was filtered through a 0.45 µm PTFE syringe filter. 5 µl samples were injected onto an HPLC column (Agilant Zorbax carbohydrate analysis column) maintained at 30°C using 75 % acetonitrile running at 1.5 ml/min as the mobile phase. Sugars were detected using a refractive index detector (Agilent 1200 refractive index detector). Data was analysed by using data system EZChrom 3.3 (Agilent).

### 3.6 Physiological assessments- sampling strategy

Twenty tubers from each replicate were sampled Tubers of Pentland Dell and Markies stored in Sutton Bridge at 3.5°C and 10°C were collected at harvest (November) and after, 2, 5 and 7 months and transported to the Produce Quality Centre. Respiration rates of tubers were measured in ‘mini-pod’ respiration chambers at either 3.5°C or 10°C in terms of rate of both $CO_2$ production and $O_2$ consumption. Subsequently, tubers were scored for sprout growth on a 3

point basis (dormant, bud movement, number of sprouts over 2 mm). Tubers were sampled for sugar analysis; core samples were taken from opposite eighths of the tuber and from the middle cortex. Samples were frozen at 80°C and freeze dried for subsequent sugar, starch and mineral analysis.

Starch content of freeze-dried potato samples from Pentland Dell, Markies (phosphate treatments P300 and P0) from samples taken at Harvest and following 2 and 5 months storage. Fry colour analysis (SBCSR) of tubers taken at harvest and at 2, 3 and 7 months storage. Additional samples of tuber tissue were frozen at -80$^{o}$C for ascorbic acid (HPLC) content and a central longitudinal slice of tuber material was sampled and stained for ROS content.

Changes in the biomechanical properties of tubers during storage was studied over storage. Segments of tuber tissue sampled from the opposite eighths and central core were taken for biomechanical measurements using a Lloyd LRX texture analyser.

### 3.6.1 Sampling method

Each variety and treatment were divided in 2 replicates of 10 tubers each. Samples of tuber cortex were taken from opposite eighths (ends), sections related to the periderm, cortex, vascular ring and outer core, and from the middle of the tuber (inner core – medulla or pith) using a cork borer size N$^{o}$ 5 (10 mm) to capture the maximum range in sugars across the tuber. All the samples were frozen and stored at -20°C and subject to 48 hours freeze drying (Supermodulyo 12 K Edwards High Vacuum International) before grinding to a fine powder in a pestle and mortar.

## 3.7 Assessment of fry colour

A 3/8th inch hand press was used to cut a single longitudinal French fry (chip) from the centre of twenty tubers, which were fried at 190°C for 90 seconds. Visual assessment was made in a light cabinet against a USDA colour chart. The USDA assessment scale used for assessing French fries (light to dark - 000, 00, 0, 1, 2, 3 & 4) has been linearized to 1 to 7 (SBCSR scale) and reported as a mean. Scores of 3 or below are considered good, 4 and 5 are acceptable/borderline, and scores of 6 or higher are unacceptable.

## 3.8 Total starch determination

Total starch was determined using 100 mg of freeze-dried potato, using enzymatic digestion of total starch (Megazyme International Ireland, 2011). The Megazyme procedure for starch determination was selected based on potato starch containing significant proportion of D-glucose and/or maltodextrins" causing potatoes to have a high content of resistant starch (Elmståhl, 2002; Chen et al., 2010).

Aliquots of 100 mg of freeze dry powdered potato was subject to a series of hot ethanol washes to remove simple sugars. Samples were incubated at 80°C for 5 min with 5 ml of aqueous

ethanol (80% v/v) followed by vortexing and the addition of 5 mL of 80% v/v aqueous ethanol and further centrifugation (1800 g) for 10 min, this process was repeated. The supernatant was discarded, a magnetic stirrer bar (5 x 15 mm) and 2 mL of 2 M KOH was added and mixed by stirring for approximately 20 min in an ice bath followed by the addition of 8 mL of 1.2 M sodium acetate buffer (pH 3.8). Immediately 0.1 mL of thermostable α-amylase and 0.1 mL of AMG was added, mixing well and incubating the tubes in a water bath at 50°C for 30 min with intermittent vortexing and transfer to a 100 mL volumetric flask; adjusting volume to 100 mL with distilled water mixed and filtered; 0.1 mL of the diluted solution was transfer in duplicate to the glass 10 ml test tube. 3 mL of GOPOD Reagent was added to each tube (including the D-glucose controls and reagent blanks) and incubated at 50°C for 20 min. D-Glucose controls consisted of 0.1 mL of D-glucose standard solution (1 mg/mL) and 3.0 mL of GOPOD Reagent. A blank solution consisted of 0.1 mL of water and 3.0 mL of GOPOD Reagent. Final absorbance of samples, and the D-glucose control was measured at 510 nm against the reagent blank. Total starch determination was calculated using the formula provided by Megazyme at https://secure.megazyme.com/Total-Starch-Assay-Kit. Starch estimation was based on the following formula:

Starch (%) = ΔA * F * (FV/0.1) * (1/1000) * (100/W) * (162/180)

= ΔA *(F/W) * FV * 0.9

Where:

ΔA = absorbance (reaction) read against the reagent blank

F = 100 (µg of D-glucose)/ absorbance for 100 µg of glucose (conversion from absorbance to µg)

FV = final volume (equals 100 mL or 10.4 mL)

0.1 = volume of sample analysed

1/1000 = conversion from µg to mg

100/W = factor to express “starch” as a percentage of flour weight

W = the weight in milligrams (“as is” basis) of the flour analysed

162/180 = adjustment from free D-glucose to anhydro D-glucose (as occurs in starch)

### 3.9 Moisture content of freeze dry samples

For the total starch determination, the % moisture content of each sample was calculated based on an adaptation of the method from ISO 1666:1998 (BS EN ISO 1666:1998 Starch.

Determination of moisture content. Oven-drying method, https://bsol.bsigroup.com/PdfViewer/Viewer?pid=000000000001264744, 29-05-18). Aluminium dishes with tight-fitting lids (lid leaning against the dish) were dried in a constant-temperature oven, electrically heated with air circulation at 130°C for 90 min, cooled in a desiccator provided with a thick perforated metal plate and weighed when cool ($m_0$). Around 0.2 g of grind freeze dry sample was weighted ($m_1$). The dish with the sample was placed open in the oven preheated to 130°C for 90 min. After this period the dishes were rapidly cover and put in the desiccator to cool down for 30 to 45 min. When the dish was at room temperature it was weight (with the lid on) ($m_2$). The moisture content was expressed as a percentage by mass and it was calculated according the formula $(m_1 - m_2) * \frac{100\%}{m_1 - m_0}$.

## 3.10 Assessment of sugar accumulation during storage

Sugars were extracted and analysed using an adaptation to the method used in Giné Bordonaba and Terry (2010) and Glowacz *et al.* (2015). Sugars were extracted from powdered potato samples (0.2 g) with 2 mL of 80:20 (ethanol:water (v/v)) for 2 hours at 70°C in a shaking water bath. Samples were centrifuged at 10,000 g for 5 minutes, the supernatant was filtered through a 0.45 µm PTFE syringe filter.

5 µL samples were injected onto an HPLC column (Agilent Zorbax Carbohydrate150 mm x 4.6 mm x 5 µm column) maintained at 30°C using 75% acetonitrile running at 2 mL $min^{-1}$ as the mobile phase. Sugars were detected using a refractive index detector (Agilent 1200 refractive index detector). Data was analysed by using data system EZChrom 3.3 (Agilent).

## 3.11 Determination and detection of ROS

Staining and quantification of ROS protocols were developed for superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) in segments of tubers. $O_2^-$ was detected by the formation of a purple/blue precipitation on addition of nitroblue tetrazolium (NBT), and when diaminobenzidine tetrahydrochloride (DAB) is used as a substrate it reacts with $H_2O_2$ forming a brown product from polymerization (Jambunathan, 2010).

At each inspection *in situ* detection of hydrogen peroxide ($H_2O_2$) was performed on three tubers per variety and treatment using 3,3'-diaminobenzidine (DAB) method adapted from Daudi and O'brian (2012). DAB oxidization via hydrogen peroxide occurs in the presence of haem peroxidases generating a dark brown precipitate (Daudi and O'Brian, 2012).

In 50 mL flask, 50 mg DAB was diluted with 45 mL of deionized $H_2O$ to a final concentration of 1 $mg^{-1}$mL DAB the pH was adjusted to 3.0 by addition of 1 M of HCl under constant stirring, and to prevent light degradation the flask was covered with aluminium foil. The reaction was activated by the addition of 2.5 mL of 1 mM $K_2HPO_4$ to DAB solution (pH ~ 6.5). Tuber slices (3-4 mm in thickness) were prepared with a mandolin slicer and placed in separate Petri dishes.

Samples were bathed in the DAB solution and gently vacuum infiltrated for 10 to 15 minutes in a desiccator, and left covered to prevent ingress of light for 4 hours. Samples were washed with sterile water, placed in a light box and the extent of staining was captured by digital photography.

Histochemical staining methods for superoxide ($O_2^-$) with Nitroblue tetrazolium chloride (NBT) was adapted from Jambunathan (2010). Three tubers per variety were sampled, Tuber slices (3-4 mm in thickness) were prepared with a mandolin slicer and placed in separate Petri dishes. Two slices were taken from each tuber, one treated the other forming the control (no NBT solution was applied). A NBT 0.1% (w/v) staining solution in 10 mM of sodium azide ($NaN_3$) was dissolved in 50 mM of potassium phosphate ($KH_2PO_4$). Potato slices were infiltrated with the $NaN_3$/NBT solution for 10 minutes followed by incubation for 2 hours at room temperature, control samples were infiltrated with water. The intensity of staining observed was photographed and images processed. The extent of precipitate/staining formation and the intensity of red, green and blue (RGB) pixels was quantified using R packages "jpeg" (https://CRAN.R-project.org/package=jpeg, 17-10-2018) and lessR (https://CRAN.R-project.org/package=lessR, 17-10-2018) ((R Core Team, 2016).

To evaluate the stain intensity formed by DAB and NBT application; the red, green and blue values (RGB) were transformed into a single value. RGB values are encoded as 8-bit integers, which range from 0 to 255. Using the formulas for RGB % for "Dark brown / #654321 hex colour" (39.6%, 26.3%, 12.9%) and "Dark violet / #6317a9 hex colour" (38.8%, 9%, 66.3%) from ColorHexa website (2012 - 2017), red, green and blue values were transformed into a decimal value of brown for DAB staining and purple (dark violet) for NBT staining. Finally, in an attempt to normalise the staining intensity data, background colour changes observed in the control slices were subtracted from those treated for DAB and NBT.

### 3.12 Assessment of tuber texture during storage

Five tubers per variety and treatment were selected for texture analysis using the wedge fracture test developed by Vincent *et al.* (1991) which mimics the action of the incisors in the propagation of the crack formation in food. A 10 mm section along the mid-part of each tuber (Plate - 1) was scored with a double-bladed knife, with blades set 10 mm apart, before being cut with a single blade-knife. A second 10 mm slice (chip section) was excised from either tuber end, a further perpendicular 10 mm section was cut creating a 1 $cm^3$ of tissue, to ensure the orientation of tissue was kept constant during analysis, cubes of tissue were marked by removing a slither from the corner of the cube representing the outer end of the potato. Due to the radial-orientation of the cells within the tuber - accurate wedge fracture analysis texture requires the same orientation of the tissue during analysis (Plate - 2).

Wedge fracture tests were carried out using a Lloyd Instruments model LRX-plus with a 50 N load cell and running with R-Control software v3.23. A wedge (30$^o$ included angle) was driven

at 33.3 x $10^{-3}$ mm $sec^{-1}$ (2 mm $min^{-1}$) into 10 mm cubes of tuber tissue orientated radially (Plate - 2, Plate - 3 and Plate - 4). Wedge movement was halted when a crack could be seen ahead of the wedge tip (after reaching the peak load) and then the total crack length was measured. The wedge was then withdrawn from the sample at the same speed so that the energy still stored in the sample could be subtracted from the total energy. Peak load, load and distance at the start of crack propagation were determined from the load-distance (from top of sample) curves (

Figure – ). In addition, work of fracture was calculated as (area under force-distance curve) / (total crack length * sample width) (Vincent et al., 1991).

The load-distance curve (

Figure – ) for each tissue was re-displayed to identify the point of first failure and the start of crack propagation (using the cursor) so that the relevant loads and distances could be recorded. R-Control was set up to produce a results table and a conversion file in “Lotus” format for each sample. These files were used to create “Excel” spreadsheets, measured crack lengths were entered and work of fracture calculated.

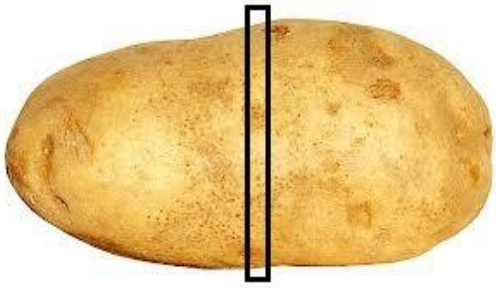


*Plate - 1. Section where a slice of 10 mm width was cut along the middle part of the tuber.*

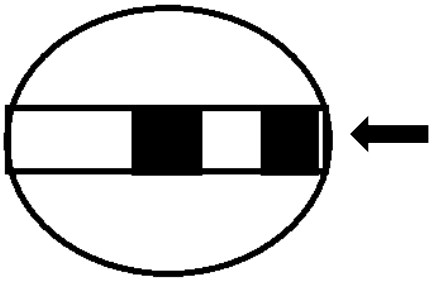


*Plate - 2. Chip shape section with two black squares representing the two 10 mm cubes from the end and middle of chip section. The arrow indicates the direction and orientation of the wedge during the wedge fracture test.*

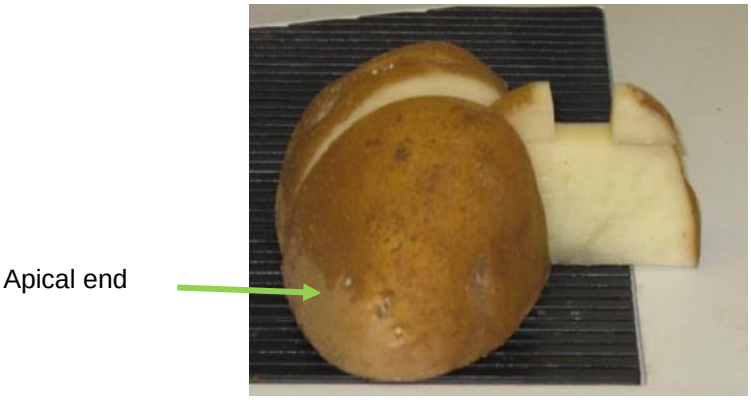



*Plate - 3. Cuttings in the tuber*

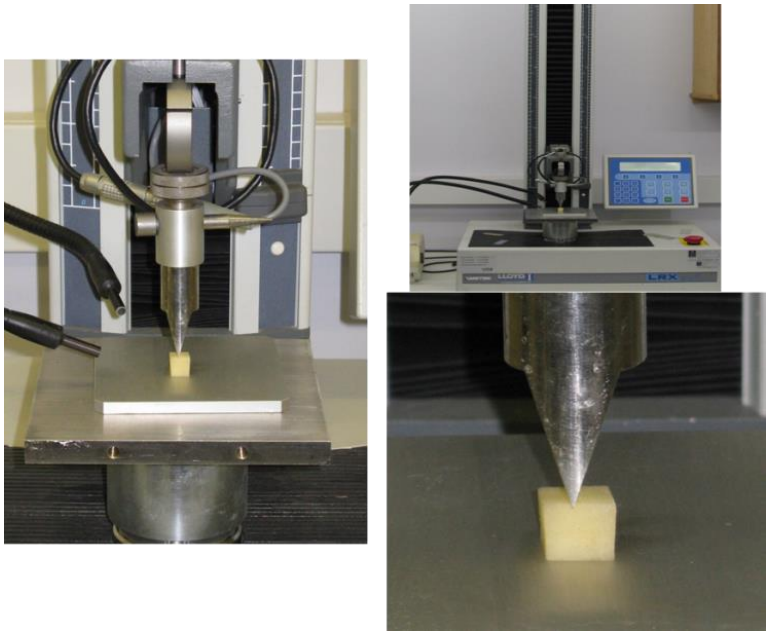


*Plate - 4. Lloyd LRX-plus texture analyser with 10 mm cubes of tuber tissue orientated radially*

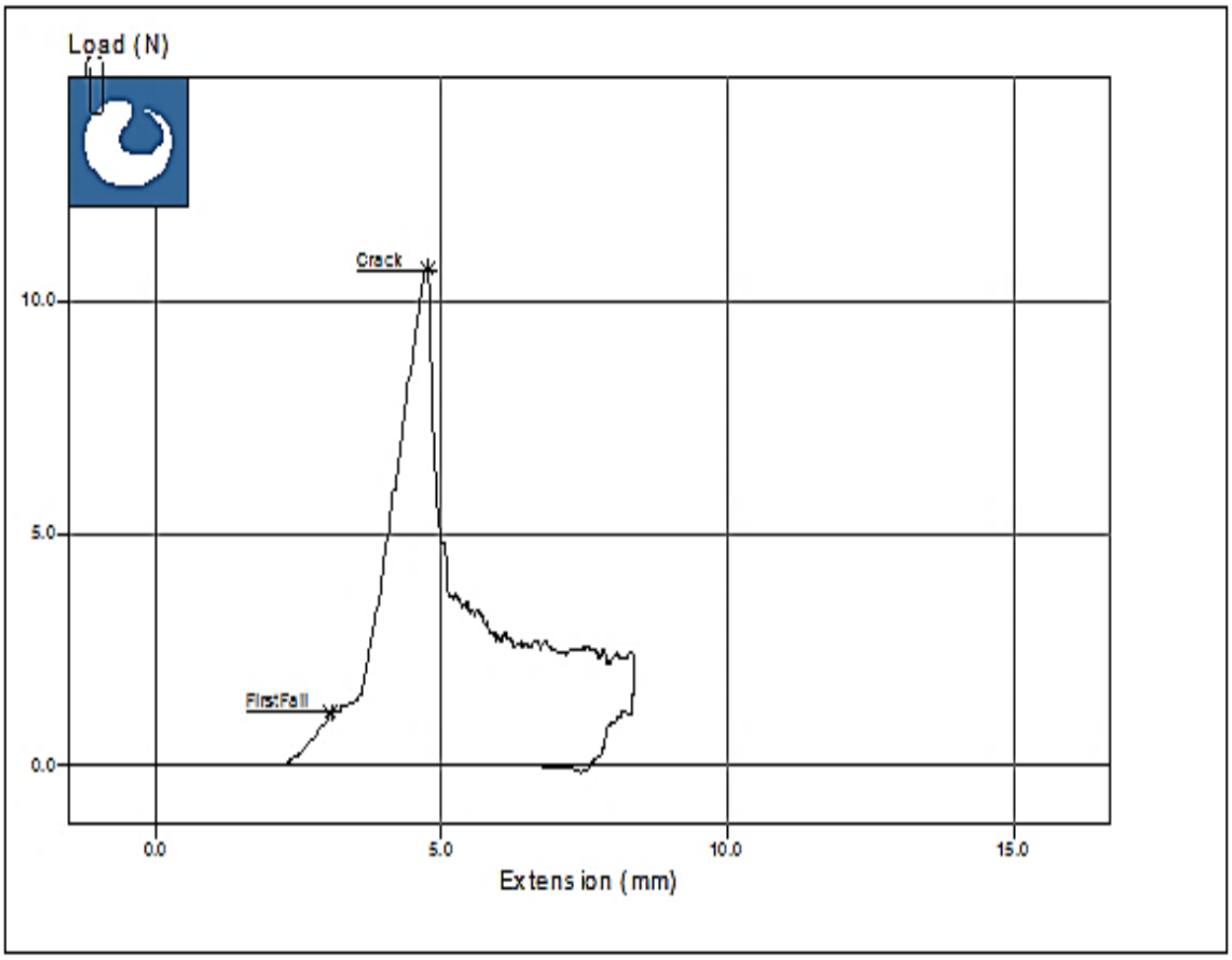



*Figure – 2.1. Example of a graph of the load-distance curve produced by Lloyd LRX-plus texture analyser in potato. The load-distance curve for each tissue was re-displayed to identify the point of first failure ("First Fall") and the start of crack propagation ("Crack") (using the cursor) so that the relevant loads and distances could be recorded to produce a results table for each sample.*

## 3.13 Mineral analyses

Samples of tuber cortex were taken from opposite eighths of potato using a cork borer (size Nº 5). Each replicate consisted of a composite of 5 tubers; 2 types of tissue were selected from each tuber: samples from apical and stolen end (opposite eighths) were combined to provide an average of the extremes in mineral profiles across the tuber and an additional sample was taken from mid-section of the tuber, with five replicates per variety for each sampling occasion. Samples were frozen and stored at -20°C and subject to 48 hours freeze drying before grinding to a fine powder with a pestle and mortar. Mineral analyses were performed (ground freeze-dried samples) by an accredited laboratory (Yara Analytical Services, York, UK).

### 3.14 Tuber sprout growth during storage

Each sample was divided into two replicates (10 potatoes per replicate) and the apical bud sprout measured with a Traceable™ Digital Calliper, 0-150 mm (Fisher Scientific, UK).

### 3.15 Assessment of tuber respiration

Approximately 3 kg of tubers per replicate with a total of 4 replicates from tubers stored at 10ºC or 3.5ºC before being placed inside a 'mini pod' (SCS Ltd) respiration chambers placed in cold rooms at either 10ºC or 3.5ºC after allowing conditions to settle for 2 hours, respiration rates were measured over a 7hour period in real-time every 30 seconds with data averaged in 15 minute segments.

### 3.16 Data analyses

Data analysis and graphical outputs were performed using Microsoft Excel 2013 and Rstudio (R Core Team, 2016). ANOVA (analyses of variance) was used to perform an analysis of the relative contributions from explained and unexplained sources of variance in a continuous response variable. Significant effects were tested with the F statistic, which assumes random sampling of independent replicates, homogeneous within-sample variances, and a normal distribution of the residual error variation around sample means (Doncaster and Davey, 2007). ANOVA was carried out to determine whether there were significant differences between samples using Rstudio (R Core Team, 2016). To determine significantly differences between means Tukey's test and LSD test were carried out using the "agricolae" package (https://cran.r-project.org/web/packages/agricolae/index.html, 26-10-2018), "Dae" package (https://cran.r-project.org/web/packages/dae/index.html, 26-10-18) and "multcomp" package ( https://cran.r-project.org/web/packages/multcomp/index.html, 26-10-2018) from R.

# 4. RESULTS

## 4.1 1st year

In the first year two trials were carried out; one to determine the effect of phosphate nutrition on low temperature sweetening (Pentland Dell, Markies and Innovator), and the other to determine the effect of calcium nutrition on senescence sweetening (Pentland Dell, Markies and Lady Rosetta).

### 4.1.1 Dry matter content at harvest – phosphate trial

The dry matter distribution within potatoes (mean of all three varieties) was affected by phosphate application. Higher application rates (200 kg $ha^{-1}$) of phosphate reduced dry matter content within the middle inner cortex tissues (Table 3.1) whereas no effect was seen in tissue sampled from opposite eighths (Table 3.1).

Varietal difference in dry matter accumulation were observed between varieties with Pentland Dell having a higher dry matter content in samples taken across the tuber than Markies or Innovator (Table 3.2). Importantly, the dry matter content of the central medulla tissue was lower than tissue sampled from the outer cortex where an opposite eighths sampling strategy was employed and this was observed in Innovator, Pentland Dell and most acutely in Markies.

Table 3.1. Distribution of dry matter (%) sampled at opposite eighths or middle section of potatoes under different application rates of Phosphate (Triple Super Phosphate) applied as a base fertiliser or foliar application (F). Data is overall means averaged over 3 varieties.

| | 0 kg $ha^{-1}$ | 100 kg $ha^{-1}$ | 200 kg $ha^{-1}$ | 300 kg $ha^{-1}$ | 200 F kg $ha^{-1}$ | F pr. | LSD | df |
|---|---|---|---|---|---|---|---|---|
| Opposite eighths | 21.1 | 21.3 | 20.5 | 20.8 | 21.1 | 0.243 | 0.81 | 101 |
| Middle | 16.2 | 16.1 | **15.1** | 15.6 | **14.9** | 0.033 | 0.945 | 101 |

*Table 3.2. Distribution of dry matter (%) found in the opposite eighths or mid-section of potatoes in varieties: Innovator, Pentland Dell and Markies. Data is the overall means averaged over all phosphate treatments.*

| Position | Innovator | Pentland Dell | Markies | F pr. | LSD | df |
|---|---|---|---|---|---|---|
| End | 19.9 | 22.2 | 20.9 | <.001 | 0.628 | 101 |
| Middle | **15.7** | **17.2** | **13.7** | <.001 | 0.732 | 101 |

The interaction between concentration of phosphate applied and potato variety was not significant (data not shown).

### 4.1.2 Dry matter content at harvest – calcium trial

No effect of calcium treatments or the interaction of treatments with variety on dry matter distribution was observed. However, the dry matter distribution was significantly influenced by variety with Markies having a lower DM content than Pentland Dell and Lady Rosetta (Table 3.3). The DM content of the inner cortex samples were lower than samples taken from the ends (opposite eighths segments) for both trials.

*Table 3.3. Distribution of % dry matter in Pentland Dell, Markies and Lady Rosetta. Data is the overall means averaged over all calcium treatments.*

| Position | Pentland Dell | Markies | Lady Rosetta | F pr. | LSD0.05 | df |
|---|---|---|---|---|---|---|
| Ends | 21.8 | 19.0 | 20.3 | <.001 | 0.806 | 116 |
| Middle | 14.6 | 13.9 | 15.1 | <.001 | 0.557 | 116 |

### 4.1.3 Tuber nutritional analysis at harvest – calcium trial

Foliar application of InCa increased calcium in tubers. Much of this increase was associated with a rise in calcium in the var. Lady Rosetta. Application of Tropicote, InCa and Gypsum to Lady Rosetta led to an increase in calcium content in tubers at harvest. Calcium fertiliser application to Pentland Dell and Markies did not lead to an increase in tuber-calcium content (Table 3.4). Application of gypsum led to a decrease in nitrogen, while Tropicote application led to a reduction in magnesium content (Table 3.5). The ratio of potassium/magnesium to calcium was lower in InCa-treated potatoes mediated by higher calcium content. Often the activity of calcium is hindered by high concentrations of potassium and magnesium that act as antagonists of calcium active sites.

*Table 3.4. The effect of calcium application on the calcium content of tubers (mg/100g dried weight)*

| | Pentland Dell | Markies | Lady Rosetta |
|---|---|---|---|
| Calcifert | 55.03 | 71.8 | 68.23 |
| Tropicote | 57.41 | 74.28 | **75.97** |
| InCa | 68.14 | 72.48 | **76.91** |
| Gypsum | 73.78 | 66.84 | **80.23** |
| Ammonium Sulphate | 68.18 | 63.37 | 67.6 |
| Control | 66.9 | 71.76 | 65.18 |
| $LSD_{0.05}$ 9.67 | | F pr. <0.001 | |

*Table 3.5. Overall effect of calcium application on mineral profile of potato tubers (average of three varieties, mg/100g dried weight)*

| | Calcifert | Tropicote | InCa | Gypsum | Ammonium sulphate | Control | F pr. | l.s.d. |
|---|---|---|---|---|---|---|---|---|
| Calcium | 65.02 | 69.22 | 72.30 | **73.60** | 63.4 | 67.9 | 0.017 | 5.59 |
| Phosphorous | 215.3 | 200.7 | 206.6 | 204.2 | 209.3 | 208.4 | 0.20 | 11.35 |
| Nitrogen | 1546 | 1565 | 1701 | **1488** | 1635 | 1607 | 0.00 | 100.60 |
| Magnesium | 108.73 | **104.44** | 111.75 | 108.27 | 109.26 | 111.28 | 0.03 | 4.57 |
| Potassium | 2199 | 2134 | 2166 | 2203 | 2177 | 2185 | 0.84 | 110.60 |
| Boron | 0.76 | 0.70 | 0.70 | 0.65 | 0.76 | 0.77 | <.001 | 0.03 |
| Zinc | 2.054 | 2.015 | 2.207 | 1.995 | 1.987 | 2.007 | <.001 | 0.11 |
| K+Mg/Ca | 35.43 | 34.21 | **30.81** | 33.26 | 35.2 | 34.38 | 0.01 | 2.66 |

### 4.1.4 Tuber nutritional analysis at harvest – phosphate trial.

Soil incorporation of Triple Super Phosphate was more effective than foliar applied phosphate. Increasing application rate from 100 to 200-300 kg $ha^{-1}$ increased incorporation of phosphate into Pentland Dell and Markies tubers (Table 3.6). In general, Markies tubers were higher in phosphate than Pentland Dell or Innovator (Table 3.7).

*Table 3.6 Phosphate content of tubers grown under different phosphate fertiliser regimes (mg/100g dried weight)*

| Phosphate | Innovator | Pentland Dell | Markies | | Mean |
|---|---|---|---|---|---|
| 0 kg ha | 194.3 | 184.0 | 230.3 | | *202.9* |
| 100 kg ha | 182.8 | 196 | 235.2 | | *204.7* |
| 200 kg ha | 202.2 | **206.2** | **251.5** | | ***220*** |
| 300 kg ha | 186.9 | **206.5** | **257** | | ***216.8*** |
| 200 F kg ha | 181.1 | 199.2 | 235.1 | | *205.2* |
| $LSD_{0.05}$ *20.10* | | | | $LSD_{0.05}$ | *11.68* |
| Mean | *189.5* | *198.4* | ***241.8*** | $LSD_{0.05}$ | *9.04* |

*Table 3.7. Overall effect of variety (averaged over all treatments) on mineral accumulation in tubers under phosphate fertiliser regimes (mg/100g dried weight)*

| | Innovator | Pentland Dell | Markies | F pr. | LSD |
|---|---|---|---|---|---|
| Calcium | 61.8 | **77.7** | 52.2 | <.001 | 4.60 |
| Phosphorous | 189.5 | 198.4 | **241.8** | <.001 | 9.04 |
| Nitrogen | **1941.0** | 1464.0 | 1494.0 | <.001 | 86.20 |
| Magnesium | **112.4** | 107.1 | **117.8** | <.001 | 4.48 |
| Potassium | 1866.0 | 1867.0 | **2202.0** | <.001 | 83.40 |
| Boron | 0.8 | **0.7** | 0.8 | <.001 | 0.03 |
| Zinc | 1.9 | 1.9 | 1.9 | 0.49 | 0.15 |
| K+Mg/Ca | 32.5 | 26.3 | **44.7** | <.001 | 2.08 |
| N/Ca | 32.0 | **19.5** | 29.0 | <.001 | 2.19 |

*Table 3.8. Overall effect of phosphate application rate on accumulation of macro and micro nutrients in potato tubers (average of Markies, Pentland Dell and Innovator) (mg/100g dried weight)*

| | 0 kg $ha^{-1}$ | 100 kg $ha^{-1}$ | 200 kg $ha^{-1}$ | 300 kg $ha^{-1}$ | 200 F kg $ha^{-1}$ | F pr. | LSD |
|---|---|---|---|---|---|---|---|
| Calcium | 64.27 | 62.89 | 67.36 | 62.91 | 62 | *0.42* | *5.93* |
| Phosphorous | 202.9 | 204.7 | **220** | **216.8** | 205.2 | *0.01* | *11.68* |
| Nitrogen | 1650 | 1530 | **1729** | **1718** | 1538 | *<.001* | *111.30* |
| Magnesium | 111.7 | 112.0 | 113.0 | 112.8 | 112.6 | *0.99* | *5.78* |
| Potassium | 1986 | 1976 | 2029 | 1978 | 1923 | *0.42* | *107.70* |
| Boron | 0.76 | 0.77 | 0.79 | 0.76 | 0.76 | *0.52* | *0.04* |
| Zinc | 1.88 | 1.79 | **2.1** | 1.8 | 1.9 | *0.04* | *0.19* |
| K+Mg/Ca | 34.6 | 35.0 | 33.4 | 35.1 | 34.4 | *0.74* | *2.68* |

### 4.1.5 Leaf-health – calcium trial

Assessment of plant health was taken soon after full canopy cover had been achieved and following the final foliar application of the calcium product; InCa. A chlorophyll content meter (Hansatech) measuring reflectance at 700 and 730 nm was used to calculate chlorophyll content and derivatives of the reflectance were used to calculate a Chlorophyll Fluorescence Ratio (CFR). Pentland Dell and Markies treated with InCa recorded a higher CFR (Table 3.9) compared to the control plots. Similarly, the same varieties also recorded higher chlorophyll concentrations (mg $m^2$) as determined by the reflectance at 700 and 720 nm (Table 3.10)

*Table 3.9 Chlorophyll fluorescence (CFR) of leaves of potato varieties Pentland Dell, Markies and Lady Rosetta under different calcium fertiliser regimes. Measurements taken at full canopy and after the final spray application (InCa)*

| | Pentland Dell | Markies | Lady Rosetta |
|---|---|---|---|
| Untreated | 832.1 | 738 | 868.8 |
| Gypsum | 701.7 | 721.7 | 761.3 |
| Calcifert | 796.6 | 745.4 | 719.1 |
| Tropicote | 675.8 | 791.1 | 793.3 |
| $NH_4SO_4$ | 797.7 | 754.1 | 745.9 |
| InCa | **912.4** | **842.1** | 796.5 |
| $LSD_{0.05}$ | 72.8 on 196 df | | |

*Table 3.10. Chlorophyll content mg/$m^2$ of leaves of potato varieties Pentland Dell, Markies and Lady Rosetta under different calcium fertiliser regimes. Measurements taken at full canopy and after the final spray application (InCa)*

| | Pentland Dell | Markies | Lady Rosetta |
|---|---|---|---|
| Untreated | 1.93 | 1.78 | 1.99 |
| Gypsum | 1.72 | 1.76 | 1.82 |
| Calcifert | 1.87 | 1.79 | 1.75 |
| Tropicote | 1.68 | 1.86 | 1.87 |
| $NH_4SO_4$ | 1.88 | 1.81 | 1.79 |
| InCa | **2.06** | **1.95** | 1.87 |
| $LSD_{0.05}$ | 0.115 on 196 df | | |

### 4.1.6 Leaf-health – phosphate trial

Assessment of plant health was also taken soon after full canopy cover had been achieved and following the final foliar application for the phosphate trial. Markies treated with foliar phosphate (200F) and the highest base phosphate (300) recorded a higher CFR and chlorophyll content (Tables 3.11, 3.12) compared to the other treatments.

*Table 3.11. Chlorophyll fluorescence (CFR) of leaves of potato varieties Pentland Dell, Markies and Innovator under different Phosphate fertiliser rates. Measurements taken at full canopy and after the final spray application (TSP).*

| Phosphate kg $ha^{-1}$ | Markies | Innovator | Pentland Dell |
|---|---|---|---|
| 0 | 784.6 | 841.4 | 691.2 |
| 100 | 768.0 | 741.7 | 708.8 |
| 200 | 793.0 | 789.6 | 746.6 |
| 200F | **889.3** | 810.6 | 736.1 |
| 300 | **852.3** | 719.3 | 717 |
| $LSD_{0.05}$ | 55.44 | | |

*Table 3.12. Chlorophyll content mg/m of leaves of potato varieties Pentland Dell, Markies and Innovator under different phosphate fertiliser regimes. Measurements taken at full canopy and after the final spray application (TSP).*

| Phosphate kg $ha^{-1}$ | Markies | Innovator | Pentland Dell |
|---|---|---|---|
| 0 | 1.85 | 1.94 | 1.71 |
| 100 | 1.83 | 1.79 | 1.73 |
| 200 | 1.87 | 1.86 | 1.79 |
| 200F | **2.02** | 1.90 | 1.78 |
| 300 | **1.96** | 1.75 | 1.75 |
| $LSD_{0.05}$ | 0.0874 | | |

### 4.1.7 Agronomic Traits - Phosphate Trial.

In general, the response to phosphate (200 kg $ha^{-1}$) application was to increase the number of tubers $ha^{-1}$ in Innovator, but no clear response was observed with the varieties Pentland Dell or Markies (Table 3.13). Phosphate application (200 kg $ha^{-1}$) increased the yield (t $ha^{-1}$) of Pentland Dell and increased the proportion of tubers in size classes: >45 mm and >50 mm while this lowered slightly the number of tubers in a 10 kg reference sample, it failed to reach significance ($P<0.05$). No effect on tuber length was noted. With Pentland Dell phosphate application increased the proportion of marketable tubers from 84% in untreated plots to between 87-90% in plots that received between 100-300 kg $ha^{-1}$.

Application of phosphate to the var. Markies led to a reduction in the number of tubers $ha^{-1}$ and a lower overall yield (tonnes $ha^{-1}$) and with fewer number of tubers in the >45 mm and > 50 mm class categories and fewer number of tubers recorded in the 10 kg reference count.

Application of phosphate (200 kg $ha^{-1}$) increased the number of Innovator tubers $ha^{-1}$ from 255.5 K tubers $ha^{-1}$ in untreated plots to 292-307 K tubers in plots receiving 200 kg $ha^{-1}$ either as a base dressing or as a foliar applied product. Moreover, phosphate (100-300 kg $ha^{-1}$) increased the yield (tonne $ha^{-1}$) and increased the proportion of tubers in the >45 mm and >55 mm category but did not significantly alter the number of tubers recorded in the 10 kg reference count. Innovator was the only variety where phosphate application altered the tuber form index (TFI=length/width) with an increase from 1.4 to 1.5. (TFI). No effect on fry colour at harvest was observed between treatments or varieties.

### 4.1.8 Agronomic Traits - calcium Trial

Calcium products applied either as base dressing or as a foliar applied product (InCa) had no effect on dry matter, yield, tuber size or shape, marketability or fry colour characteristics at harvest for all three varieties: Pentland Dell, Markies and Lady Rosetta (Table 3.14).

*Table 3.13. Impact of Phosphate application on yield, tuber size and dry matter content of potato vars. Pentland Dell, Markies and Innovator.*

| Treatment | Dry Matter (%) | Tuber No (000/Ha) | Total Yield (T/Ha) | Yield >45mm (T/Ha) | Yield >50mm (T/Ha) | Tuber count 10kg | Average length (mm) | Tuber form index (lgth/wdth) | Market-able (%) | Fry Index USDA |
|---|---|---|---|---|---|---|---|---|---|---|
| **Pentland Dell** | | | | | | | | | | |
| P0 | 24.4 | 439.6 | 47.4 | 39.9 | 29.5 | 65.5 | 92.2 | 1.4 | 84% | 1.9 |
| P100 | 24.0 | 390.1 | 51.2 | **46.4** | **38.1** | 60.3 | 94.8 | 1.4 | **90%** | 1.95 |
| P200 | 24.4 | 428.6 | 49.9 | 43.2 | 35.2 | 61.6 | 90.3 | 1.4 | **87%** | 1.85 |
| P300 | 24.3 | 393.8 | **53.0** | **47.0** | **38.4** | 60.7 | 91.7 | 1.4 | **89%** | 1.85 |
| P200+F | 23.7 | 469.8 | **57.6** | **50.9** | **40.0** | 61.6 | 94.5 | 1.4 | **88%** | 1.75 |
| $LSD_{0.05}$ | 0.78 | 102.93 | 4.50 | 4.26 | 7.77 | 11.62 | 7.13 | 0.08 | 0.07 | 0.45 |
| **Markies** | | | | | | | | | | |
| P0 | 23.6 | 370.9 | 66.7 | 65.0 | 62.1 | 51.1 | 106.9 | 1.4 | 97% | 0.275 |
| P100 | 23.6 | 348.9 | 62.7 | 60.3 | 57.1 | 48.5 | 103.6 | 1.4 | 97% | 0.25 |
| P200 | 23.1 | **302.2** | **52.2** | **50.1** | **46.1** | **42.8** | **96.1** | 1.4 | 94% | 0 |
| P200+F | 23.3 | **335.2** | **60.5** | **58.7** | **55.3** | **45.7** | 102.6 | 1.4 | 96% | 0 |
| P300 | 23.3 | **305.9** | **58.9** | **55.5** | **50.2** | **44.9** | 99.3 | 1.4 | 96% | 0 |
| $LSD_{0.05}$ | 0.65 | 34.68 | 5.47 | 5.46 | 6.45 | 4.19 | 8.41 | 0.07 | 0.02 | 0.66 |
| **Innovator** | | | | | | | | | | |
| P0 | 20.4 | 255.5 | 51.7 | 49.6 | 46.1 | 42.5 | 100.1 | 1.4 | 96.0% | 0.25 |
| P100 | 20.6 | 275.6 | 56.2 | 54.5 | 51.7 | 41.3 | **110.3** | **1.5** | **97.3%** | 0 |
| P200 | 21.0 | **307.7** | **59.9** | **56.8** | **52.9** | 42.9 | 102.0 | **1.5** | 94.8% | 0.5 |
| P200+F | 21.0 | **292.1** | **56.8** | 53.9 | 48.2 | 41.5 | 106.8 | **1.5** | 95.0% | 0.5 |
| P300 | 21.0 | 272.9 | **60.4** | **58.8** | **55.9** | **38.3** | **111.5** | **1.5** | **97.3%** | 0 |
| | 0.65 | 34.68 | 5.47 | 5.46 | 6.45 | 4.19 | 8.41 | 0.07 | 0.02 | 0.66 |

*Table 3.14. Impact of Calcium application on yield, tuber size and dry matter content of potato vars. Pentland Dell, Markies and Lady Rosetta.*

| Treatment | Dry Matter (%) | Tuber number (1000/Ha) | Total Yield (T/Ha) | Yield >45mm (T/Ha) | Yield >50mm (T/Ha) | 10kg tuber count | Average length mm | Tuber form index (lgth/wdth) | Market-able (%) | Fry Index USDA |
|---|---|---|---|---|---|---|---|---|---|---|
| **Pentland Dell** | | | | | | | | | | |
| Untreated | 23.2 | 485.3 | 54.6 | 44.4 | 32.4 | 60.0 | 88.4 | 1.4 | 0.8 | 1.9 |
| Tropicote | 23.2 | 486.3 | 55.4 | 44.9 | 34.9 | 61.7 | 88.7 | 1.4 | 0.8 | 1.9 |
| InCa | 23.4 | 522.9 | 56.8 | 47.9 | 37.0 | 62.2 | 89.7 | 1.4 | 0.8 | 2.0 |
| Gypsum | 23.3 | 516.5 | 56.6 | 46.5 | 35.7 | 61.9 | 89.3 | 1.4 | 0.8 | 2.0 |
| CalciFert | 23.2 | 453.3 | 54.3 | 43.2 | 30.9 | 57.2 | 86.1 | 1.4 | 0.8 | 1.8 |
| AS | 23.4 | 533.9 | 57.6 | 48.7 | 39.9 | 64.6 | 90.8 | 1.4 | 0.9 | 2.0 |
| $LSD_{0.05}$ | **0.61** | **112.69** | **5.64** | **5.03** | **6.24** | **6.37** | **3.20** | **0.03** | **0.06** | **0.21** |
| **Markies** | | | | | | | | | | |
| Untreated | 21.6 | 358.1 | 64.3 | 62.2 | 58.2 | 44.7 | 99.2 | 1.3 | 1.0 | 0.1 |
| Tropicote | 21.7 | 365.4 | 65.9 | 62.8 | 60.3 | 45.1 | 99.4 | 1.3 | 1.0 | 0.1 |
| InCa | 21.4 | 329.7 | 63.5 | 61.6 | 57.8 | 41.6 | 97.9 | 1.3 | 1.0 | 0.0 |
| Gypsum | 21.8 | 396.5 | 67.6 | 65.1 | 61.9 | 46.6 | 104.6 | 1.4 | 1.0 | 0.3 |
| CalciFert | 21.8 | 368.1 | 67.3 | 64.3 | 60.8 | 45.1 | 99.6 | 1.4 | 1.0 | 0.1 |
| AS | 21.5 | 353.5 | 64.0 | 61.8 | 58.1 | 44.0 | 99.0 | 1.3 | 1.0 | 0.1 |
| $LSD_{0.05}$ | **1.16** | **80.28** | **8.62** | **8.56** | **8.10** | **6.17** | **6.47** | **0.07** | **0.03** | **0.36** |
| **Lady Rosetta** | | | | | | | | | | |
| Untreated | 24.2 | 505.5 | 49.7 | 38.8 | 27.2 | * | * | * | * | * |
| Tropicote | 24.7 | 510.1 | 50.1 | 42.4 | 34.1 | * | * | * | * | * |
| InCa | 24.2 | 465.2 | 49.3 | 37.7 | 27.1 | * | * | * | * | * |
| Gypsum | 24.9 | 572.3 | 51.5 | 43.5 | 35.3 | * | * | * | * | * |
| CalciFert | 25.2 | 598.9 | 53.6 | 44.2 | 35.7 | * | * | * | * | * |
| AS | 24.7 | 568.7 | 50.4 | 42.6 | 34.9 | * | * | * | * | * |
| $LSD_{0.05}$ | **1.04** | **83.68** | **6.50** | **9.76** | **11.13** | * | * | * | * | * |

## 4.19 Impact of Phosphate treatments on sugar concentration and location at harvest

Assessment of postharvest and storage quality focused on only two varieties Markies and Pentland Dell and did not include foliar phosphate application.

Fructose content of tubers at harvest was very low and barely detectable in Markies, and while twenty-fold higher in Pentland Dell at harvest still represented very low concentrations when averaged across treatments (0.0038% FW) (Table 3.15 and Figure 3.2).

Core samples taken from the middle cortex (Peri-medulla) of the tuber were higher in fructose than samples taken from opposite eighths. However, phosphate had no effect on fructose content of tubers from either variety (Table 3.15 and Figure 3.1).

Glucose was significantly higher in Pentland Dell at harvest, and neither effects of phosphate application nor positional effects in either variety was found.

Sucrose content was not affected by variety but was influenced by the rate of phosphate application in the field with lower sucrose present in plots pre-treated with 100 and 300 kg $ha^{-1}$ but not at 200 kg $ha^{-1}$. The strongest influence on sucrose content was sampling position of tissue within the tuber with a near doubling of sucrose in the middle cortex compared to the outer edges. This effect was observed in Markies and Pentland Dell.

Sucrose content of Markies and Pentland Dell was higher in the central medulla compared to the outer edges of the tuber, whereas fructose and glucose content were more evenly distributed.

There was a positional effect of sucrose and glucose based on response to preharvest phosphate application; application of phosphate 200-300 kg $ha^{-1}$ at planting led to lower glucose in Markies in the central medulla and lower sucrose in the opposite eighths' sections of tuber; there were positional effects with lower sucrose present within the central medulla where tubers received 300 kg $ha^{-1}$ sucrose (Table 3.15 and Figure 3.1).

*Table 3.15. The effect of phosphate fertiliser application at planting on the accumulation of sugars at harvest on potato varieties Markies and Pentland Dell in the opposite eights (Ends) versus middle section (Peri-medulla).*

| | | | Variety | | | |
|---|---|---|---|---|---|---|
| | Phosphate | | Markies | | Pentland Dell | |
| | kg ha$^{-1}$ | Position | Ends | Middle | Ends | Middle |
| Fructose | 0 | | 0.00003 | 0.0005 | 0.0039 | 0.0045 |
| | 100 | | ND | ND | 0.0034 | 0.0043 |
| | 200 | | ND | ND | 0.0037 | 0.0043 |
| | 300 | | ND | ND | 0.0031 | 0.0039 |
| LSD | 0.000895 | | | | | |
| Glucose | 0 | | 0.0012 | 0.0017 | 0.0054 | 0.0051 |
| | 100 | | 0.0007 | 0.0006 | 0.0044 | 0.0064 |
| | 200 | | 0.0004 | **0.0002** | 0.0053 | 0.0055 |
| | 300 | | 0.0000 | **0.0000** | 0.0053 | 0.0059 |
| LSD | 0.001453 | | | | | |
| Sucrose | 0 | | 0.030 | 0.049 | 0.024 | 0.045 |
| | 100 | | **0.023** | 0.047 | 0.031 | 0.048 |
| | 200 | | 0.027 | 0.045 | 0.030 | 0.039 |
| | 300 | | **0.022** | **0.040** | 0.026 | 0.041 |
| LSD | 0.006481 | | | | | |

*N.B* figure in bold are significant from the control for each individual sugar. Sugar concentrations given as %FW

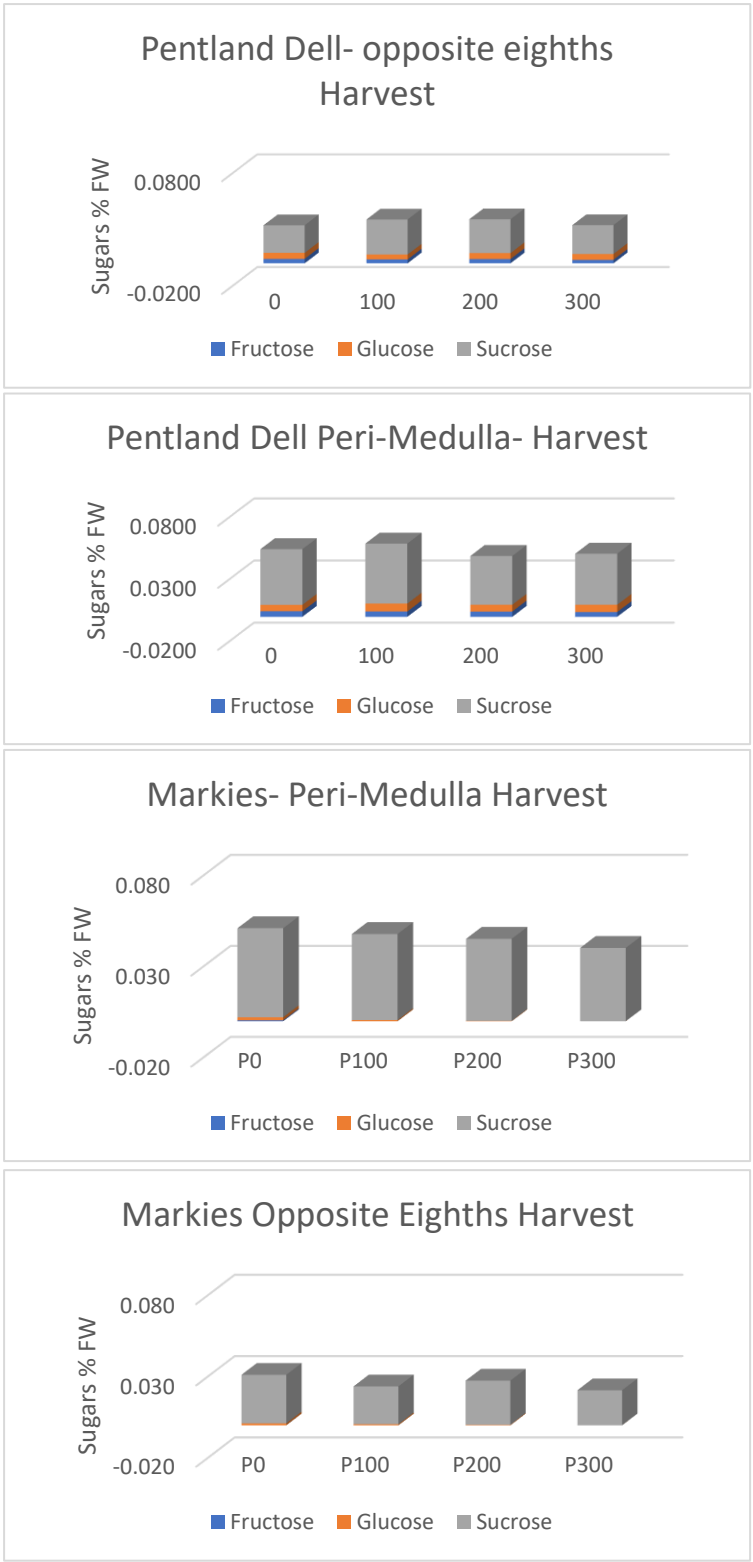



*Figure 3.1- Combined (Sucrose, Fructose & Glucose) profiles of Markies and Pentland Dell tubers at harvest grown under different phosphate fertiliser regimes*

## 4.20 Storage Assessments: Impact of Temperature

Tubers (CIPC-treated) were stored for 7 months at 5 and 10°C and sampled after 2 (January), 5 (April) and 7 (June) months of storage and assessed for dormancy/sprout vigour. Tissues were also sampled across the tuber and analysed for fructose, glucose and sucrose.

Under storage conditions of 5˚C Pentland Dell exhibited a minor rise in fructose content at the end of 7 months storage (Figure 3.1), while Markies showed a transitory increase at month 5 then decreased by month 7. Glucose accumulation increased over time was mirrored between varieties. Sucrose content in Pentland Dell stored at 5ºC declined over the first 2 months then increased thereafter while in Markies slowly declined over the storage period. The glucose and fructose content of Markies stored at 10ºC were low across the whole storage period, while in Pentland Dell a steep rise in fructose accumulation was seen after 2 months and glucose content remained low until 5 months before a small rise was observed. The pattern of sucrose accumulation in Markies and Pentland Dell at 10ºC were similar, declining over the first 2 months of storage before rising until the end of storage.

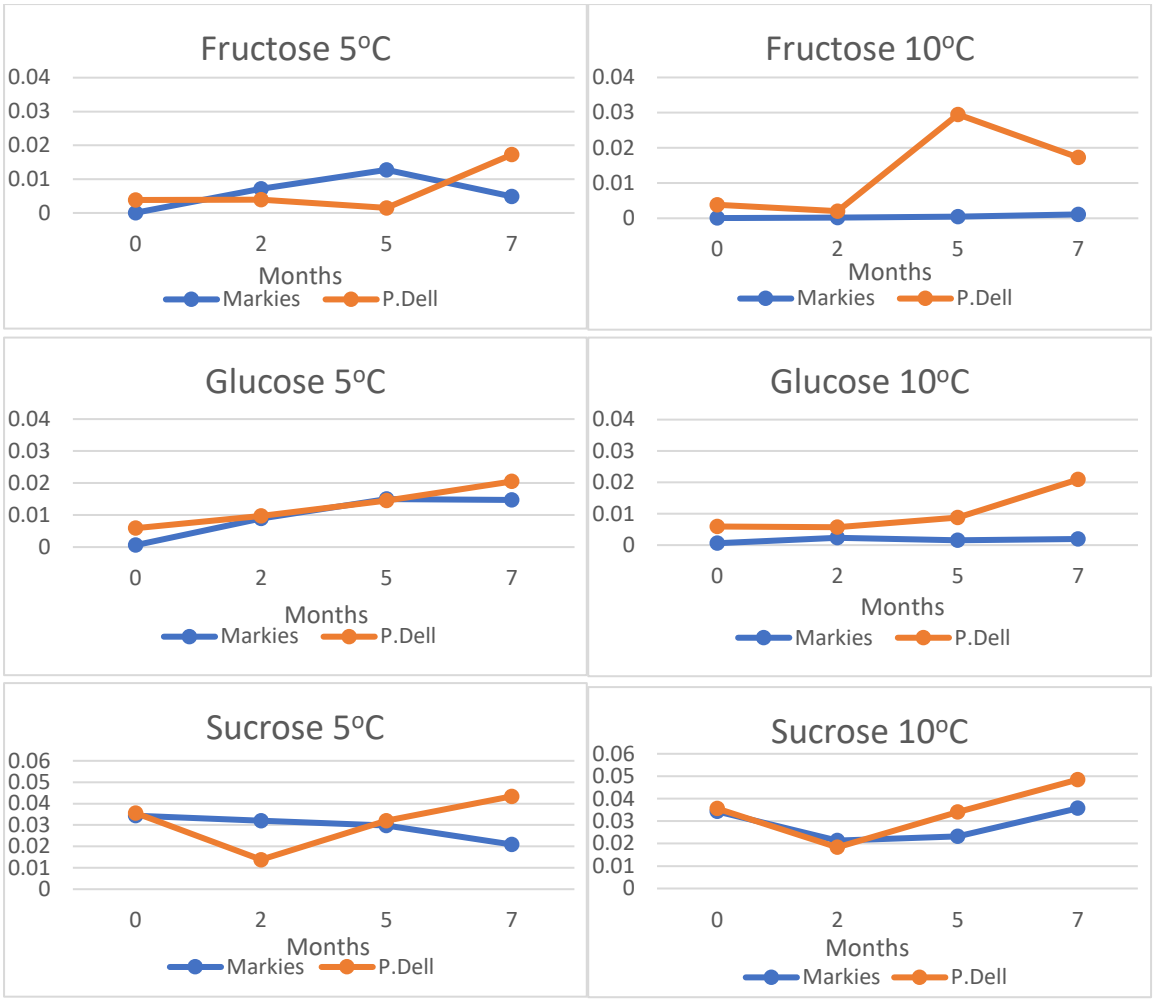



*Figure 3.2. Sugar profiles of Markies and Pentland Dell stored at 5 and 10°C and sampled out of store after 0, 2, 5 and 7 months. Data averaged across all phosphate treatments and positional effects Sugar concentrations given as %FW*

### 4.21 Impact of pre-harvest phosphate application on sweetening

Storage of Markies at 5°C for 2 months led to a significant rise in fructose, with smaller but significant increases in glucose and sucrose. Smaller increases in fructose were observed in Pentland Dell, while glucose and sucrose content in tubers were similar across both temperature regimes.

Increasing tuber phosphate content failed to protect tubers from a rise in low-temperature sweetening, and all three sugars increased at lower temperatures (Table 3.16).

*Table 3.16. Phosphate application failed to reduce sugar accumulation (%FW) in response to low-temperature storage Markies and Pentland Dell after 2 months storage (January 2016) in the opposite eights (ends)*

| | | | Variety | |
|---|---|---|---|---|
| | Phosphate kg ha$^{-1}$ | Position | Markies | P. Dell |
| Fructose | 5°C | P0 | 0.0075 | 0.0034 |
| | | P100 | 0.0065 | 0.0038 |
| | | P200 | 0.0082 | 0.0042 |
| | | P300 | 0.0067 | 0.0044 |
| | 10°C | P0 | 0.0001 | 0.0015 |
| | | P100 | 0.0002 | 0.0021 |
| | | P200 | 0.0007 | 0.0021 |
| | | P300 | 0.0000 | 0.0024 |
| | $LSD_{0.05}$ | | 0.00134 | |
| Glucose | 5°C | P0 | 0.010 | 0.009 |
| | | P100 | 0.008 | 0.010 |
| | | P200 | 0.010 | 0.010 |
| | | P300 | 0.008 | 0.010 |
| | 10°C | P0 | 0.002 | 0.005 |
| | | P100 | 0.002 | 0.006 |
| | | P200 | 0.003 | 0.006 |
| | | P300 | 0.002 | 0.006 |
| | $LSD_{0.05}$ | | 0.00194 | |
| Sucrose | Temperature | Phosphate Variety | Markies | Pentland D |
| | 5°C | P0 | 0.030 | 0.016 |
| | | P100 | 0.033 | 0.014 |
| | | P200 | 0.034 | 0.013 |
| | | P300 | 0.031 | 0.011 |
| | 10°C | P0 | 0.018 | 0.017 |
| | | P100 | 0.026 | 0.020 |
| | | P200 | 0.022 | 0.017 |
| | | P300 | 0.020 | 0.020 |
| | $LSD_{0.05}$ | | 0.008793 | |

The elevation in fructose concentration in Markies and Pentland Dell tubers increased after 5 months storage at 5°C (Table 3.17); fructose content had increased generally between 2 (January) and 5 (April) months storage. The glucose concentrations in each variety were significantly higher in 5°C stored tubers with a general increase in glucose occurring between January and April. The sucrose content in Markies increased between 2 and 5 months storage. In contrast, the sucrose content of Pentland Dell sucrose remained stable between the two sampling points. A small increase in sucrose was observed in Markies tubers stored at 5°C which was the case in tubers sampled in January. No effect of phosphate on sugar accumulation in response to storage in low-temperatures was observed.

*Table 3.17. The impact of phosphate fertilisers on the accumulation of sugars (% FW) in varieties Markies and Pentland Dell after 5 months (April 2016) storage at 5 and 10°C.*

| Temperature | Phosphate | Markies | Pentland Dell |
|---|---|---|---|
| Fructose | | | |
| 5°C | P0 | 0.014 | 0.010 |
| | P100 | 0.012 | 0.012 |
| | P200 | 0.012 | 0.012 |
| | P300 | 0.013 | 0.012 |
| 10°C | P0 | 0.001 | 0.005 |
| | P100 | 0.000 | 0.005 |
| | P200 | 0.001 | 0.005 |
| | P300 | 0.000 | 0.007 |
| | | $LSD_{0.05}$ | 0.00158 |
| Glucose | | | |
| 5°C | P0 | 0.016 | 0.013 |
| | P100 | 0.014 | 0.015 |
| | P200 | 0.014 | 0.015 |
| | P300 | 0.016 | 0.015 |
| 10°C | P0 | 0.002 | 0.008 |
| | P100 | 0.001 | 0.008 |
| | P200 | 0.002 | 0.009 |
| | P300 | 0.001 | 0.010 |
| | | $LSD_{0.05}$ | 0.00201 |
| Sucrose | | | |
| 5°C | P0 | 0.029 | 0.034 |
| | P100 | 0.030 | 0.033 |
| | P200 | 0.027 | 0.028 |
| | P300 | 0.033 | 0.033 |
| 10°C | P0 | 0.022 | 0.035 |
| | P100 | 0.026 | 0.036 |
| | P200 | 0.023 | 0.030 |
| | P300 | 0.022 | 0.035 |
| | | $LSD_{0.05}$ | 0.00565 |

After 7 months (June 2016) storage, the overall sugar profile (combined sucrose, fructose and glucose) of Pentland Dell had doubled over time from 0.04% FW at harvest to nearly 0.08% FW (Table 3.18). At the end of storage, the sugar profiles of Pentland Dell for sucrose, glucose and fructose were similar for tubers stored at 5 or 10°C. The overall total sugar profile of Pentland Dell (0.08% FW) was approximately double that of Markies (0.04% FW). A significant difference in the overall total sugar concentration between tubers of Markies stored at 5°C and 10°C with tubers stored at 5°C retaining a greater proportion of glucose and fructose and less sucrose compared to tubers stored at 10°C, suggests the breakdown of sucrose was greater at lower storage temperatures. No significant effect of phosphate on sugar profiles in either variety was seen during storage.

*Table 3.18. The impact of phosphate fertilisers on the accumulation of sugars (% FW) in varieties Markies and Pentland Dell during storage after 7 months (June 2016) storage at 5 and 10°C*

| Sugars | Temp | Phosphate kg $ha^{-1}$ | Markies | Pentland Dell |
|---|---|---|---|---|
| Fructose | | | | |
| | 5ºC | P0 | 0.005 | 0.017 |
| | | P100 | 0.005 | 0.016 |
| | | P200 | 0.005 | 0.018 |
| | | P300 | 0.006 | 0.019 |
| | 10ºC | P0 | 0.002 | 0.015 |
| | | P100 | 0.001 | 0.019 |
| | | P200 | 0.001 | 0.018 |
| | | P300 | 0.001 | 0.017 |
| | $LSD_{0.05}$ | | 0.003337 | |
| Glucose | | | | |
| | 5ºC | P0 | 0.016 | 0.020 |
| | | P100 | 0.017 | 0.017 |
| | | P200 | 0.016 | 0.021 |
| | | P300 | 0.015 | 0.024 |
| | 10ºC | P0 | 0.002 | 0.016 |
| | | P100 | 0.002 | 0.022 |
| | | P200 | 0.002 | 0.024 |
| | | P300 | 0.002 | 0.022 |
| | $LSD_{0.05}$ | | 0.004391 | |
| Sucrose | | | | |
| | 5ºC | P0 | 0.020 | 0.048 |
| | | P100 | 0.021 | 0.039 |
| | | P200 | 0.020 | 0.042 |
| | | P300 | 0.016 | 0.045 |
| | 10ºC | P0 | 0.039 | 0.049 |
| | | P100 | 0.047 | 0.048 |
| | | P200 | 0.020 | 0.048 |
| | | P300 | 0.037 | 0.050 |
| | $LSD_{0.05}$ | | 0.01287 | |

## 4.22 Improving calcium nutrition to reduce the onset of senescent sweetening

Detailed assessment of tuber quality after harvest and during storage in order to determine the impact of calcium nutrition focused on Markies and Lady Rosetta. Application of calcium in the form of Gypsum, InCa and Tropicote to Lady Rosetta resulted in reduced glucose content (%FW) of tubers at harvest. Sucrose content of tubers were relatively high at harvest (Table 3.19) while tubers from plants treated with InCa and Tropicote were approximately 60% lower in sucrose compared to tubers that received no calcium supplementation during the growing season. Fructose content at harvest was low across both varieties and no treatment effects were observed. Markies tubers did not show a similar response to calcium application.

*Table 3.19. Impact of calcium fertilisers (Gypsum, InCa and Tropicote) on the sugar profile of Lady Rosetta and Markies at harvest. Data averaged across opposite eighths and middle cortex samples (%FW).*

| | Fructose | | Glucose | | Sucrose | |
|---|---|---|---|---|---|---|
| | L.Rosetta | Markies | L.Rosetta | Markies | L.Rosetta | Markies |
| Control | 0.00031 | 0.0005 | 0.00275 | 0.002444 | 0.04431 | 0.03769 |
| Gypsum | 0.00019 | 0.0003 | **0.00119** | 0.001994 | 0.05425 | 0.03648 |
| InCa | 0.00025 | 0.00037 | **0.00106** | 0.002237 | **0.03169** | 0.03792 |
| Tropicote | 0.00019 | 0.00032 | **0.00119** | 0.001631 | **0.0325** | 0.03689 |
| $LSD_{0.05}$ on 112 df | 0.0001885 | | 0.0005002 | | 0.004842 | |

The reduction in glucose was observed in tissues sampled across the tuber (peri-medulla: middle and the opposite eighths: ends).

Analysis of sugars in the middle (medulla) of the tuber and the outer edges (opposite eighths) indicated a heterogeneous distribution of sucrose content across the tuber, with the middle (peri-medulla) region of tubers significantly higher in sucrose than the composite samples taken from the opposite eighths' sections in Lady Rosetta and Markies (Table 3.20).

*Table 3.20. The effect of calcium fertigation on the sugar composition of tubers varieties Markies and Pentland Dell at harvest.*

| | | | Variety | | | |
|---|---|---|---|---|---|---|
| | Calcium | | Lady Rosetta | | Markies | |
| | kg ha$^{-1}$ | Position | Ends | Middle | Ends | Middle |
| Fructose | Control | | 0.0004 | 0.0003 | 0.0006 | 0.0004 |
| % FW | Gypsum | | 0.0003 | 0.0001 | 0.0003 | 0.0003 |
| | Inca | | 0.0001 | 0.0004 | 0.0004 | 0.0004 |
| | Tropicote | | 0.0003 | 0.0001 | 0.0004 | 0.0003 |
| $LSD_{0.05}$ | 0.00038 | | | | | |
| Glucose | Control | | 0.0029 | 0.0026 | 0.0023 | 0.0026 |
| % FW | Gypsum | | **0.0011** | **0.0013** | 0.0019 | 0.0021 |
| | Inca | | **0.0010** | **0.0013** | 0.0022 | 0.0023 |
| | Tropicote | | **0.0013** | **0.0013** | 0.0015 | 0.0018 |
| $LSD_{0.05}$ | 0.00100 | | | | | |
| Sucrose | Control | | 0.0394 | 0.0493 | 0.0264 | 0.0490 |
| % FW | Gypsum | | 0.0423 | 0.0663 | 0.0243 | 0.0487 |
| | Inca | | **0.0243** | **0.0391** | 0.0260 | 0.0498 |
| | Tropicote | | **0.0253** | **0.0398** | 0.0257 | 0.0480 |
| $LSD_{0.05}$ | 0.00969 | | | | | |

Data in bold is significantly different ($p<0.05$) between data in the same column.

Subsequent sampling of tuber tissue for sugar analysis during storage experiments was restricted to opposite eighths sections.

After 2 months storage at 10°C, the concentration of individual sugars measured in Lady Rosetta and Markies were at similar concentrations to samples taken at harvest. No effect of calcium application was observed in changes in sugars (Table 3.21).

*Table 3.21. The impact of calcium fertilisers on sugar accumulation (%FW) of tubers varieties Markies and Lady Rosetta in tubers sampled after 2 months (January 2016) storage at 10°C.*

| | | | Variety | |
|---|---|---|---|---|
| | Calcium | | Lady Rosetta | Markies |
| | kg ha$^{-1}$ | Position | Ends | Ends |
| Fructose | Control | | 0.0011 | 0.0009 |
| % FW | Gypsum | | 0.0013 | 0.0009 |
| | Inca | | 0.0013 | 0.0011 |
| | Tropicote | | 0.0009 | 0.0011 |
| LSD | 0.000474 | | | |
| Glucose | Control | | 0.0018 | 0.0021 |
| % FW | Gypsum | | 0.0013 | 0.0023 |
| | Inca | | 0.0014 | 0.0019 |
| | Tropicote | | 0.0014 | 0.0021 |
| LSD | 0.000594 | | | |
| Sucrose | Control | | 0.0260 | 0.0291 |
| % FW | Gypsum | | 0.0268 | 0.0300 |
| | Inca | | 0.0267 | 0.0278 |
| | Tropicote | | 0.0244 | 0.0295 |
| LSD | 0.004583 | | | |

By the final inspection after 7 months storage (June 2016), glucose concentrations in Lady Rosetta tubers had increased 3-fold over samples taken after 2 months storage. Markies maintained a stable glucose concentration throughout storage. Sucrose concentrations in Lady Rosetta and Markies increased significantly by the end of storage indicating increased rates of starch breakdown. There were no obvious effects of calcium products on the long-term storage behaviour of tubers (Table 3.22).

*Table 3.22. The effect of calcium fertilisers on the accumulation of sugars in tubers varieties Markies and Lady Rosetta 7 months storage (10°C).*

| | | | Variety | |
|---|---|---|---|---|
| | | | Lady Rosetta | Markies |
| | Calcium | Position | Ends | Ends |
| Fructose | Control | | 0.0017 | 0.0003 |
| | Gypsum | | 0.0014 | 0.0004 |
| | Inca | | 0.0018 | 0.0005 |
| | Tropicote | | 0.0013 | 0.0003 |
| $LSD_{0.05}$ Var x Treat. | | | 0.00046 | |
| Glucose | Control | | 0.00612 | 0.00205 |
| | Gypsum | | 0.00617 | 0.00121 |
| | Inca | | 0.00692 | 0.00184 |
| | Tropicote | | 0.00586 | 0.00149 |
| $LSD_{0.05}$ Var x Treat. | | | 0.00303 | |
| Sucrose | Control | | 0.0468 | 0.0414 |
| | Gypsum | | 0.0432 | 0.0398 |
| | Inca | | 0.0479 | 0.0352 |
| | Tropicote | | 0.0453 | 0.0457 |
| $LSD_{0.05}$ Var x Treat. | | | 0.01266 | |

## 4.3 2nd year

### 4.3.1 Agronomic traits and mineral analysis at harvest – Calcium trial

Application of calcium nitrate (19% Ca 15.5% N) between 50-200 kg $ha^{-1}$ to the seed bed at planting did not lead to an increase in the number of tubers harvested per plant or the size distribution or yield per hectare (Table 3.23).

*Table 3.23. Yield of tubers of Markies and Pentland Dell treated with calcium nitrate (Tropicote) to the seed bed at planting.*

| Variety | $Ca(NO_3)_2$ t $ha^{-1}$ | No tubers per plant | Total Yield (t/Ha) | Yield >45mm (t/Ha) |
|---|---|---|---|---|
| Markies | 0 | 11 | 67.2 | 66.8 |
| | 50 | 10 | 66.6 | 66.4 |
| | 100 | 12 | 65.8 | 65.5 |
| | 200 | 11 | 68.1 | 67.5 |
| Pentland Dell | 0 | 14 | 57.8 | 52.4 |
| | 50 | 15 | 56.5 | 50.4 |
| | 100 | 15 | 56.8 | 51.2 |
| | 200 | 14 | 58.1 | 53.3 |
| $LSD_{0.05}$ | Variety | 2.8 | 5.7 | 6.3 |
| | Treatment | 3.6 | 6.4 | 7.2 |
| | Variety x treatment | 7.8 | 9.2 | 10.1 |

Calcium nitrate application to the seedbed at planting did not lead to increase in calcium or other nutrients in tubers of Markies or Pentland Dell at harvest and actually led to a reduction in uptake of iron and manganese (Table 3.24). The distribution of nutrients across the tuber favoured accumulation within the central medulla (middle) for all but manganese and molybdenum (Tables 3.25 and 3.26); nitrogen, phosphorus, potassium, boron, copper and zinc were particularly abundant in the central medulla compared to the outer edges (Table 3.26) with Pentland Dell accumulating more nitrogen, calcium, sulphur; Markies was higher in magnesium and molybdenum (Table 3.25 and 3.26).

*Table 3.24. Increasing calcium rate to the seedbed at planting failed to increase calcium content of tubers (averaged across varieties) and decreased iron and manganese uptake.*

| Calcium kg $ha^{-1}$ | 0 | 50 | 100 | 200 | F.prob | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|
| Iron (ppm) | 127.1 | 112.8 | **108.4** | **98.6** | 0.022 | 17.912 |
| Manganese (ppm) | 9.31 | **8.42** | **8.16** | **7.83** | <.001 | 0.621 |
| Calcium (%) | 0.0631 | 0.0625 | 0.0581 | 0.065 | 0.259 | 0.007 |

*Table 3.25. Overall effect of sampling position and variety on macro nutrient uptake and distribution across Markies and Pentland Dell treated at planting with Calcium Nitrate (Tropicote).*

| | Position | | | Variety | | | Variety and Position |
|---|---|---|---|---|---|---|---|
| Mineral (%) | End | Middle | f.prob | Markies | P. Dell | f.prob | $LSD_{0.05}$ |
| Nitrogen | 1.57 | **2.08** | *<.001* | 1.76 | **1.89** | *0.004* | *0.0818* |
| Calcium | 0.06 | **0.07** | *0.015* | 0.06 | **0.07** | *<.001* | *0.005* |
| Magnesium | 0.11 | **0.12** | *<.001* | **0.12** | 0.11 | *<.001* | *0.0051* |
| Sulphur | 0.11 | **0.12** | *<.001* | 0.10 | **0.13** | *<.001* | *0.0053* |
| Phosphorous | 0.20 | **0.30** | *<.001* | 0.25 | 0.25 | *0.331* | *0.016* |
| Potassium | 2.01 | **3.00** | *<.001* | 2.40 | **2.61** | *0.009* | *0.0157* |

*Table 3.26. Overall effect of sampling position and variety on macro nutrient uptake and distribution across Markies and Pentland Dell treated at planting with Calcium Nitrate (Tropicote).*

| | Position | | | Variety | | | Variety and Position |
|---|---|---|---|---|---|---|---|
| Mineral (ppm) | End | Middle | f.prob | Markies | P. Dell | f.prob | $LSD_{0.05}$ |
| Iron | 107.1 | 116.3 | *0.153* | 112.8 | 110.7 | *0.741* | *12.67* |
| Manganese | 8.42 | 8.45 | *0.887* | 8.54 | 8.32 | *0.336* | *0.439* |
| Boron | 5.82 | **8.91** | *<.001* | 7.36 | 7.37 | *0.968* | *0.464* |
| Copper | 12.4 | **28.5** | *0.012* | 16.1 | **24.8** | *0.164* | *6.16* |
| Molybdenum | 0.25 | 0.22 | *0.125* | **0.29** | 0.181 | *<.001* | *0.0427* |
| Zinc | 19.2 | **27.02** | *<.001* | 21.95 | 24.27 | *0.131* | *3.03* |

### 4.3.2 Dry matter levels and distribution – Calcium trial

Dry matter distribution (sugars, starches, alcohol insoluble fibres) varied across the tuber and between varieties. Across the two varieties %DM content sampled from tissue from the opposite eighths (OE) of control samples was significantly higher in comparison with samples taken from middle-cortex (MC). Markies %DM of tissue sampled across the tuber averaged 16.9% however, this value comprised 3.7% DM in the MC compared to 20.1 % in OE region. In Pentland Dell the average % DM was 13.1 % was lower with 11.3% in MC compared to 14.9% (OE).

The application calcium nitrate to the seed bed ahead of planting at 50-200 kg $ha^{-1}$ to Pentland Dell increased the dry matter content of tubers (Table 3.27). Untreated tubers averaged 13.1% DM while increasing rates of calcium nitrate applied to the seed bed raised % DM content of tubers at harvest; with the highest value of 16.6% achieved with the application of 200 kg $ha^{-1}$. When % DM data from the opposite eighths or middle cortex were analysed the largest increase in % DM was observed in the opposite eighths region with controls recording 14.5% DM opposed to calcium nitrate treated tubers recording 19.5-20.1% DM. Samples from the middle cortex averaged 11.3% DM, tubers from plants receiving 100-200 kg $ha^{-1}$ increased %DM to 12.1 and 12.9% respectively. No effects of applying calcium on increased % DM were observed with Markies.

It is important to note that % DM values reported here are considerably lower than %DM recorded using displacement techniques. Here the % DM data was measured directly from tissue sampled from the different regions of the tuber and the values do not take into account the different proportions of tissue density across the tuber that make up whole tuber % DM data.

The distribution of dry matter from the opposite eighth segments and middle cortex will influence the processing quality of tubers. Standard DM assessments using water displacement may under-estimate low DM content of the inner cortex as a proportion of the overall tuber.

### 4.3.3 Sugar concentrations at harvest and during storage – calcium trial

On average, P. Dell had twice the sugar content at harvest (0.06% FW) than Markies (0.03%). Moreover, the predominant sugar of P. Dell was glucose, with equal amounts of sucrose and fructose, while 2/3rds of the sugar content of Markies was sucrose (Figure 3.3 and 3.4).
The influence of calcium application on sugar was less clear and dependent of variety and the position of tissue sampled from the tuber. Sugar profiles from the middle cortex tissue sampled from Markies showed a slight but significant rise in glucose content in tubers taken at harvest when tubers were grown under a 100 kg $ha^{-1}$ $Ca(NO_3)_2$ regime but not at the higher rate of 200 kg $ha^{-1}$ (Figure 3.3) P. Dell was not affected by pre-harvest calcium application.

*Table 3.27. Dry weights of Markies tubers grown under increasing supplementation with Calcium nitrate (Tropicote 19% Ca, 15.5% N), measured at harvest in November and after storage at 10°C for 3, 5, 7 months*

| **Markies** | | | | | |
|---|---|---|---|---|---|
| | Position | 0 | 50 kg $ha^{-1}$ | 100 kg $ha^{-1}$ | 200 kg $ha^{-1}$ |
| November | Opposite Eighths | 20.1 | 21.7 | 20.7 | 20.2 |
| | Mid Cortex | 13.7 | 14.2 | 13.4 | 13.0 |
| | **Average** | **16.9** | **18.0** | **17.1** | **16.6** |
| Feb | Opposite Eighths | 22.72 | 25.84 | 26.92 | 26.33 |
| | Mid Cortex | 19.44 | 23.97 | 24.36 | 24.50 |
| | **Average** | **21.08** | **24.91** | **25.64** | **25.42** |
| April | Opposite Eighths | 21.1 | 20.0 | 23.7 | 19.2 |
| | Mid Cortex | 14.5 | 14.8 | 15.1 | 15.1 |
| | **Average** | ***17.8*** | ***17.4*** | ***19.4*** | ***17.1*** |
| June | Opposite Eighths | 20.4 | 19.8 | 19.9 | 19.4 |
| | Mid Cortex | 13.2 | 14.5 | 14.4 | 13.4 |
| | **Average** | ***16.8*** | ***17.1*** | ***17.2*** | ***16.4*** |

| **Pentland Dell** | | | | | |
|---|---|---|---|---|---|
| | | 0 | 50 kg $ha^{-1}$ | 100 kg $ha^{-1}$ | 200 kg $ha^{-1}$ |
| November | Opposite Eighths | 14.9 | 20.1 | 19.5 | 20.0 |
| | Mid Cortex | 11.3 | 11.0 | 12.1 | 12.9 |
| | **Average** | **13.1** | **15.6** | **15.8** | **16.6** |
| Feb | Opposite Eighths | 22.24 | 26.13 | 24.78 | 24.77 |
| | Mid Cortex | 21.59 | 22.91 | 21.45 | 21.68 |
| | **Average** | **21.92** | **24.52** | **23.12** | **23.23** |
| April | Opposite Eighths | 19.4 | 19.8 | 19.1 | 19.8 |
| | Mid Cortex | 13.3 | 13.7 | 13.4 | 14.4 |
| | **Average** | ***16.3*** | ***16.9*** | ***16.3*** | ***17.1*** |
| June | Opposite Eighths | 18.3 | 19.7 | 19.1 | 20.9 |
| | Mid Cortex | 12.9 | 13.0 | 12.4 | 13.1 |
| | **Average** | ***15.6*** | ***16.3*** | ***15.8*** | ***17.0*** |

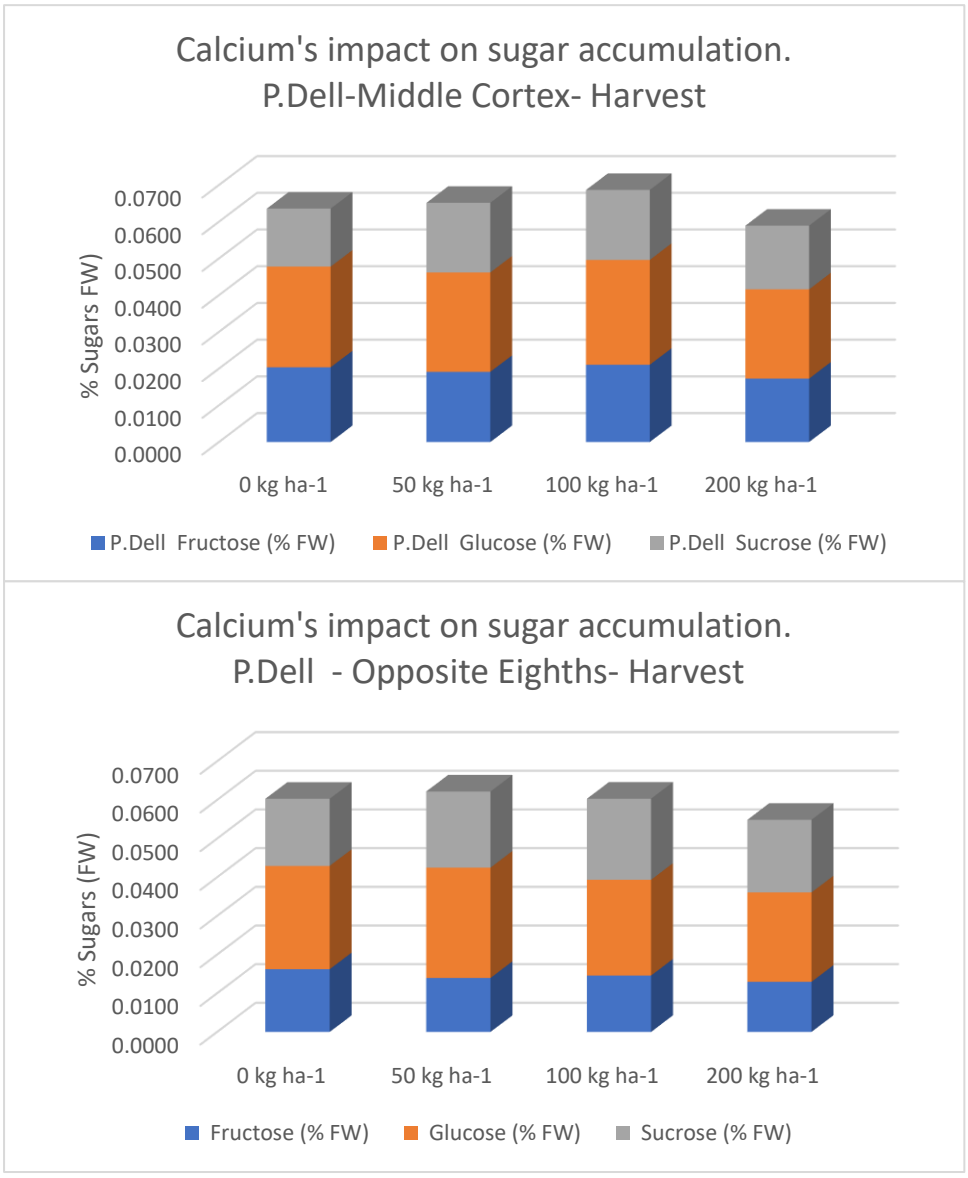



*Figure 3.3. Sugar analysis of Pentland Dell sampled form the middle cortex and opposite eighths segments of the tuber at harvest for a range of calcium treatments.*

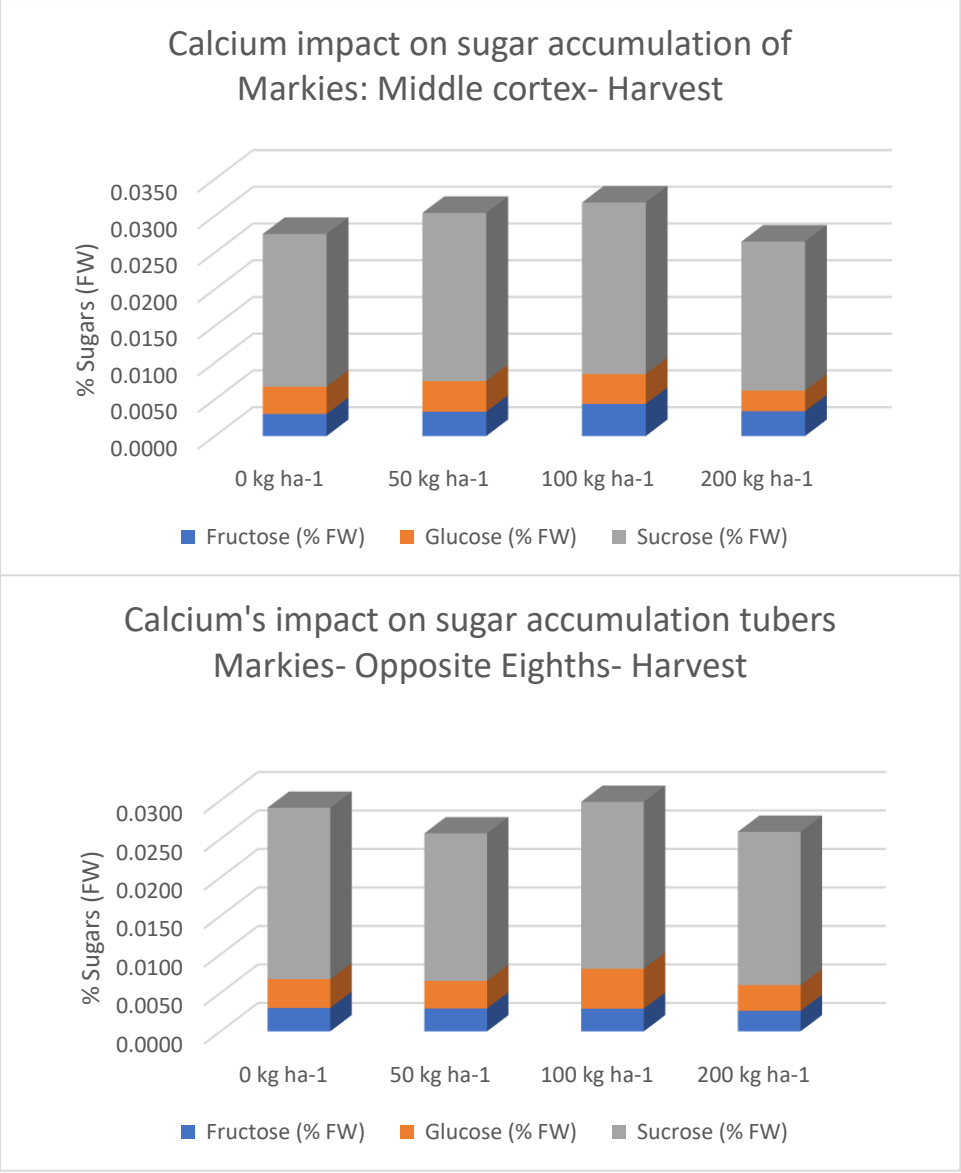



*Figure 3.4. Sugar analysis of Markies sampled form the middle cortex and opposite eighths segments of the tuber at harvest for a range of calcium treatments..*

In the second year of the trial, calcium pre-treatments in the field to Pentland Dell (Table 3.28) and Markies (Table 3.29) failed to alter tubers sugar profile at harvest or alter tubers propensity to sweeten during storage with the exception that calcium treatments (50-200 kg $ha^{-1}$) transiently reduced sucrose content in Pentland Dell after 5 month storage but then increased by month 7.

*Table 3.28. P. Dell season 2016/17 impact of calcium fertilisers 50-200 kg ha$^{-1}$ on sugars accumulation on tubers stored at 10°C. Means with the same letter are not significantly different (Tukey test "multcomp" package p < 0.05).*

| | | Treatment kg ha$^{-1}$ | | | |
|---|---|---|---|---|---|
| **Sugar** | **Month** | **0** | **50** | **100** | **200** |
| Fructose | 0 | 0.018$^{c}$ | 0.017$^{c}$ | 0.018$^{c}$ | 0.015$^{c}$ |
| | 3 | 0.021$^{c}$ | 0.023$^{c}$ | 0.024$^{c}$ | 0.022$^{c}$ |
| | 5 | 0.001$^{a}$ | 0.006$^{b}$ | 0.004$^{a}$ | 0.006$^{b}$ |
| | 7 | 0.086$^{d}$ | 0.090$^{d}$ | 0.091$^{d}$ | 0.092$^{d}$ |
| | p-value | | <0.001 | | |
| Glucose | 0 | 0.027$^{c}$ | 0.028$^{c}$ | 0.027$^{c}$ | 0.024$^{c}$ |
| | 3 | 0.024$^{c}$ | 0.030$^{c}$ | 0.030$^{c}$ | 0.027$^{c}$ |
| | 5 | 0.004$^{a}$ | 0.006$^{b}$ | 0.005$^{ab}$ | 0.007$^{b}$ |
| | 7 | 0.088$^{d}$ | 0.097$^{d}$ | 0.092$^{d}$ | 0.099$^{d}$ |
| | p-value | | <0.01 | | |
| Sucrose | 0 | 0.017$^{cd}$ | 0.019$^{de}$ | 0.020$^{de}$ | 0.018$^{de}$ |
| | 3 | 0.021$^{de}$ | 0.027$^{e}$ | 0.024$^{de}$ | 0.024$^{de}$ |
| | 5 | 0.016$^{c}$ | **0.005$^{b}$** | **0.003$^{a}$** | **0.004$^{ab}$** |
| | 7 | 0.074$^{f}$ | 0.077$^{f}$ | 0.083$^{f}$ | 0.079$^{f}$ |
| | p-value | | <0.001 | | |

*Table 3.29. Markies season 2016/17 impact of calcium fertilisers 50-200 kg ha$^{-1}$ on sugars accumulation on tubers stored at 10°C. Means with the same letter are not significantly different (Tukey test "multcomp" package p < 0.05).*

| | | Treatment kg ha$^{-1}$ | | | |
|---|---|---|---|---|---|
| | **Months** | **0** | **50** | **100** | **200** |
| Fructose | 0 | 0.003$^{ac}$ | 0.003$^{ac}$ | 0.004$^{acd}$ | 0.003$^{ac}$ |
| | 3 | 0.008$^{de}$ | 0.007$^{ce}$ | 0.007$^{de}$ | 0.006$^{bce}$ |
| | 5 | 0.009$^{de}$ | 0.004$^{a}$ | 0.011$^{e}$ | 0.014$^{e}$ |
| | 7 | 0.003$^{ab}$ | 0.006$^{ce}$ | 0.008$^{de}$ | 0.004$^{acd}$ |
| | p-value | | <0.001 | | |
| Glucose | 0 | 0.004$^{cd}$ | 0.004$^{cd}$ | 0.005$^{cdf}$ | 0.003$^{bcd}$ |
| | 3 | 0.009$^{ef}$ | 0.011$^{ef}$ | 0.010$^{f}$ | 0.007$^{df}$ |
| | 5 | 0.001$^{ab}$ | 0.001$^{a}$ | 0.0003$^{a}$ | 0.005$^{ac}$ |
| | 7 | 0.001$^{a}$ | 0.000$^{a}$ | 0.006$^{cd}$ | 0.000$^{a}$ |
| | p-value | | <0.001 | | |
| Sucrose | 0 | 0.022$^{c}$ | 0.021$^{c}$ | 0.023$^{c}$ | 0.020$^{c}$ |
| | 3 | 0.023$^{c}$ | 0.017$^{bc}$ | 0.022$^{c}$ | 0.021$^{c}$ |
| | 5 | 0.005$^{a}$ | 0.024$^{b}$ | 0.004$^{a}$ | 0.004$^{a}$ |
| | 7 | 0.025$^{c}$ | 0.022$^{c}$ | 0.029$^{c}$ | 0.018$^{bc}$ |
| | p-value | | <0.001 | | |

### 4.3.4 Agronomic traits and mineral analysis at harvest – phosphate trial

The application of additional phosphate to a seed bed with a phosphate index of 1 failed to increase the number of tubers or the yield of tubers for either Markies or Pentland Dell in the second year of the trial (Table 3.30). In P. Dell there was a slight increase in tuber numbers per plant and yield but just failed to reach significance at ($p<0.05$). Application of higher phosphate rates of 150-300 kg $ha^{-1}$ increased the accumulation of phosphate in tissues along with zinc, copper, sulphur and calcium. (Table 3.31). Distribution of nutrients across the tuber favoured accumulation in the central region of the tuber with most macro and micronutrients higher in the centre compared to the opposite eighths' sections (Tables 3.32 and 3.33).

*Table 3.30. Increasing Phosphate application to the seed bed at planting failed to increase yield of Markies and Pentland Dell tubers at harvest (2017).*

| Variety | $PO_3^-$ | Number tubers per plant | Total Yield (t/Ha) | Yield >45mm (t/Ha) |
|---|---|---|---|---|
| Markies | 0 | 9 | 64.2 | 63.9 |
| | 75 | 12 | 65.6 | 64.3 |
| | 150 | 12 | 65.8 | 64.2 |
| | 300 | 11 | 66.1 | 65.3 |
| Pentland Dell | 0 | 12 | 48.8 | 47.3 |
| | 75 | 15 | 52.5 | 50.3 |
| | 150 | 15 | 51.8 | 49.8 |
| | 300 | 14 | 53.1 | 51.2 |
| $LSD_{0.05}$ | Variety | 2.8 | 7.7 | 9.5 |
| | Treatment | 3.2 | 6.8 | 8.9 |
| | Variety x treatment | 5.5 | 9.1 | 14.2 |

*Table 3.31. Higher rate Phosphate application to the seed bed increased phosphate content of Markies and Pentland Dell (overall average) tubers at harvest (2017).*

| Phosphate kg $ha^{-1}$ | 0 | 75 | 150 | 300 | f.prob | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|
| Phosphorous (%) | 0.24 | 0.26 | **0.28** | **0.29** | 0.007 | 0.033 |
| Boron (ppm) | 6.24 | 6.06 | 6.68 | 6.61 | 0.046 | 0.498 |
| Zinc (ppm) | 20.74 | 20.11 | **22.84** | **22.01** | <.001 | 1.187 |
| Sulphur (%) | 0.12 | 0.12 | **0.13** | **0.13** | <.001 | 0.0058 |
| Calcium (%) | 0.06 | 0.05 | **0.07** | 0.06 | <.001 | 0.0031 |
| Magnesium (%) | 0.11 | 0.11 | 0.12 | 0.11 | 0.008 | 0.0672 |

*Table 3.32. Macro nutrient distribution across the tuber and overall comparison of nutrient profile between Markies and Pentland Dell (average across all treatments).*

| | Position | | | Variety | | | Position, Variety |
|---|---|---|---|---|---|---|---|
| Macro (%) | End | Middle | F prob | Markies | P. Dell | F.frob | $LSD_{0.05}$ |
| Calcium | 0.06 | 0.06 | *0.257* | 0.06 | 0.06 | *0.257* | 0.0022 |
| Magnessium | 0.11 | **0.12** | *<.001* | **0.12** | 0.1 | *<.001* | 0.0036 |
| Nitrogen | 1.66 | **2.0** | *<.001* | 1.9 | 1.77 | *0.018* | 0.1045 |
| Phosphorous | 0.24 | **0.3** | *<.001* | 0.28 | 0.25 | *0.008* | 0.0234 |
| Potassium | 2.3 | **2.79** | *<.001* | 2.59 | 2.5 | *0.203* | 0.1045 |
| Sulphur | 0.12 | **0.13** | *<.001* | 0.12 | **0.13** | *<.001* | 0.412 |

*Table 3.33. Micro nutrient distribution across the tuber and overall comparison of nutrient profile between Markies and Pentland Dell (average across all treatments).*

| | Position | | | Variety | | | Position, Variety |
|---|---|---|---|---|---|---|---|
| Micro (ppm) | End | Middle | F prob | Markies | P. Dell | F.frob | $LSD_{0.05}$ |
| Boron | 5.6 | **7.2** | *<.001* | 6.45 | 6.35 | *0.571* | 0.352 |
| Copper | 9.59 | **14.89** | *<.001* | 11.39 | 13.09 | *0.111* | 2.1 |
| Iron | 124.6 | 126.6 | *0.796* | **135** | 116.2 | *0.019* | 15.5 |
| Manganese | 10.26 | 10.13 | *0.798* | 10.69 | 9.69 | *0.051* | 1 |
| Molybdenum | 0.16 | 0.14 | *0.497* | **0.21** | 0.09 | *<.001* | 0.038 |
| Zinc | 19.2 | **27.02** | *<.001* | 21.95 | **24.27** | *0.003* | 0.838 |

### 4.3.5 Sugar concentrations at harvest and during storage – phosphate trial

In the second year of the trial the sugar profile of P. Dell and Markies tubers grown under phosphate supplementation (75, 150 and 300 kg $ha^{-1}$) at harvest were similar across treatments.

Storage of Pentland Dell at 3.5°C led to a rapid rise in glucose and fructose (Table 3.34) within the first 3 months of sampling. Applying phosphate at the highest rate 300 kg $ha^{-1}$ at planting slowed the rate of reducing sugar accumulation in tubers stored between 3-5 months but by 7 months the rate of reducing sugar accumulation were similar across treatments (Table 3.34). Pentland Dell stored at 10°C maintained stable fructose and glucose concentrations for the first 5 months of storage and then by the end of the 7 month trial reducing sugars increased due to senescent sweetening. Phosphate pre-treatments failed to influence the late season rise in sugars.

*Table 3.34. Sugars accumulation for Pentland Dell during season 2016/17 for different phosphate fertilizations, stored at 10°C and 3.5°C. Means with the same letter are not significantly different (Tukey test; "multcomp", p < 0.05).*

| | | Storage Temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10°C | | | | 3.5°C | | | |
| **Sugar** | **Month** | **P0** | **P75** | **P150** | **P300** | **P0** | **P75** | **P150** | **P300** |
| | **0** | $0.018^{ab}$ | $0.016^{ab}$ | $0.017^{ab}$ | $0.015^{ab}$ | na | na | na | na |
| | **3** | $0.005^{a}$ | $0.018^{a}$ | $0.020^{ab}$ | $0.023^{ab}$ | $0.131^{f}$ | $0.028^{f}$ | $0.123^{f}$ | $0.036^{f}$ |
| **Fructose** | **5** | $0.020^{ab}$ | $0.006^{ab}$ | $0.010^{ab}$ | $0.008^{ab}$ | $0.159^{ef}$ | $0.154^{bc}$ | $0.142^{e}$ | **$0.116^{ab}$** |
| | **7** | $0.059^{cd}$ | $0.074^{cd}$ | $0.073^{d}$ | $0.072^{d}$ | $0.155^{ef}$ | $0.159^{ef}$ | $0.136^{ef}$ | $0.137^{e}$ |
| | ***p-value*** | ***<0.001*** | | | | ***<0.001*** | | | |
| | **0** | $0.027^{ab}$ | $0.026^{ab}$ | $0.025^{ab}$ | $0.023^{ab}$ | na | na | na | na |
| | **3** | $0.008^{a}$ | $0.023^{ab}$ | $0.024^{ab}$ | $0.029^{abcd}$ | $0.146^{efg}$ | $0.026^{efg}$ | $0.134^{fg}$ | $0.038^{g}$ |
| **Glucose** | **5** | $0.025^{ab}$ | $0.008^{a}$ | $0.012^{a}$ | $0.009^{a}$ | $0.166^{ef}$ | $0.132^{ab}$ | $0.149^{ef}$ | **$0.111^{abc}$** |
| | **7** | $0.057^{bcd}$ | $0.064^{d}$ | $0.061^{bcd}$ | $0.068^{cd}$ | $0.147^{efg}$ | $0.180^{ef}$ | $0.127^{efg}$ | $0.146^{e}$ |
| | ***p-value*** | ***<0.001*** | | | | ***<0.001*** | | | |
| | **0** | $0.016^{ab}$ | $0.019^{abc}$ | $0.016^{abc}$ | $0.020^{abc}$ | na | na | na | na |
| | **3** | $0.009^{a}$ | $0.029^{c}$ | $0.026^{bc}$ | $0.025^{bc}$ | $0.068^{fghi}$ | $0.015^{ghi}$ | $0.064^{hi}$ | $0.019^{i}$ |
| **Sucrose** | **5** | $0.030^{c}$ | **$0.015^{ab}$** | **$0.015^{ab}$** | **$0.015^{ab}$** | $0.076^{fghi}$ | $0.065^{ab}$ | $0.068^{efg}$ | **$0.061^{ab}$** |
| | **7** | $0.051^{d}$ | $0.060^{efg}$ | $0.055^{de}$ | $0.058^{def}$ | $0.070^{fghi}$ | $0.078^{efgh}$ | $0.066^{efgh}$ | $0.068^{defg}$ |
| | ***p-value*** | ***<0.001*** | | | | ***<0.001*** | | | |

*Table 3.35. Sugars accumulation for Markies during season 2016/17 for different phosphate fertilizations, stored at 10°C and 3.5°C. Means with the same letter are not significantly different (Tukey test; "multcomp", p < 0.05).*

| | | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10°C | | | | 3.5°C | | | |
| **Sugar** | **Month** | **P0** | **P75** | **P150** | **P300** | **P0** | **P75** | **P150** | **P300** |
| | **0** | $0.003^{ab}$ | $0.004^{ab}$ | $0.004^{ab}$ | $0.003^{ab}$ | na | na | na | na |
| | **3** | $0.001^{ab}$ | $0.001^{a}$ | $0.001^{a}$ | $0.002^{ab}$ | $0.099^{def}$ | $0.101^{def}$ | $0.081^{ef}$ | $0.073^{f}$ |
| **Fructose** | **5** | $0.026^{ab}$ | $0.030^{b}$ | $0.022^{ab}$ | $0.016^{ab}$ | $0.118^{cdef}$ | $0.124^{cdef}$ | $0.104^{cd}$ | $0.097^{c}$ |
| | **7** | $0.002^{ab}$ | $0.004^{ab}$ | $0.004^{ab}$ | $0.004^{ab}$ | $0.109^{cdef}$ | $0.107^{def}$ | $0.100^{cdef}$ | $0.092^{cde}$ |
| | ***p-value*** | **<0.05** | | | | **<0.05** | | | |
| | **0** | $0.003^{a}$ | $0.006^{ab}$ | $0.005^{a}$ | $0.007^{ab}$ | na | na | na | na |
| | **3** | $0.000^{a}$ | $0.000^{a}$ | $0.001^{a}$ | $0.001^{a}$ | $0.101^{ef}$ | $0.100^{ef}$ | $0.080^{ef}$ | $0.072^{f}$ |
| **Glucose** | **5** | $0.008^{a}$ | $0.006^{a}$ | $0.011^{a}$ | $0.016^{a}$ | $0.084^{def}$ | $0.098^{def}$ | $0.083^{cde}$ | $0.079^{cd}$ |
| | **7** | $0.006^{a}$ | $0.000^{a}$ | $0.045^{bc}$ | $0.003^{a}$ | $0.092^{cde}$ | $0.068^{de}$ | $0.118^{cde}$ | **$0.057^{cde}$** |
| | ***p-value*** | **<0.001** | | | | **<0.001** | | | |
| | **0** | $0.020^{bcd}$ | $0.021^{cd}$ | $0.022^{cd}$ | $0.021^{bcd}$ | na | na | na | na |
| | **3** | $0.002^{a}$ | $0.003^{a}$ | $0.003^{a}$ | $0.004^{ab}$ | $0.120^{hi}$ | $0.144^{i}$ | $0.093^{j}$ | **$0.077^{k}$** |
| **Sucrose** | **5** | $0.014^{abcd}$ | $0.020^{abcd}$ | $0.009^{abc}$ | $0.009^{abc}$ | $0.100^{gh}$ | $0.094^{h}$i | $0.079^{fgh}$ | **$0.078^{e}$** |
| | **7** | $0.025^{cd}$ | $0.022^{cd}$ | $0.023^{cd}$ | $0.027^{d}$ | $0.070^{fgh}$ | $0.068^{efg}$ | $0.059^{efg}$ | $0.062^{ef}$ |
| | ***p-value*** | **<0.05** | | | | **<0.05** | | | |

Markies stored at 3.5°C showed a rapid rise in fructose, glucose and sucrose within the first 3 months of storage and thereafter the accumulation of sugars remained constant (Table 3.35). Phosphate applied pre-harvest at 300 kg $ha^{-1}$ to Markies lowered glucose content at the end of 7 months storage and sucrose was transiently reduced between 3-5 months before rising in line with other treatments at the end of the storage trial. Storing tubers at 10°C reduced significantly the amount of sugars present.

### 4.3.6 Tuber Starch content – phosphate trial

Starch content of freeze-dried tuber material using an α-amylase digestion assay found no significant difference between varieties, phosphate treatments or the length of storage.

In both varieties, phosphate supplementation during cultivation did not influence starch accumulation (Figure 3.5) in tubers at 10°C, however these tubers were lower in sugar accumulation than the ones stored at 3.5°C.

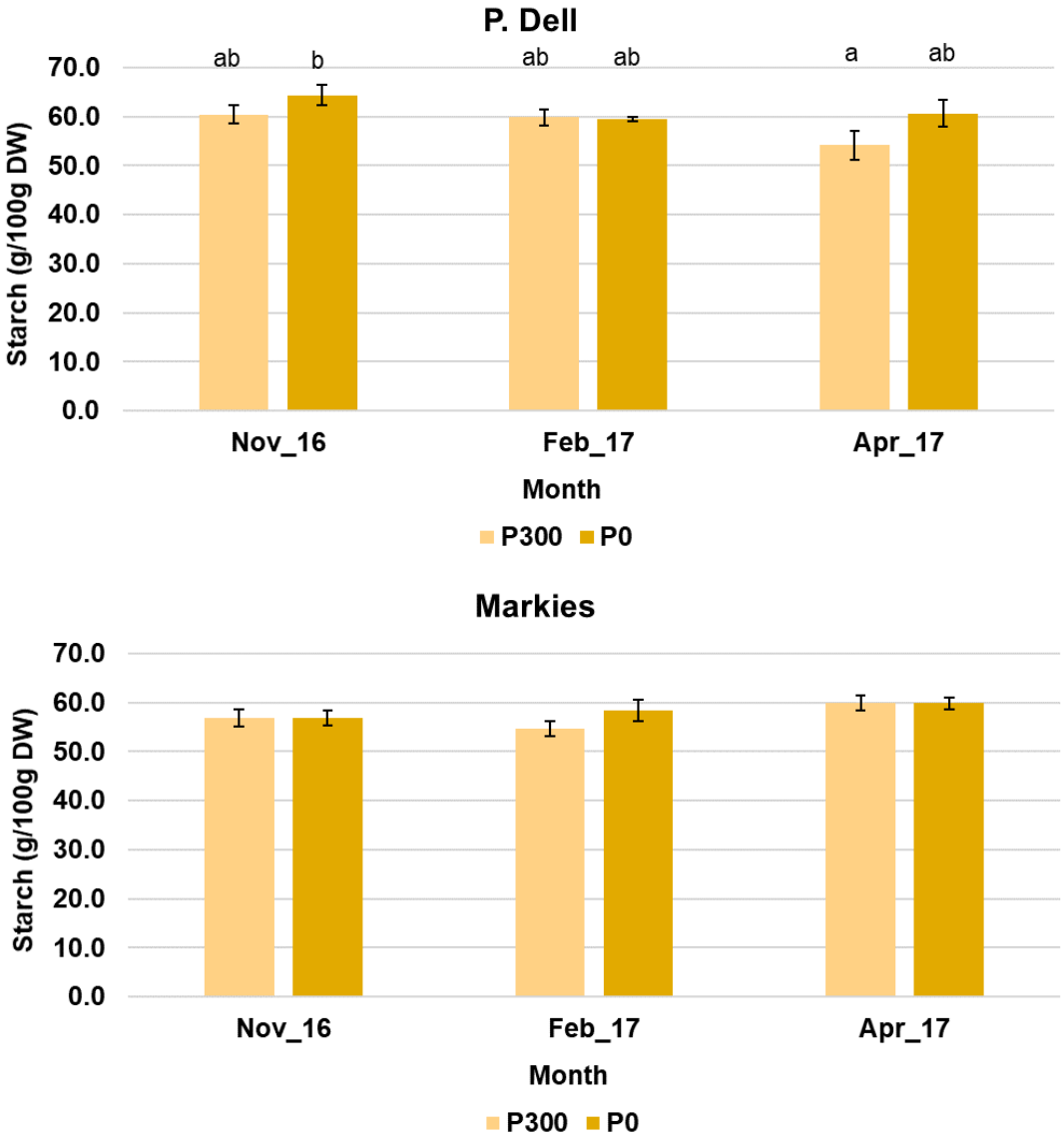



*Figure 3.5. Starch content (g/100g DW) for Markies and Pentland Dell grown under phosphate fertilization of 0 and 300 kg/ha regimes and stored at 10°C, with SE bars. Mean values with different letters are significantly different according to Tukey p<0.05 test.*

### 4.3.7 Fry Colour analysis – calcium and phosphate trials

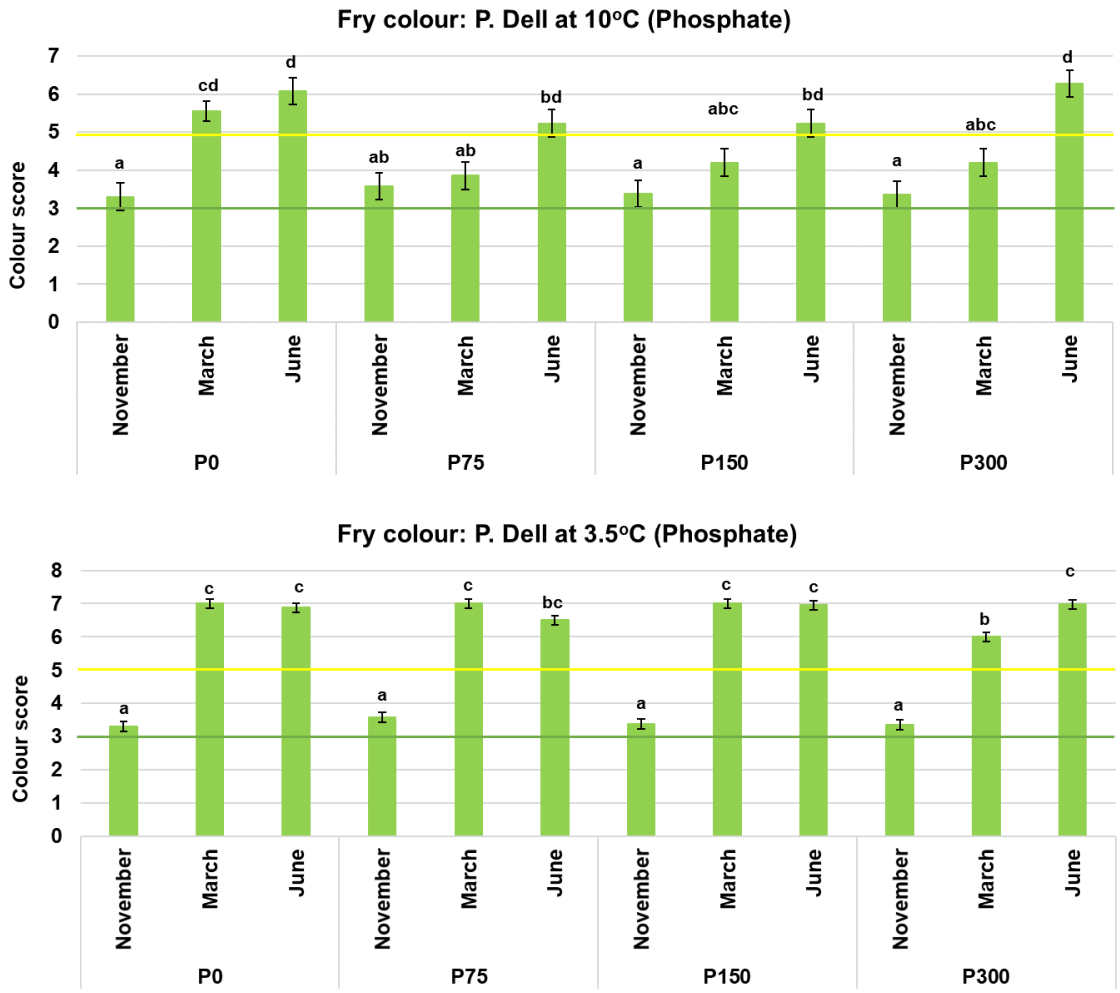
Fry colour analysis scores at intake (November) using the SBCSR fry colour test for chips showed that all consignments were below the threshold score of 4 at harvest (Figure 3.6, 3.7 and 3.8). No effects of pre-harvest calcium or phosphate treatments were observed at harvest. Half of the phosphate-treated crop was stored at 3.5°C while the rest remained at 10°C along with all of the calcium treated tubers after crop pull down. Fry colour analysis of Markies stored

at 10°C between 3 and 7 months remained stable (fry colour value 2) and below the quality thresholds by the trial termination date of 7 months (June 2017; Figure 3.6 and 3.8).
Pentland Dell tubers had a darker fry colour at harvest, averaging 3.4 (SBCSR-Chip fry colour) but maintained stable fry colour for 4 months at 10°C, however by 7 months of storage, fry colour had darkened substantially to 6.5-6.7 well above the quality threshold (Figure 3.6 and 3.7).

### 4.3.8 Fry colour – phosphate trial

Tubers generated from phosphate-treated plants and stored at 3.5°C exhibited LTS symptoms within 3 months of storage, resulting in fry colour scores of 5-6 (SBCSR-chip) for Markies and 6-7 for Pentland Dell (Figure 3.6 and 3.7). In both cases, phosphate treated tubers under a 300 kg $ha^{-1}$ regime showed lower fry colour scores for Markies, (5) and Pentland Dell (6), suggesting high phosphate supplementation had reduced susceptibility to LTS. After 7 months storage at 3.5°C, the fry colour of Markies was significantly lower in tubers grown under phosphate supplementation of 200-300 kg $ha^{-1}$ with scores of 5.1-5.5, compared to non-supplemented plots where fry colour was 6.7. Fry colour analysis for Pentland Dell tubers after 7 months storage at 3.5°C found chip colour had darkened with SBCSR scores of (6.5-7.0), in this case added phosphate during the growing season failed to prevent the decrease in fry quality late in the season. While fry-colour scores above 4 are considered commercially unacceptable, higher phosphate supplementation was able to lower tubers sensitivity to chilling damage. The impact of adding phosphate on fry colour needs to be considered in relation to phosphate's impact on crop cultivation on tuber size and dry matter content.

Meanwhile, storage of Markies tubers at 10°C maintained acceptable fry colours of 2.1-2.2 throughout the 7 months of the storage trial and no additional benefit on fry colours were gained by the addition of phosphate. In contrast, P. Dell, sweetened rapidly returning unacceptable fry colour scores after 3 months storage of 3.8-4.2 increasing to (5.2-6.3) by 7 months storage at 10°C. While no trend in fry colours against phosphate applied during cultivation was observed, phosphate-treated tubers grown under 75-150 kg $ha^{-1}$ phosphate regimes returned lower fry colour values (5.2) than untreated crop (6), however, tubers growing under 300 kg $ha^{-1}$ P yielded the highest fry colour values (6.3).




*3.6. Fry colour analysis (SBCSR) for chip samples taken from a 20 tuber sample Pentland Dell grown under increasing phosphate regimes and stored at 3.5°C and 10°C, with SE bars. Mean values with different letters are significantly different according to Tukey p<0.05 test.*

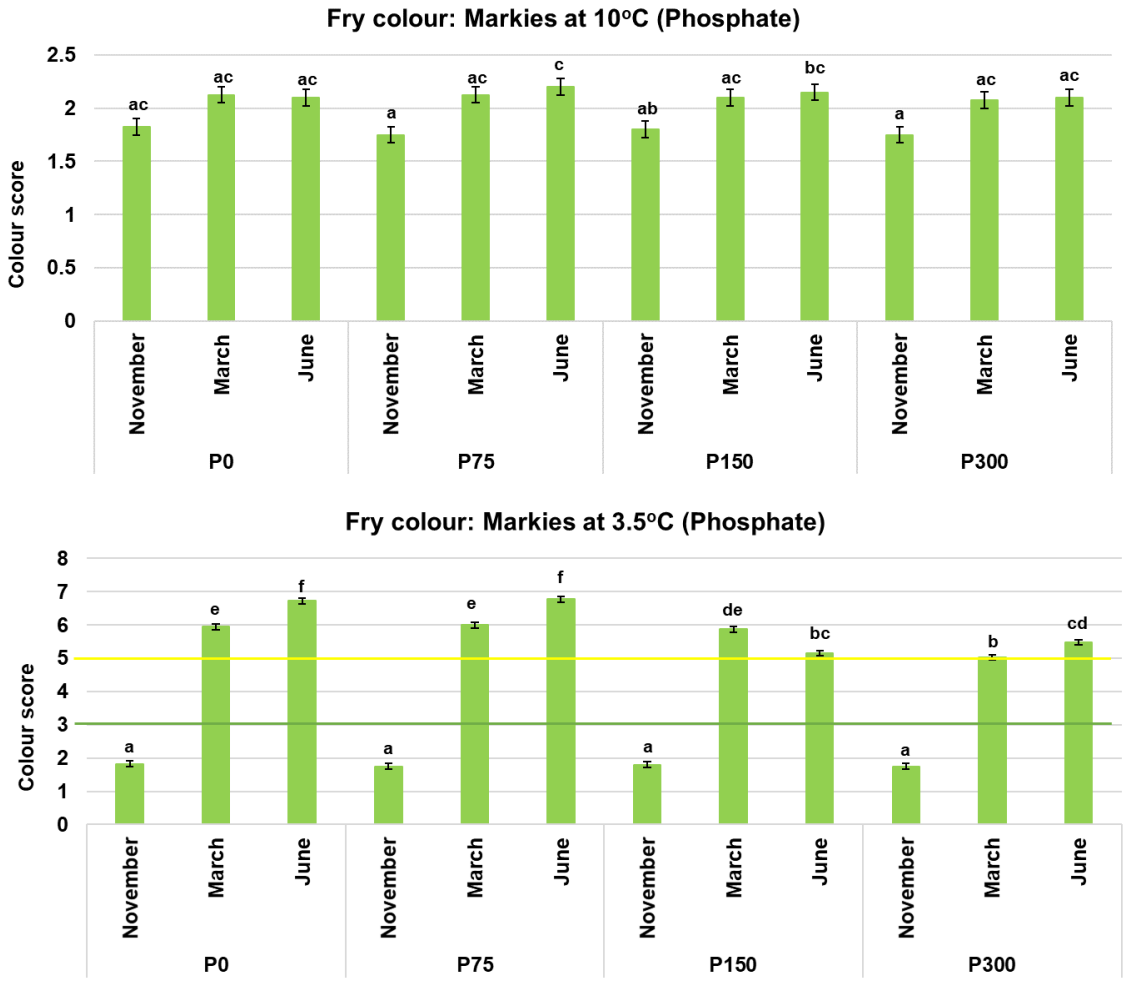



*Figure 3.7. Fry colour analysis (SBCSR) for chip samples taken from a 20 tuber sample Markies grown under increasing phosphate regimes and stored at 3.5°C and 10°C, with SE bars. Mean values with different letters are significantly different according to Tukey p<0.05 test.*

### 4.3.9 Fry colour – calcium trial

Markies stored at 10°C retained fry colours of 2-2.1 throughout the 7 months of storage, while fry colours for Pentland Dell were maintained below 4 for the first 3-4 months of storage thereafter tubers started to sweeten and by the end of storage. Pre-harvest treatment with calcium failed to improve on fry colour scores (Figure 3.8).


*Figure 3.8. Fry colour analysis (SBCSR) for chip samples taken from a 20 tuber sample Markies and Pentland Dell grown under calcium fertigation regimes and stored at 10°C, with SE bars. Mean values with different letters are significantly different according to Tukey p<0.05 test.*

## 4.4 3rd year

### 4.4.1 The relationship between tuber respiration and tuber quality – year 3

The purpose of year three was to correlate the use of detailed analysis of respiration data with changes in sugar content, texture changes and biochemical markers for ageing (ROS and ascorbic acid).

### 4.4.2 Sugars

Analysis of sugars sampled from the opposite eighths and middle-cortex found no significant difference ($p < 0.05$) in the distribution of fructose, glucose and sucrose in Markies and P. Dell. Hence, subsequent data analysis was restricted to storage temperature and sampling times.

As expected, an impact of temperature and length of storage on the amount of sugars accumulating in tubers ($p<0.001$) was observed for both varieties.

The sucrose, glucose and fructose content of Markies increased rapidly in response to storage at 3.5°C. Sucrose content of tubers increased from 0.015% DW at harvest (Figure 3.11, Table 3.37)) to 0.82% DW after 3 months storage before dropping back to 0.52% DW at the end of storage at 7 months. Fructose and glucose content of Markies at harvest was 0.0012% and 0.0023% DW respectively, rising rapidly to 0.26 and 0.25 % DW after 4 months storage then dropping back to 0.06% and 0.12% DW at the end of 7 months storage at 3.5°C (Table 3.36 and 3.37).

*Table 3.36. Dry weights of Markies and P. Dell tubers stored at 3.5 and 10 °C (season 2017/18).*

| | | Markies | | | P. Dell | | |
|---|---|---|---|---|---|---|---|
| | | Position | | | | | |
| **Months in storage** | | **Apical** | **Middle** | **Bottom** | **Apical** | **Middle** | **Bottom** |
| **0** | **10°C** | 21.2 | 15.4 | na | 20.8 | 13.5 | na |
| **1** | | 21.3 | 16.5 | na | 21.8 | 17.3 | na |
| **2** | | 21.8 | 14.0 | 22.0 | 20.0 | 13.6 | 22.1 |
| **3** | | 22.0 | 16.0 | 23.8 | 21.8 | 14.8 | 24.3 |
| **4** | | 21.0 | 13.5 | 22.7 | 21.3 | 14.8 | 22.2 |
| **5** | | 18.1 | 13.3 | 19.7 | 19.8 | 14.1 | 22.1 |
| **6** | | 21.9 | 13.5 | 22.4 | 22.7 | 16.4 | 23.1 |
| **7** | | 21.6 | 14.1 | 23.2 | 22.6 | 16.6 | 24.6 |
| **0** | **3.5°C** | 23.3 | 13.7 | na | 20.71 | 14.1 | na |
| **1** | | 22.3 | 17.9 | na | 21.9 | 17.8 | na |
| **2** | | 22.1 | 14.3 | 22.5 | 20.8 | 14.1 | 21.2 |
| **3** | | 22.2 | 14.5 | 22.4 | 21.6 | 15.8 | 22.6 |
| **4** | | 22.5 | 14.3 | 23.2 | 21.8 | 16.8 | 22.4 |
| **5** | | 21.0 | 14.7 | 21.4 | 20.3 | 15.3 | 21.9 |
| **6** | | 23.2 | 14.9 | 23.9 | 20.5 | 15.6 | 22.8 |
| **7** | | 23.7 | 15.0 | 24.2 | 22.1 | 15.4 | 22.8 |
| ***p-value*** | | | ***>0.05*** | | | ***>0.05*** | |

*Table 3.37. Sugar content of Markies and P. Dell tubers stored at 3.5 and 10 °C (season 2017/18). Mean values with different letters are significantly different according to Tukey$_{p<0.05}$ test.*

| Months in storage | | Markies | | | P. Dell | | |
|---|---|---|---|---|---|---|---|
| | | Fructose | Glucose | Sucrose | Fructose | Glucose | Sucrose |
| **0** | **10°C** | 0.001$^{a}$ | 0.002$^{a}$ | 0.01$^{a}$ | 0.01$^{a}$ | 0.01$^{a}$ | 0.01$^{a}$ |
| **1** | | 0.01$^{b}$ | 0.01$^{bc}$ | 0.07$^{c}$ | 0.05$^{c}$ | 0.06$^{def}$ | 0.03$^{b}$ |
| **2** | | 0.01$^{bc}$ | 0.01$^{c}$ | 0.06$^{bc}$ | 0.04$^{b}$ | 0.04$^{c}$ | 0.08$^{d}$ |
| **3** | | 0.01$^{bc}$ | 0.01$^{bc}$ | 0.06$^{bc}$ | 0.03$^{b}$ | 0.03$^{b}$ | 0.06$^{c}$ |
| **4** | | 0.01$^{b}$ | 0.02$^{d}$ | 0.05$^{b}$ | 0.07$^{d}$ | 0.06$^{cde}$ | 0.12$^{ef}$ |
| **5** | | 0.01$^{bc}$ | 0.01$^{b}$ | 0.06$^{bc}$ | 0.05$^{c}$ | 0.05$^{cd}$ | 0.12$^{e}$ |
| **6** | | 0.02$^{c}$ | 0.01$^{bc}$ | 0.07$^{c}$ | 0.07$^{d}$ | 0.08$^{ef}$ | 0.15$^{f}$ |
| **7** | | 0.02$^{c}$ | 0.04$^{de}$ | 0.22$^{d}$ | 0.06$^{cd}$ | 0.09$^{f}$ | 0.22$^{g}$ |
| **0** | **3.5°C** | 0.001$^{a}$ | 0.002$^{a}$ | 0.02$^{a}$ | 0.01$^{a}$ | 0.01$^{a}$ | 0.01$^{a}$ |
| **1** | | 0.05$^{d}$ | 0.05$^{ef}$ | 0.74$^{ef}$ | 0.33$^{fg}$ | 0.33$^{hi}$ | 0.22$^{gh}$ |
| **2** | | 0.07$^{d}$ | 0.07$^{fg}$ | 0.80$^{ef}$ | 0.28$^{f}$ | 0.28$^{h}$ | 0.27$^{hij}$ |
| **3** | | 0.14$^{e}$ | 0.14$^{h}$ | 0.82$^{f}$ | 0.27$^{f}$ | 0.29$^{h}$ | 0.24$^{ghi}$ |
| **4** | | 0.26$^{f}$ | 0.25$^{i}$ | 0.60$^{ef}$ | 0.44$^{g}$ | 0.42$^{i}$ | 0.29$^{ij}$ |
| **5** | | 0.18$^{ef}$ | 0.17$^{hi}$ | 0.58$^{e}$ | 0.32$^{f}$ | 0.31$^{hi}$ | 0.32$^{j}$ |
| **6** | | 0.19$^{ef}$ | 0.17$^{hi}$ | 0.56$^{ef}$ | 0.29$^{f}$ | 0.29$^{h}$ | 0.29$^{ij}$ |
| **7** | | 0.06$^{d}$ | 0.13$^{gh}$ | 0.52$^{e}$ | 0.12$^{e}$ | 0.19$^{g}$ | 0.33$^{j}$ |
| ***p-value*** | | ***<0.001*** | ***<0.001*** | ***<0.001*** | ***<0.001*** | ***<0.001*** | ***<0.001*** |

Markies stored at 10˚C led to a gradual and small increase of sugars over the storage period. Sucrose at harvest 0.012% DW rose to 0.22% DW by the end of storage at 7 months. Fructose and glucose started at harvest at 0.0013% and 0.0024% DW respectively, rising gradually to 0.018 and 0.035 % DW at the end of 7 months storage.

The sucrose profile of P. Dell stored at 10˚C increased from 0.0098% DW at harvest to 0.21% DW after 7 months (Figure 3.9, Table 3.37) while glucose rose from 0.011% DW at harvest to 0.091% DW after 7 months; fructose content was 0.0104% DW at harvest peaking in March at 0.07% DW before dropping to 0.057% DW after 7 months storage (Table 3.37).

Storage of Pentland Dell at 3.5˚C led to an increase in glucose and fructose but not sucrose which is in contrast to Markies. Sucrose content rose from 0.008% DW at harvest rising to 0.29% DW after 7 months; glucose content was 0.014% DW at harvest peaking at 0.416% after 4 months and thereafter declining to 0.28% DW after 7 months; fructose 0.0129 at harvest peaking in March at 0.44% DW dropping to 0.285% DW after 7 months.

Sprout growth increased between 1 and 2 month storage despite 2 applications of CIPC at intake and in December (Figure 3.9 Bottom and 3.11 Bottom).

As expected low-temperature sweetening was observed with higher fructose and glucose content in Markies and Pentland Dell ($p<0.05$) present in tubers stored at 3.5˚C. In response to storage at low temperatures, Markies accumulated 2.8 times more sucrose than Pentland Dell, but the latter variety contained 2 fold more fructose and glucose suggesting that the cold-induced invertases were highly inducible in Pentland Dell.

### 4.4.3 Respiration rates

The respiration rates of Markies stored at 10°C measured as production rate of $CO_2$ remained fairly constant over time, settling at 0.8 mL/g/h for the first 5 months of storage then rising after 6 months of storage to 1.5 ml/g/h with a final increase to just over 2 ml/g/h by the end of storage at 7 months. Interestingly, the respiration rate of tubers stored at 3.5˚C followed the same production pattern as tubers stored at 10ºC (Figure 3.12 Top).

The respiration rate of Pentland Dell followed a similar pattern of respiration to Markies over the season with rates rising from 1ml/g/hr during the first 5 months of storage to 2 ml after 6 months and 3 ml/g/hr by the end of storage. The respiration rate of tubers stored at 3.5˚C were similar to those stored at 10˚C (Figure 3.10 Top).

In many horticultural produce an increase of 10˚C in storage temperature results in a doubling of respiration rate; the lack of difference between rates at two contrasting temperatures suggests respiration at 3.5˚C is supplemented by low-temperature stress respiration.

The respiratory quotient (RQ) values that represent the ratio of $[CO_2]/[O_2]$ for tubers is generally reported to be 0.8 (Burton, 1974) and results from this trial confirm this, with RQ's from P. Dell and Markies registering 0.7-0.8, suggesting that a proportion of the $CO_2$ evolved is dissolved in the cell cytosol or that respiration is being driven by lipid and protein breakdown. However, considering potatoes have relatively little fats and protein reserves, it is most probably associated with a proportion of dissolved $CO_2$ remaining in the cytosol, lowering the overall measurable $CO_2$ content. The use of $O_2$ consumption rather than $CO_2$ evolution may provide a more informative marker for tuber respiration characteristics. Moreover, a reduction in RQ observed in tubers stored at 10°C in January/February (2/3 months in storage) and similarly in tubers stored at 3.5°C in February (3 months in storage) correspond to application of CIPC, inhibiting sprout growth.

### 4.4.4 Fry colour analysis

Markies, was more resistant to induction of fructose and glucose accumulation at 3.5°C than P. Dell.

Markies processed as chips after 2 months storage at 3.5°C remained acceptable with a SBCSR score of 4. Fry colours increased to borderline acceptability score of 5 after 5 months storage before the degree of darkening of the chips became unacceptable (6) after 7 months storage. Tubers stored at 10°C retained acceptable fry colour quality for the entire 7 months of storage in air (Figure 3.13 and 3.14).

There was a poor correlation between sugar content and fry colour development in Markies, particularly with tubers stored at 10ºC. There was a stronger correlation for tubers stored at 3.5ºC with an increase in fry colours between harvest and after 2 months of storage in-line with increasing concentrations of reducing sugars. However, later in storage, reducing sugars declined while fry colours remained high suggesting the presence of other factors such as asparagine become more of an important factor in fry colour development.

Fry colour analysis of chips sampled from P. Dell tubers stored at 10°C deteriorated from a score of 2.8 at harvest to 3.5 after 2 months storage before rising to a borderline score of 4.0- after 5 months storage and became unacceptable (5) when stored for 7 months. There was a poor correlation between increasing fry colour ('sweetening') and the presence of reducing sugars at 10ºC. However, a stronger relationship between was observed for Pentland Dell at 3.5ºC: a rapid increase in fry colour to unacceptable levels (SBCSR score 6.5) was observed after 2 months storage concurrent with an increase in reducing sugar concentration. At the end of 7 months storage reducing sugars were declining in a background of high fry colour values.

There was a poor correlation between fry colour and respiration rate, $CO_2$ production in Pentland Dell at 10°C rose steadily over the storage season as did fry colours (Figure 3.13).

### 4.4.5 Texture

The biomechanical properties of tuber tissue were measured in samples of Markies and P. Dell removed from storage at 3.5°C and 10°C over a 7 month period. In general, the higher the energy (N) required to slice into the potato segments (Load at first failure) the more 'spongey' the tissue, leading to a greater force requirement to penetrate tissue. Tubers stored at 3.5°C were less resistant to penetration than tubers stored at 10°C for Markies

For P. Dell, the degree of resistance to tissue fracture (Load at first failure) was similar in tubers stored at 3.5°C and 10°C (Figure 3.17 and 3.18). The increases in resistance to tissue fracture will in part be due to a loss of moisture during storage but also changes in cell turgor, cell wall strength and properties of the middle lamella; the region of the cell wall that interacts with adjacent cell walls to impart cell to cell cohesion.

### 4.4.6 Weight loss

Trays of tubers were weighed each month coming out of store and weights were adjusted for sample removal and rots. Weight loss for Markies averaged 3-4 % between 2-5 months of storage (January-April) in tubers stored at 10°C dropping to <2% for 6-7 months storage (May-June). Higher initial weight loss (~4%) was observed in tubers stored at 3.5°C corresponding to higher respiration rates recorded during exposure to lower temperatures (Figure 3.18). P. Dell weight loss averaged 5% between the first 2-5 months of storage (January-April). Interestingly, P. Dell did not exhibit significantly higher weight loss when stored at 3.5°C (~3%) compared to 10°C (~2%), however, in the later stage of the storage trial (April-June) increased moisture loss were observed (6-8%) in tubers stored at 3.5°C (Figure 3.17).

### 4.4.7 ROS accumulation and tissue aging

In both varieties there are an increase of ROS accumulation after 4 months storage (March). However, in P. Dell, $O_2^-$ rose at both temperatures and just $H_2O_2$ at 10°C (Figure 3.15), whereas in Markies, $H_2O_2$ rose at both temperatures and just $O_2^-$ at 3.5°C (Figure 3.16). At the same time there was a rise in the tuber RQ and glucose and fructose content. With the increase in ROS accumulation there was a concomitant increase in the amount of sucrose present in P. Dell tubers.

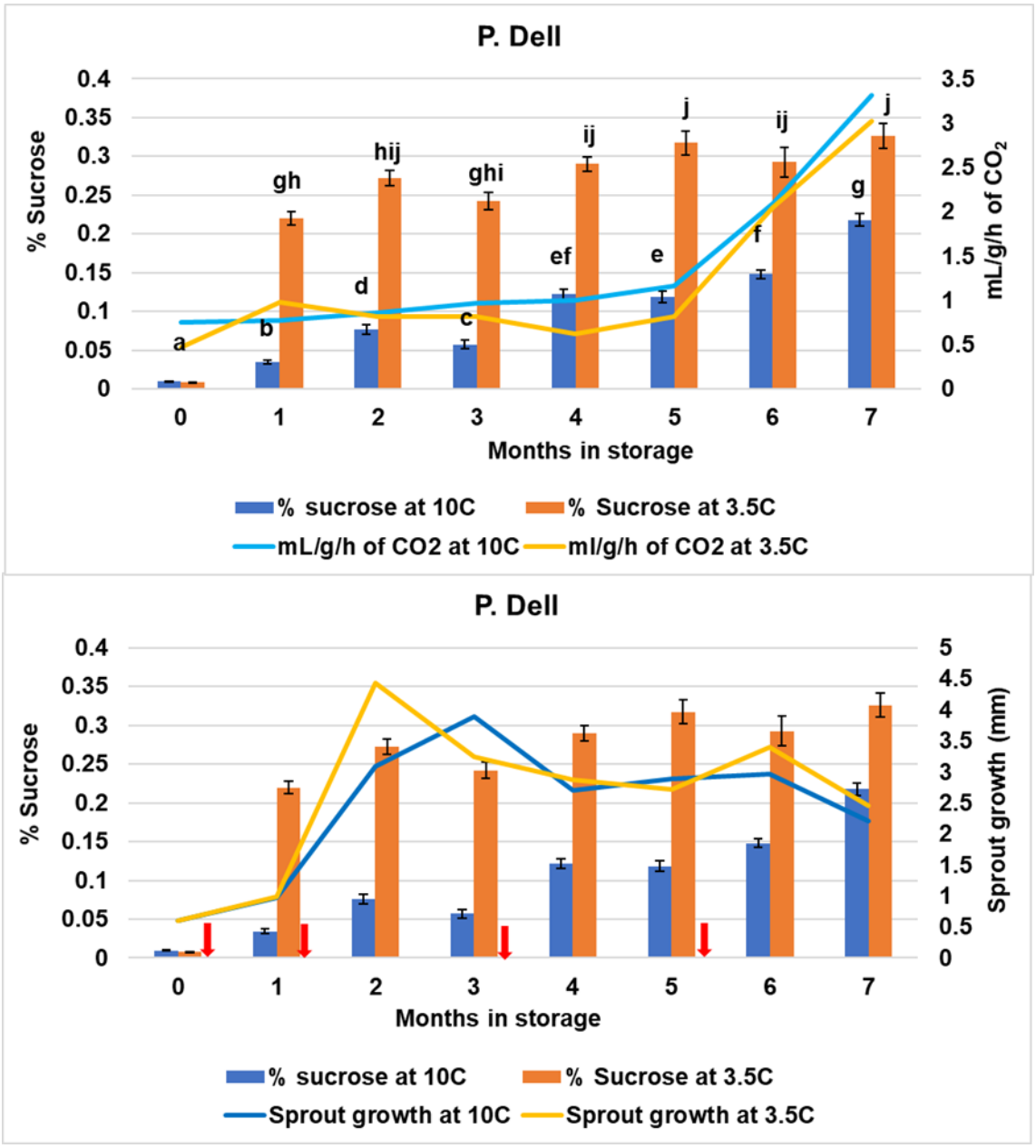



*Figure 3.9.* ***Top****: %DM of sucrose and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars and LSD p<0.05 test assigned letters for %DM of sucrose).* ***Bottom****: %DM of sucrose and sprout growth (mm) from season 2017/18 from P. Dell (with SE bars for %DM of sucrose). Red arrows mark CIPC application. Mean values with different letters are significantly different according to LSD p<0.05 test.*

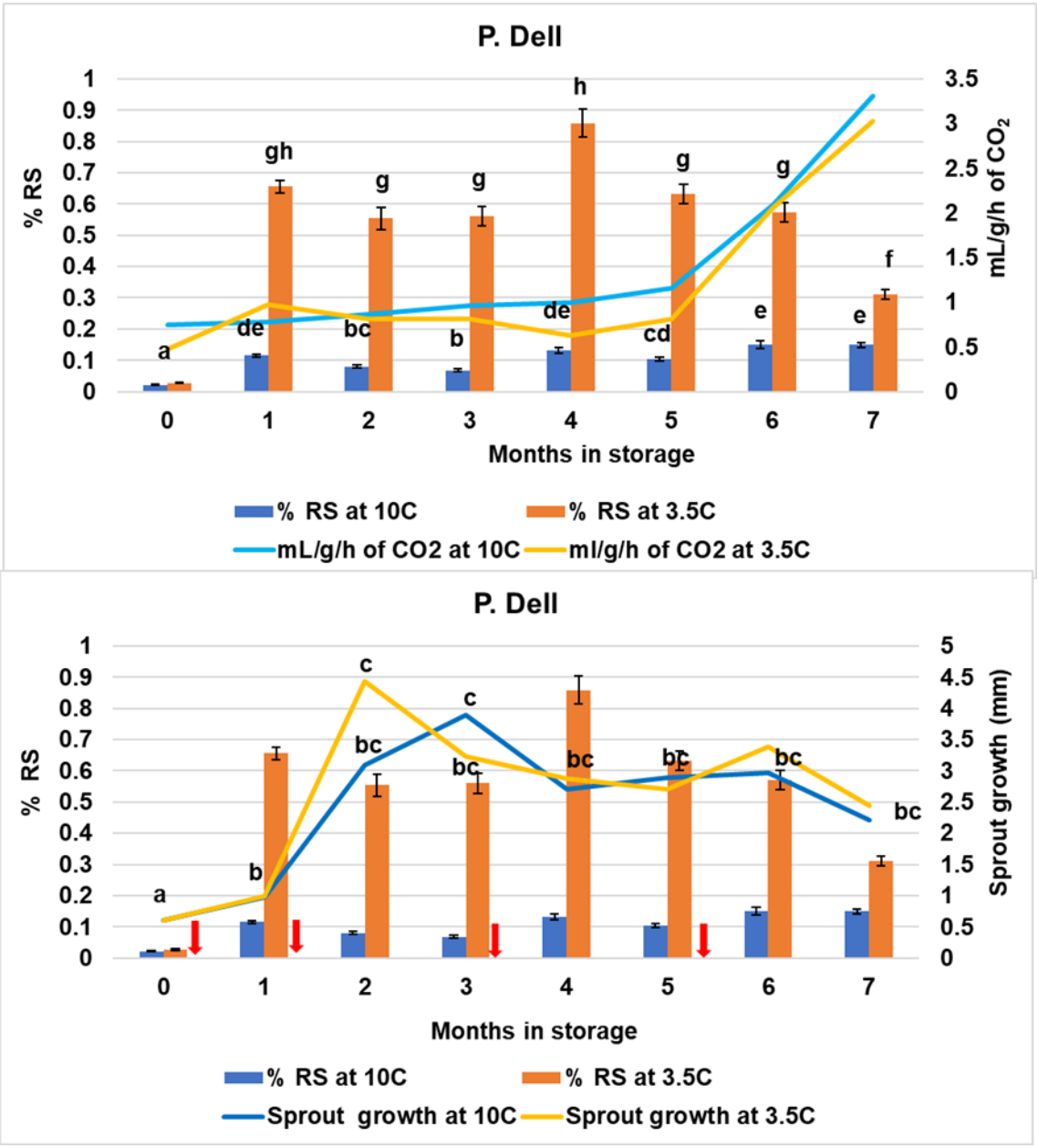



*Figure 3.10.* ***Top****: %DM of reducing sugars (RS) and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars and LSD p<0.05 test assigned letters for %DM of RS).* ***Bottom****: %DM of RS and sprout growth (mm) from season 2017/18 from P. Dell (with SE bars for %DM of RS and LSD p<0.05 test assigned letters for sprout growth). Red arrows mark CIPC application. Mean values with different letters are significantly different according to LSD p<0.05 test.*

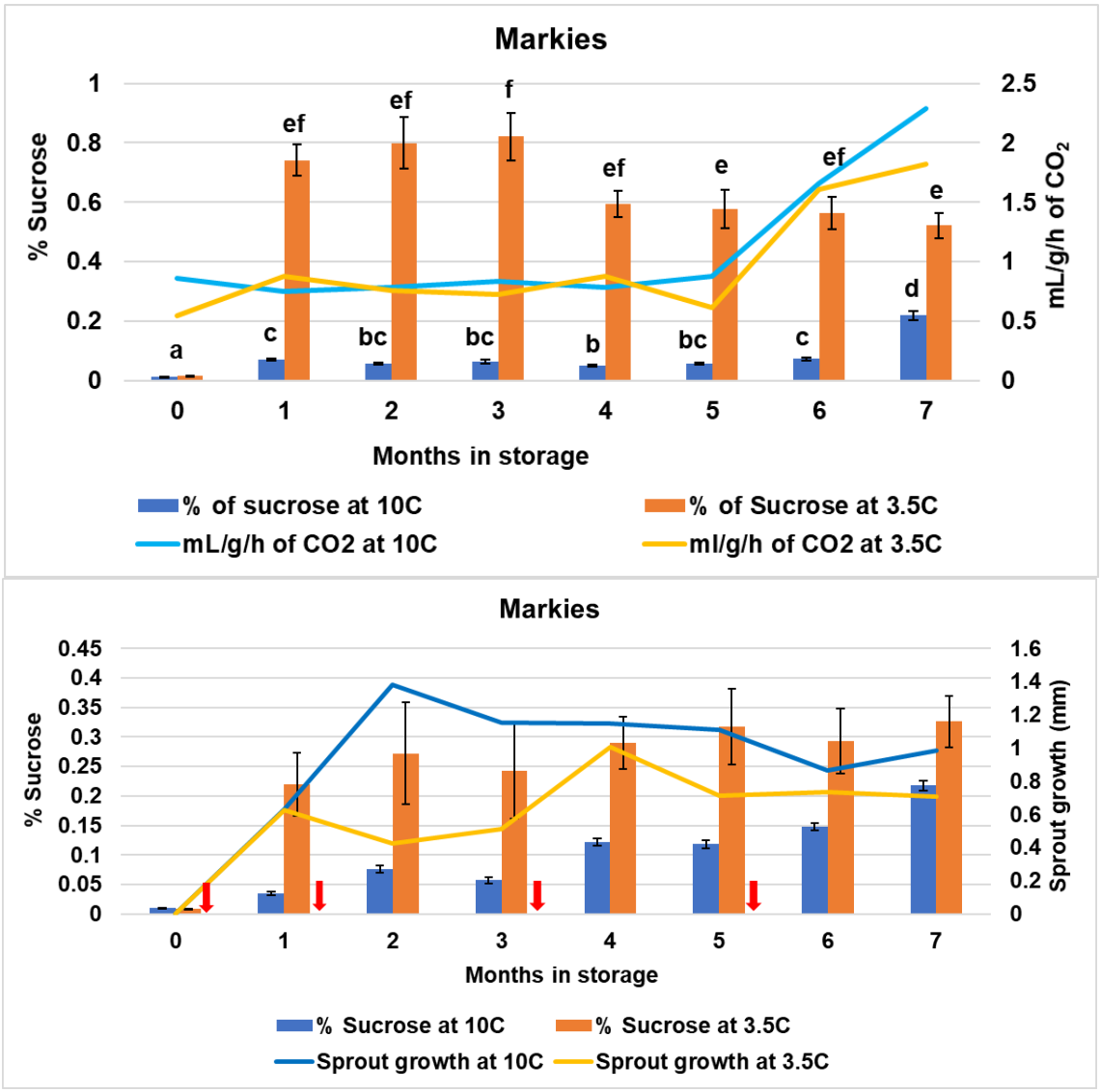



*Figure 3.11.* ***Top****: %DM of sucrose and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from Markies (with SE bars and LSD p<0.05 test assigned letters for %DM of sucrose).* ***Bottom****: %DM of sucrose and sprout growth (mm) from season 2017/18 from Markies (with SE bars for %DM of sucrose). Red arrows mark CIPC application. Mean values with different letters are significantly different according to LSD p<0.05 test.*

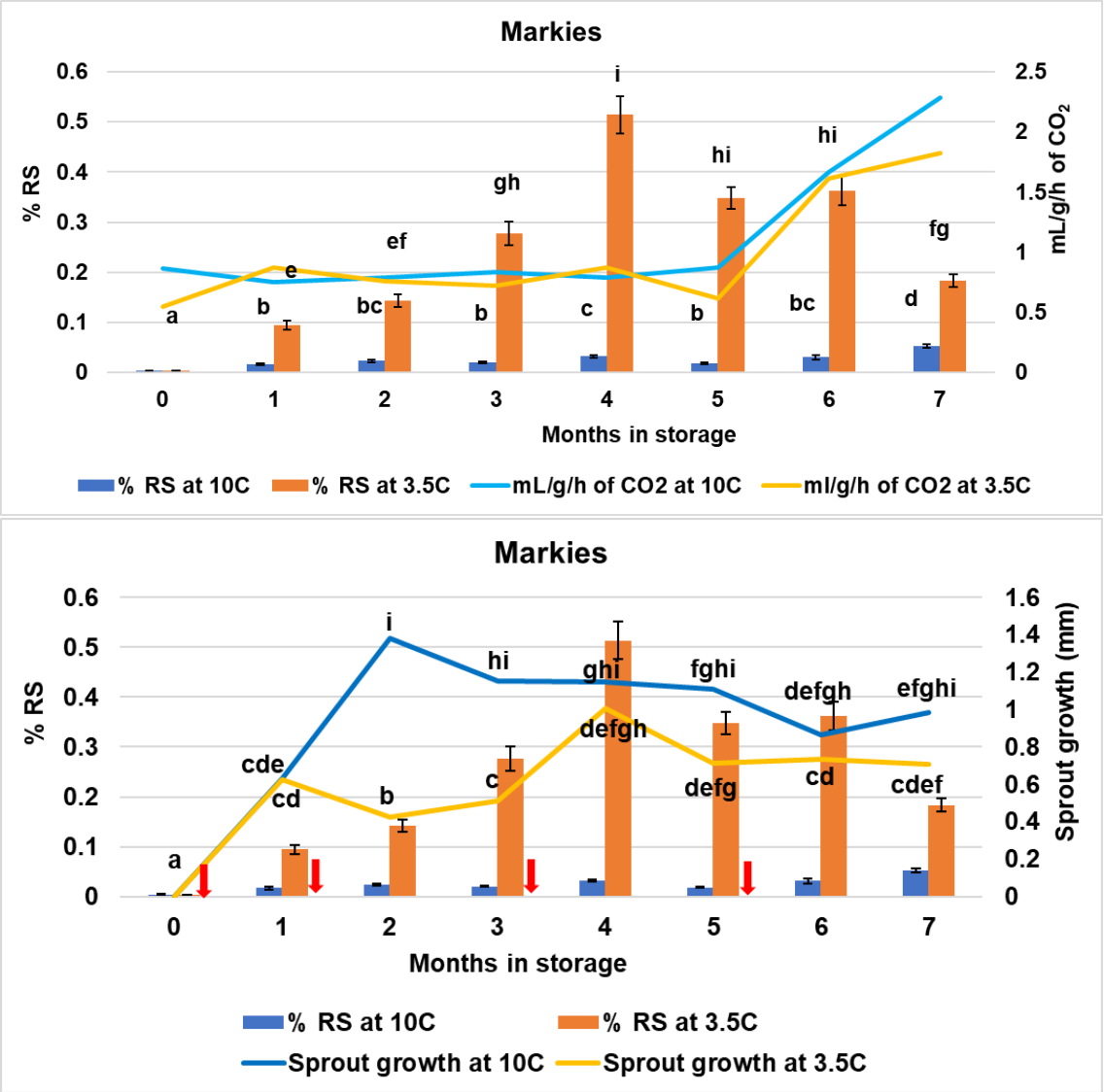



*Figure 3.12.* ***Top****: %DM of reducing sugars (RS) and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from Markies (with SE bars and LSD p<0.05 test assigned letters for %DM of RS).* ***Bottom****: %DM of RS and sprout growth (mm) from season 2017/18 from Markies (with SE bars for %DM of RS and LSD p<0.05 test assigned letters for sprout growth). Red arrows mark CIPC application. Mean values with different letters are significantly different according to LSD p<0.05 test.*

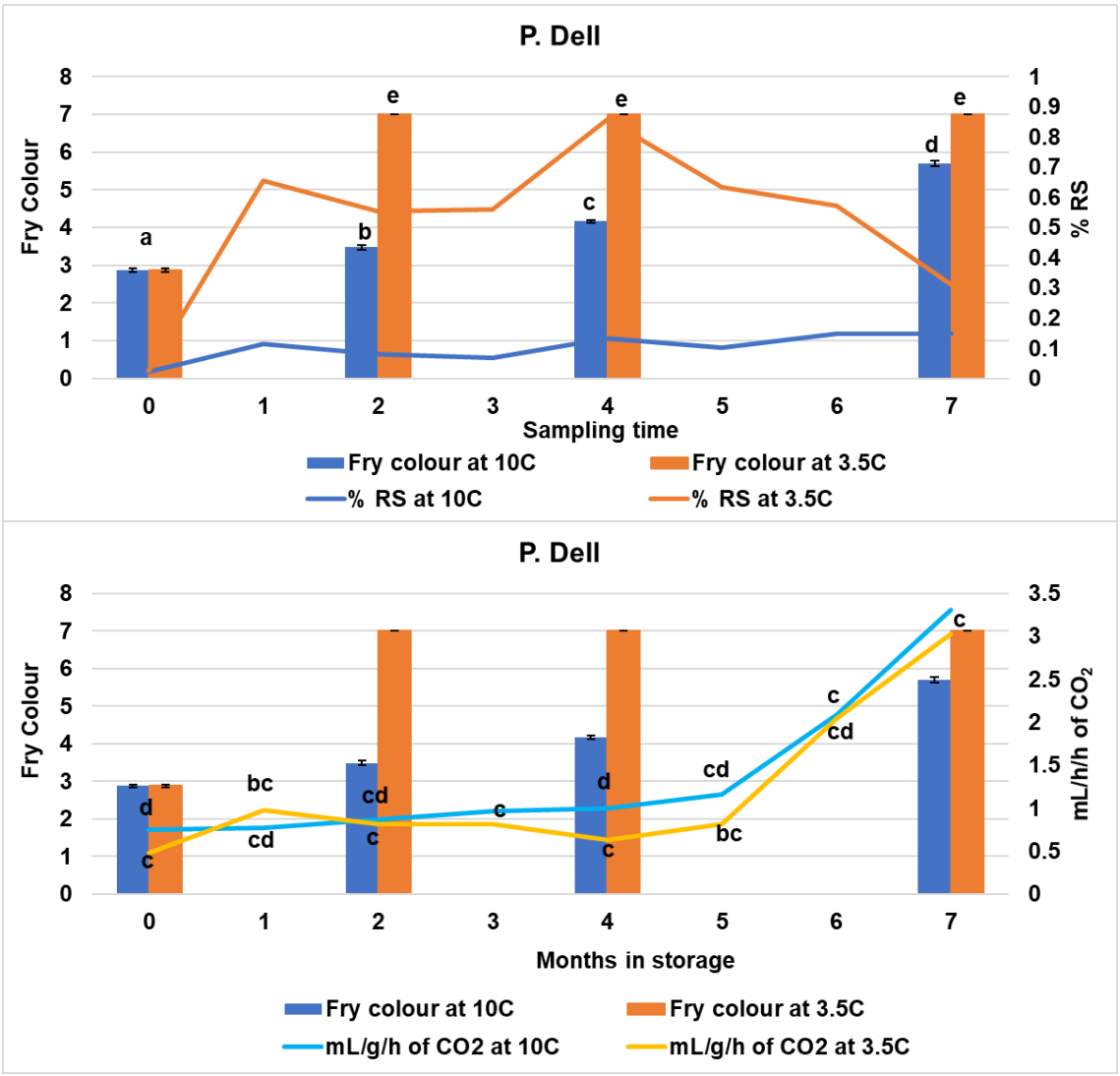



*Figure 3.13.* ***Top****: Fry colour and %DM of reducing sugars from season 2017/18 from P. Dell (with SE bars and Tukey p<0.05 test assigned letters for fry colour).* ***Bottom****: fry colour and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars for fry colour and LSD p<0.05 test assigned letters for production of $CO_2$ (ml $CO_2$ ml/g/h)). Mean values with different letters are significantly different according to LSD p<0.05 test and Tukey p<0.05 test.*

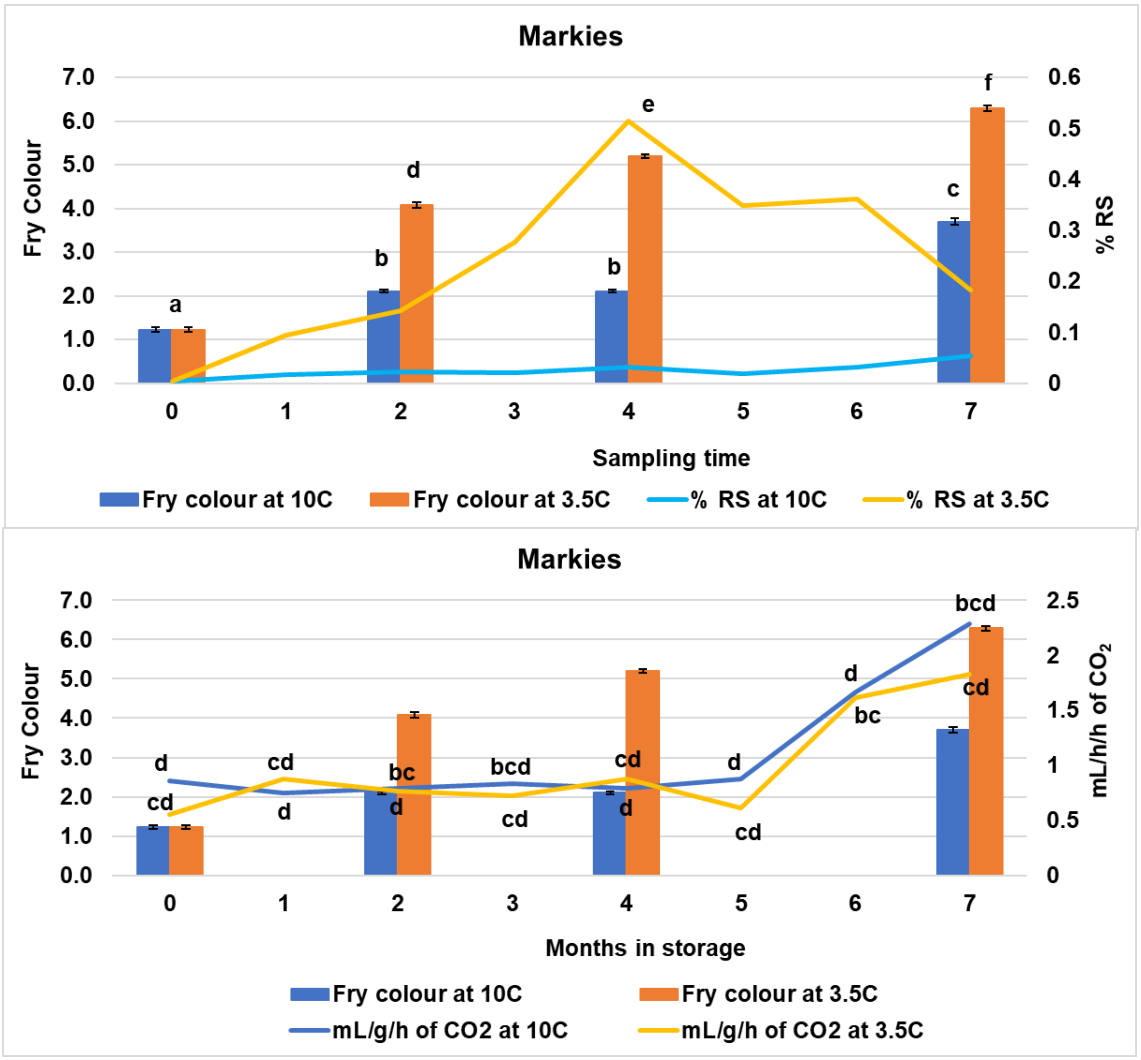



*Figure 3.14.* ***Top****: Fry colour and %DM of reducing sugars from season 2017/18 from Markies (with SE bars and Tukey p<0.05 test assigned letters for fry colour).* ***Bottom****: fry colour and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from Markies (with SE bars for fry colour and LSD p<0.05 test assigned letters for production of $CO_2$ (ml $CO_2$ ml/g/h)). Mean values with different letters are significantly different according to LSD p<0.05 test and Tukey p<0.05 test.*

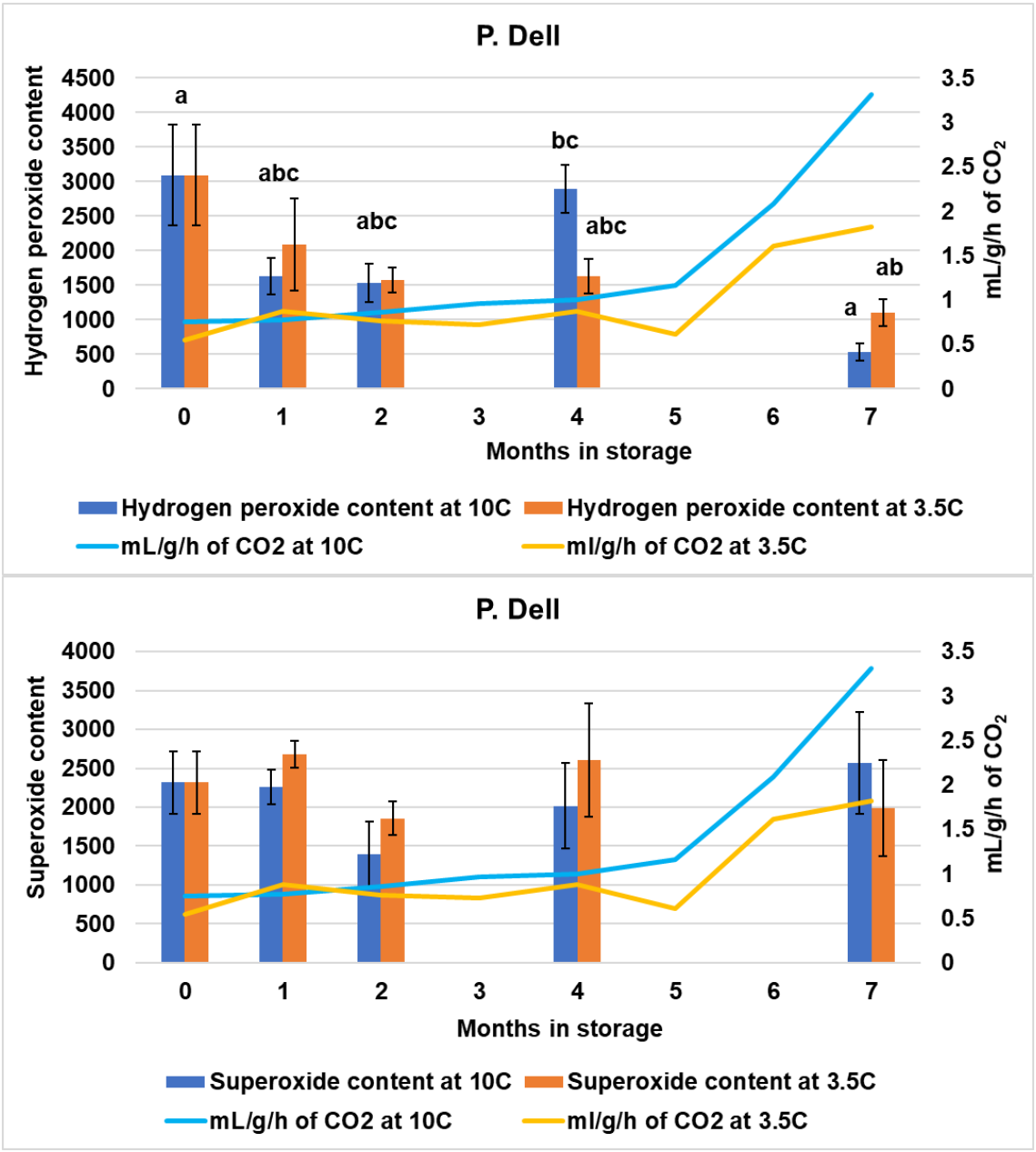



*Figure 3.15.* ***Top****: Hydrogen peroxide content and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars and LSD p<0.05 test for duration of storage (p<0.001) assigned letters for hydrogen peroxide content).* ***Bottom****: Superoxide content and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars for superoxide content and LSD p<0.05 test assigned letters for superoxide content are not present because there was no statistically significant effect of storage duration and temperature in this situation). Mean values with different letters are significantly different according to LSD p<0.05 test.*

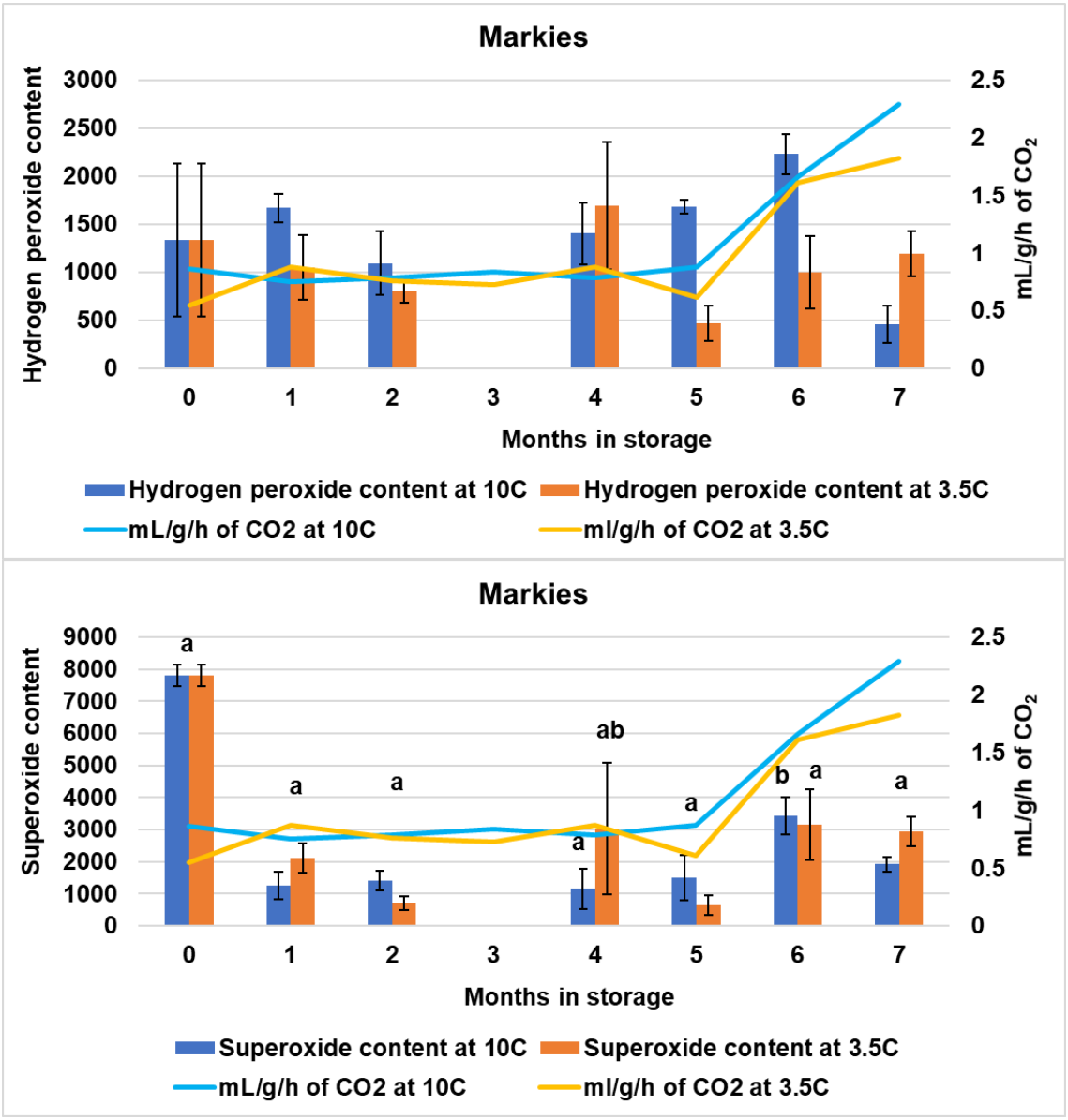



*Figure 3.16.* ***Top****: Hydrogen peroxide content and production of CO2 (ml CO2 ml/g/h) from season 2017/18 from Markies (with SE bars for hydrogen peroxide content and LSD p<0.05 test assigned letters for hydrogen peroxide content are not present because there was no statistically significant effect of storage duration and temperature in this situation).* ***Bottom****: Superoxide content and production of CO2 (ml CO2 ml/g/h) from season 2017/18 from Markies (with SE bars and LSD p<0.05 test for duration of storage (p<0.001) assigned letters for superoxide content). Mean values with different letters are significantly different according to LSD p<0.05 test.*

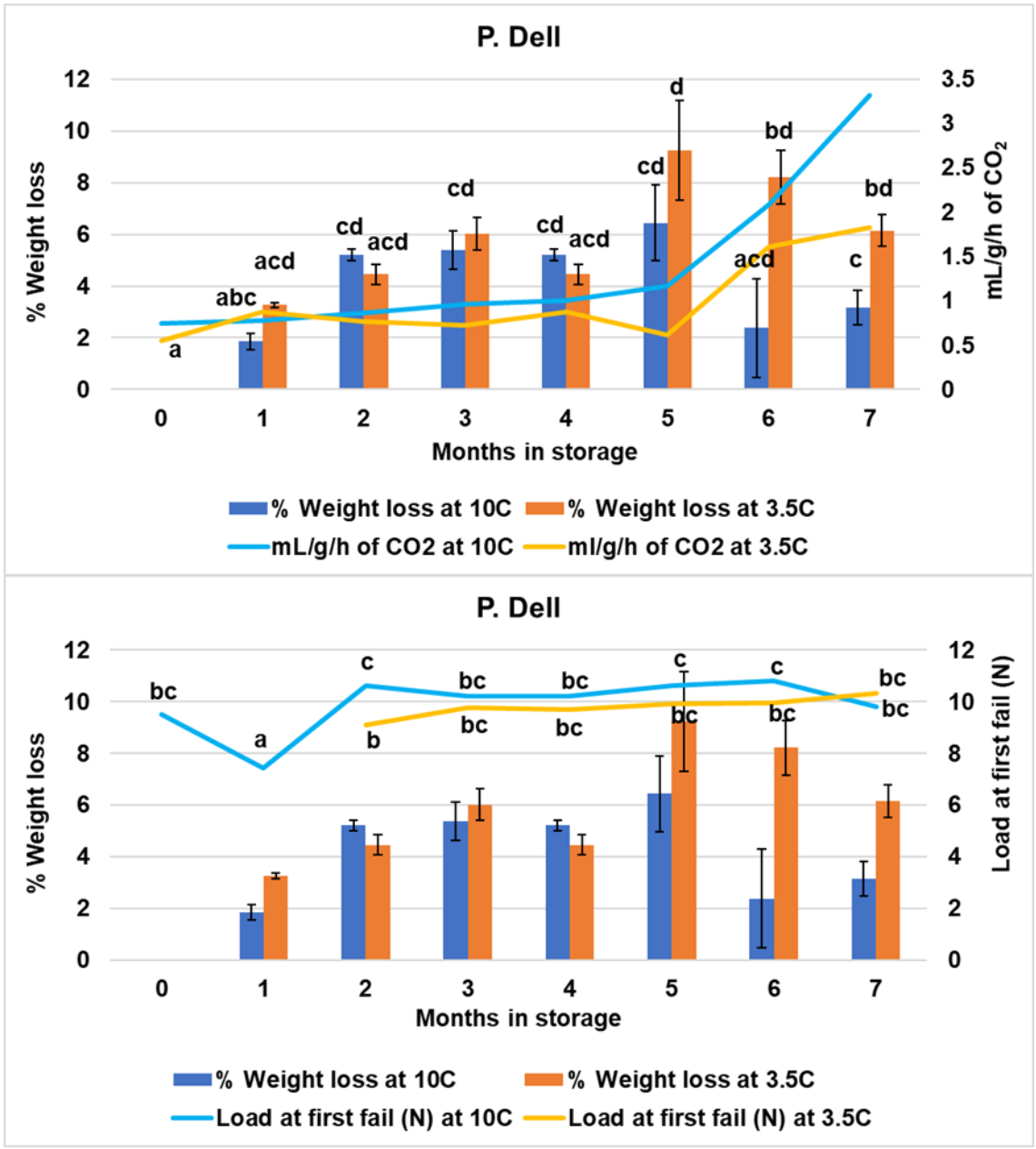



*Figure 3.17.* ***Top****: % Weight loss (WL) and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from P. Dell (with SE bars and LSD p<0.05 test assigned letters for %weight loss).* ***Bottom****: %WL and load at first fail (N) from season 2017/18 from P. Dell (with SE bars for %WL and Tukey p<0.05 test assigned letters for load at first fail (N)). Mean values with different letters are significantly different according to LSD p<0.05 test and Tukey p<0.05 test.*

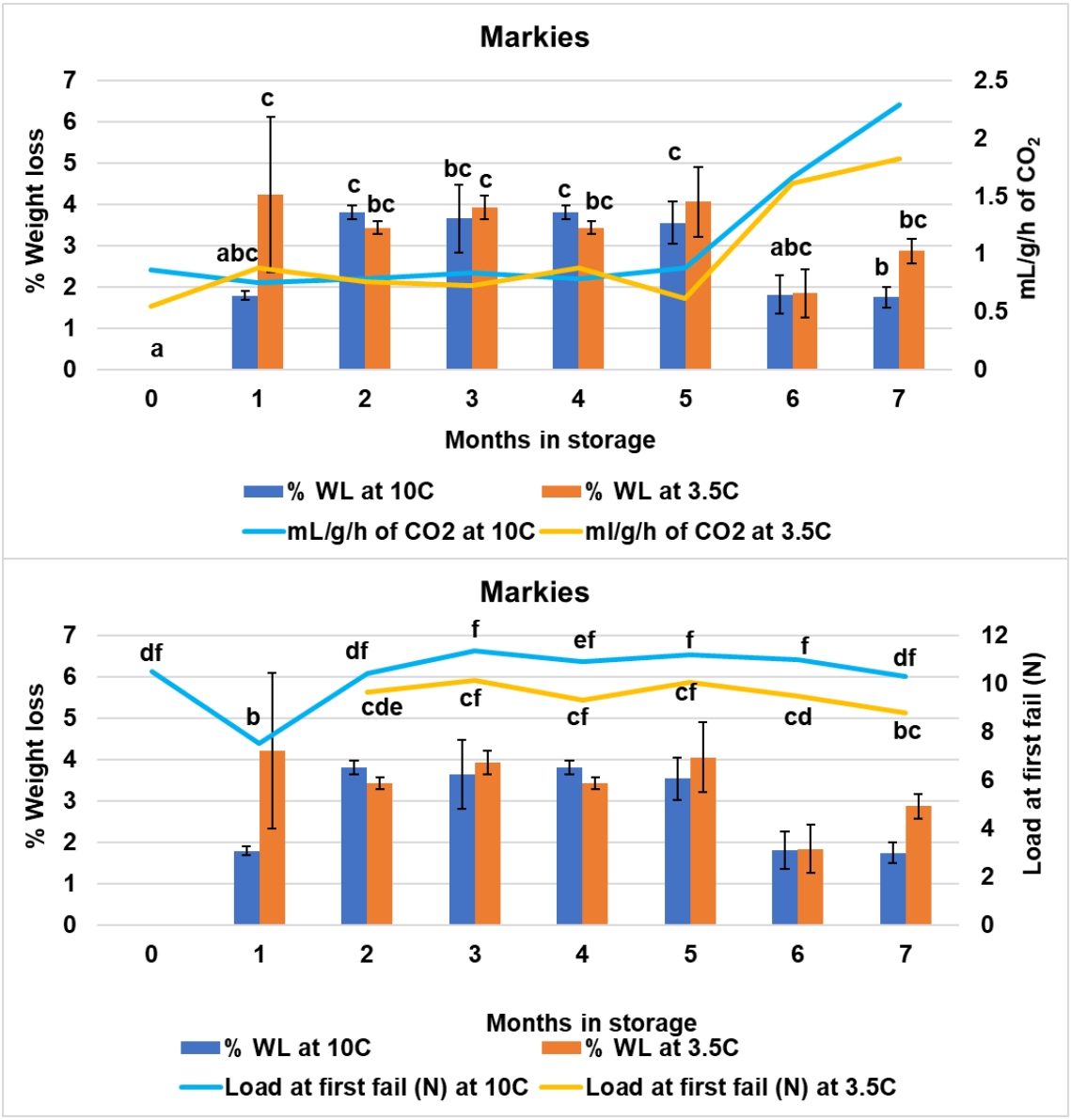



*Figure 3.18.* ***Top****: % Weight loss (WL) and production of $CO_2$ (ml $CO_2$ ml/g/h) from season 2017/18 from Markies (with SE bars and LSD p<0.05 test assigned letters for %weight loss).* ***Bottom****: %WL and load at first fail (N) from season 2017/18 from Markies (with SE bars for %WL and Tukey p<0.05 test assigned letters for load at first fail (N)). Mean values with different letters are significantly different according to LSD p<0.05 test and Tukey p<0.05 test.*

# 5. DISCUSSION

## 5.1 Phosphate and low temperature sweetening

Phosphate is an important element that encourages increased yields (Freeman et al 2008, Rosen and Bierman 2008) optimising nutritional quality and resistance to certain diseases. It is essential for promotion of rapid canopy development, root cell division, tuber set, and starch synthesis (Rosen et al 2014). This is especially true in soils categorised with a low $P_{index}$ (P0 or P1) where phosphorus is considered limiting, however, a positive response to additional phosphate application in potato yields have been reported in soils with a high P testing soils (Rosen et al 2014).

Phosphorus stimulates cell division and the synthesis and storage of starch and in doing so can increase tuber size and influence dry matter partitioning within the tuber. At high soil P tuber dry matter can decline and from the work presented here in some years phosphate applied at 200-300 kg $ha^{-1}$ led to a lowering of dry matter in the medulla region of the tuber while the outer cortex remained unaffected. Markies were particularly low in %DM in tissues taken from the medulla region of the tuber.

In the first year of the trial, Markies grown under phosphate fertiliser regimes yielded tubers higher in phosphate but responded to storage at lower temperatures through raised fructose and sucrose synthesis. In contrast, Pentland Dell accumulated less phosphate and less sugars when stored at low-temperature; suggesting phosphate may not be directly linked to increasing resistance to low temperature sweetening (LTS).

The propensity to undergo LTS depends of the expression of acid invertase enzymes (β fructofuranosidase). Different forms are present within the cell wall and vacuole of potato cells (Xuemei et al 2005), with at least 4 found in the cell wall and 2 in the vacuole; each having a unique expression pattern (Xun et al 2011); the vacuole invertases are highly expressed under conditions of low temperature.

The propensity of tubers to undergo LTS will depend on the induction of acid invertases as well as the presence of co-factors and inhibitors of enzyme function. A study (Matsourae-Endo et al, 2009) of 6 Japanese potato varieties stored at 4˚C or 20°C found cultivars could be divided into 3 response types; those that responded to low-temperature with a large increase in reducing sugars, type 2 defined as tubers where an increase in reducing sugars was observed, but at a 4-6 fold lower rate of induction and a third category of tubers where an increase in sucrose was observed but reducing sugars remained low. Here it was found that elevated acid invertase expression was only detected in the sub-types that responded with a large increase in reducing sugars (Matsourae-Endo et al, 2009).

Numerous yeast derived studies on β fructofuranosidase activity have reported enzyme sensitivity to certain divalent ions such as $Cu^{2+}$ but not $Mg^{2+}$ Fe or Mn. $Cu^{2+}$ interacts with sulfhryl (-SH) groups on cysteine and methionine residues of the proteins sub-units making up the enzyme, interfering with the tertiary structure of the enzyme causing conformational changes and inhibiting activity.

While there is no direct evidence to link higher phosphorous content with a reduction in acid invertase activity, it is clear from the mineral analysis data that increasing tuber P content also led to higher rates of $Cu^{2+}$ and $Zn^{2+}$. Moreover, $Cu^{2+}$ and $Zn^{2+}$ were higher in the medulla compared to the outer cortex, and $Cu^{2+}$ and $Zn^{2+}$ were found in higher concentrations in Pentland Dell compared to Markies. Furthermore, it is known that phosphate can encourage root hair formation and thus increase the uptake of other nutrients (Foehse and Jungk 1983)

A number of guidelines for applying phosphate to horticultural and agricultural crops exists. The Olsen P chemical extraction of soil samples is a well defined and provides a routine test. Olsen P method can be used as an indicator of a site's potential yield response to applied P fertiliser. However, at those soil P concentrations where a yield response is expected, Olsen P is of little value to predict the amount of additional P fertiliser required to achieve maximum yields (Freeman et al 1998). Petiolar phosphate analysis is another method for assessing phosphate requirement during the growing season. Critical petiole P ranges have been proposed to assist in the assessment of the P status of developing tubers above 5-10 mm in length but are specific to each variety and soil type. Phosphate application should be calibrated to local soil conditions and band-apply fertilizer P at least 5 cm from the seed piece, Petiole P analysis can be used during the season to top up the plant's requirements (Rosen et al. 2014)

## 5.2 Calcium and senescent sweetening

In general, application of calcium at recommended rates increased calcium content of Lady Rosetta tubers but not those of Pentland Dell or Markies. Averaged over all three varieties, gypsum was the only product that raised calcium content. However, the application rate of gypsum to the seed bed was the equivalent to 500 kg $ha^{-1}$ available calcium compared to 100 kg $ha^{-1}$ for Tropicote and Calcifert. While few soils are considered low in calcium, its movement *in-planta* is restricted to the water conducting vessels (xylem) resulting in limited uptake into tubers. Most of the soil-applied calcium is transported through the transpiration stream to leaves. Rapidly expanding leaves and stems act as a major sink for calcium at the expense of storage organs. Most calcium accumulating in tubers is derived from calcium absorbed via stolon or tuber root hairs. Calcium distribution in potato is not uniform, with higher

concentrations in the outer cortex decreasing towards the central medulla (Subramanian et al 2009). Calcium and dry matter have a similar distribution which is not unexpected considering the cell wall matrix is a major reserve of calcium, where it aids the cross-linking of homogalalcturonan components in pectin molecules.

Foliar applied calcium is often restricted to leaves as once it is absorbed it is transported to outer margins through transpiration. InCa formulation is reported to facilitate some phloem mobility and hence increase the propensity of calcium to accumulate in underground organs. In these trials some increase in calcium in Lady Rosetta was recorded. It is not clear whether the resultant increase in tuber-calcium was due to translocation in the phloem or if additional phosphate encouraged earlier and greater leaf canopy establishment drawing more nutrients from root system.

Interestingly, both Lady Rosetta and P. Dell are varieties that are lower in calcium and have a greater propensity to develop senescent sweetening compared to Markies which is more resistant to sweetening. InCa is a formulation of 5% Ca and 4% nitrogen (nitrate) and it is therefore not unexpected to see higher chlorophyll content on InCa treated leaves as nitrogen will stimulate chlorophyll synthesis and leaf growth.

The extent to which calcium distribution within tubers impacts on tuber physiology is not well documented. At a localized level, regulation of calcium within the cell wall, plasma membrane, and the cytoplasm and vacuole, affect the propensity for localized cell necrosis and senescence (de Freitas et al 2013). Calcium contributes to cellular structure and by binding to membrane bound phospholipids and proteins (Hirschi 2004). Maintaining a concentration of free calcium above 0.1 mM $Ca^{2+}$ in the cell wall (apoplastic pool) has been reported to maintain plasma membrane structure and function (Plieth 2001) and delay the onset of senescence (de Frietas 2013). In contrast, calcium inside the cell (cytosolic calcium $[Ca^{2+}]_{cyt}$ ) is highly regulated, with concentrations maintained at 0.1-0.2 µm, through the activity of Ca-ATPase and $Ca^{2+}/H^{+}$ antiporters located in the cell membrane (White 2000). Vacuolar $[Ca^{2+}]$ is maintained at mM concentrations (Berridge 1997), and the steep gradient between the calcium concentration in the vacuole and cytoplasm is maintained by proton-cation exchange. Release of calcium from the vacuole through calcium channels plays a crucial role in calcium influx into the cytoplasm and leads to oscillations in the $[Ca^{2+}]_{cyt}$. The magnitude of these spatio-temporal oscillations is controlled by calcium efflux mechanisms (Allen et al 2001).

On a whole tuber level, it was interesting to observe that Lady Rosetta, a variety with a lower calcium content has a shorter storage life than Pentland Dell and Markies. While it was possible to raise internal calcium content of Lady Rosetta, higher calcium content did not

retard the propensity to undergo senescent sweetening. With many aspects of calcium activity, the bioavailability and localisation within the cell which is more important than overall concentration. Calcium is inactivated via conjugation to oxalates and phytates while binding to calmodulin and calmodulin-like proteins act as calcium receptors relaying downstream signalling events.

There is some debate if sweetening in long-term stored tubers is truly a senescent process; many of the molecular mechanisms involved are not fully elucidated. Kumar *et al.* (1999) identified protein modifications related to tuber aging (increase in protein glycation, oxidation and deamidation/isomerization/racemization) and suggested that aging is accompanied by increased respiration rate, oxidative stress, lipid peroxidation and decreased protein content in the tubers.

## 5.3 Reactive oxygen species

Increase in Reactive Oxygen Species (ROS) which are oxygen free radicals ($O_2^-$) generated as a biproduct of respiration as respiration rates increase so does the production of ROS (Spychalla and Desborough (1990), tissues that become stressed accumulate ROS and are key co-ordinators of senescence. Oxidative and free radical stresses, such as $O_2^-$ production are cumulative over time (Coleman, 2000; Rughani *et al.*, 2015). These products can be neutralized during aging by intracellular compartimentalisation, protective enzymes, such as SOD and CAT, and naturally occurring antioxidants (α-tocopherols, ascorbic acid). Carvalho (2017) found increasing patterns of staining assocaited with an accumulation of ROS the longer tubers were stored. As ROS levels increased antioxidant content decreased. Analysis of Pentland Dell samples treated with calcium (Tropicote 200 kg $ha^{-1}$) increased the ascorbic acid content of tubers but in this case did not alter ROS activity..

In general, as tissues age, cell membranes weaken, rates of electrolyte leakage from within the cell increases and its sugeested to be a realtively easy indicator of physiological aging in potato tubers, nevertheless, the efficacy of this technique remains uncertain, since leakage responses can be small, and dependent on storage temperature and not consistent among samples (Coleman, 2000 and references therein).

More detailed analysis of potato cell membranes under the Scanning Electron Microscopy (Carvalho, 2017) revealed the first visible signs of starch degradation are changes to the membrane encapsulating the starch granule. Changes in whole starch granule appearance was observed in this particular study early in storage in the senescent sweetening prone variety Lady Rosetta, while the process was delayed in VR808 a variety with longer storage life. Two water dikinase enzymes (GWD) have been indicated in having a putative role in

disrupting amyloplast surface polymers organization (Smith, 2012). Water dikinases are key enzyme of starch metabolism (Mahlow *et al.*, 2014), catalysing starch phosphorylation in a range of plant storage organs (Bansal and Das, 2013). Glucan, water dikinase (GWD1) phosphorylates glucose residues at the C-6 position and phosphoglucan, water dikinase (GWD3) phosphorylates the glucose residues at the C-3 position in amylopectin chains (Ritte *et al.*, 2006). Those enzymes facilitates the attack of amylases on the starch granule (Orzechowski *et al.*, 2013).

## 5.4 Respiration rate to monitor tuber status

Wills *et al.* (2007) reported that respiration rate of tubers was an excellent indicator of the metabolic activity and a useful guide for predicting their storage life. When stored for long periods at high temperature (> 4 $^{o}$C) there is an increase in basal metabolism that can possibly accelerate tuber aging and suggested tuber respiration rate was the “pacemaker” of aging in potato tubers (Kumar and Knowles, 1996a, Kumar and Knowles, 1996b). In these trials the respiration rate of Pentland Dell tubers increased as tubers aged and increased in sugar content. According to Copp *et al.* (2000) the onset in the decline of chip colour quality coincides with the increase in the respiration rate of stored tubers. It is also important to note that respiration rises are often observed at the onset of sprouting and after treatment with CIPC, respiration rates decline (Carvalho 2017).

In addition to respiration rates measured by $CO_2$ production, the respiratory quotient (RQ) provides additional measures of the changes in the type respiration activity over time. Under normal aerobic respiration the $CO_2$ production is equilivant to consumption of $O_2$ $[CO_2]/[O_2]$ providing an RQ of 1 (Burton, 1989). However, Burton (1974) reported RQ values of 0.8-0.9 in the early storage period when the tubers were still dormant, and RQ=1.3 later when tubers were sprouting (Burton *et al.*, 1992). According to Isherwood and Burton (1975) senescent potato tubers have a higher production of $CO_2$ when compared with the $O_2$ consumption. RQ analysis of the respiration data (data not provided) from these trial shows that RQ values remained at ~ 0.8. Where genunine RQ’s are less than 1, it suggest that either fats and proteins are being preferentially respired or that a proportion of $CO_2$ is absorbed into the cell sap. At the stage where RQ’s increase above 1.3 either localised anearobic respiration due to water-soaking of tissues has occurred or organic acids are being preferentially respired compared to simple sugars. As potatoes have little, fat, protein or acids it is less likely that changes in carbon sources utilised for respiration and that physical differences are the basis for changes in RQ, theses are either due to $CO_2$ adsorption or localised anaerobsis that can lead to damage such as blackheart.

Whether, respiration rates can be used practically for determining the onset of dormancy break in commercial stores is currently unclear, but is a potential important tool for store managers. Accurate measurements of RQ have in the past been difficult to determine due in the most part to sampling methods and sensitivity of $O_2$ and $CO_2$ detectors. The advent of SafePod technology (http://www.storagecontrol.ltd/ ) currently employed in over 130 apple stores globally to measure in real-time storage respiration rates and RQ measurements may have utility in potato stores. In these trials, a smaller lab-based version “mini-pod” have shown promise but requires further work. An additional trial  funded by AHDB with SafePods is currently underway at SBCSR with support from NRI.

## 6. REFERENCES


Allen, G. J., Chu, S. P., Harrington, C. L., Schumacher, K., Hoffmann, T., Tang, Y. Y., et al. (2001). A defined range of guard cell calcium oscillation parameters encodes stomatal movements. *Nature* 411, 1053–7. doi:10.1038/35082575.

Bansal, A. and Das, N. 2013. Molecular cloning and sequence comparison of a cDNA encoding α-glucan, water dikinase (GWD) from potato (Solanum tuberosum L.), and analysis of gene expression. *Journal of Plant kll and Biotechnology,* 22(4)**,** pp 441-452.

Berridge, M. J. (1997). Elementary and global aspects of calcium signalling. *J. Exp. Biol.* 200, 315–9. doi:10.1042/BST20110766.

Burton, W. G. 1974. Requirements of the users of ware potatoes. *Potato Research,* 17(4)**,** pp 374-409.

Burton, W. G. 1989. *The Potato*, 3rd ed.: Longman Scientific and Technical.

Burton, W. G., Es, A. and Hartmans, K. J. 1992. The physics and physiology of storage. *In:* Harris, P. (ed.) *The Potato Crop.* Springer Netherlands.

Carvalho, C.A.G.S (2017) Understanding mechanisms and identifying markers for the onset of senescent sweetening of potato (*Solanum tuberosum*) PhD Thesis, University of Greenwich.

Chen, L.-Y., Liu, R.-P., Qin, C.-Y., Meng, Y., Zhang, J., Wang, Y. & Xu, G.-F. 2010. Sources and intake of resistant starch in the Chinese diet. *Asia Pacific journal of clinical nutrition,* 19(2)**,** pp 274-282.

Coleman, W. K. 2000. Physiological ageing of potato tubers: A review. *Annals of Applied Biology,* 137(2)**,** pp 189-199.

ColorHexa. 2012 - 2017. *Color encyclopedia: Information and conversion* [Online]. Available: http://www.colorhexa.com [Accessed 02-04-2017.

Copp, L. J., Blenkinsop, R. W., Yada, R. Y. and Marangoni, A. G. 2000. The relationship between respiration and chip color during long-term storage of potato tubers. *American Journal of Potato Research,* 77(5)**,** pp 279-287.

Daudi, A. & O'Brian, J. A. 2012. *Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves* [Online]. Bio-Protocol. Available: http://www.bio-protocol.org/wenzhang.aspx?id=263 [Accessed 17-06-2014.

de Freitas, S. T., Amarante, C. V. T. d., Dandekar, A. M., and Mitcham, E. J. (2013). Shading affects flesh calcium uptake and concentration, bitter pit incidence and other fruit traits in “Greensleeves” apple. *Sci. Hortic. (Amsterdam).* 161, 266–272. doi:10.1016/j.scienta.2013.07.019.

Doncaster, C. P. & Davey, A. J. H. 2007. *Analysis of variance and covariance: how to choose and construct models for the life sciences*: Cambridge University Press.

Elmståhl, H. L. 2002. Resistant starch content in a selection of starchy foods on the Swedish market. European Journal of Clinical Nutrition, 56(6)**,** pp 500.

Foehse, D. & Jungk, A. Plant Soil (1983) Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants 74: 359. https://doi.org/10.1007/BF02181353

Freeman, K.L., Franz, P. R  de Jong, R. W. (1998) Effect of phosphorus on the yield, quality and petiolar phosphorus concentrations of potatoes (cvv. Russet Burbank and Kennebec) grown in the krasnozem and duplex soils of Victoria. Australian Journal of Experimental Agriculture 38(1) 83 - 93

Giné Bordonaba, J. & Terry, L. A. 2010. Manipulating the taste-related composition of strawberry fruits (Fragaria × ananassa) from different cultivars using deficit irrigation. *Food Chemistry,* 122(4)**,** pp 1020-1026.

Glowacz, M., Colgan, R. & Rees, D. 2015. Influence of continuous exposure to gaseous ozone on the quality of red bell peppers, cucumbers and zucchini. *Postharvest Biology and Technology,* 99(1-8.

Hirschi, K. D. (2004). The Calcium Conundrum. Both Versatile Nutrient and Specific Signal.

*PLANT Physiol.* 136, 2438–2442. doi:10.1104/pp.104.046490.

Jambunathan, N. 2010. Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. *Methods in molecular biology (Clifton, N.J.),* 639(292-8).

Kumar, G. N. M. and Knowles, N. R. 1996a. Nature of enhanced respiration during sprouting of aged potato seed-tubers. *Physiologia Plantarum,* 97(2)**,** pp 228-236.

Kumar, G. N. M. and Knowles, N. R. 1996b. Oxidative stress results in increased sinks for metabolic energy during aging and sprouting of potato seed-tubers. *Plant Physiology,* 112(3)**,** pp 1301-1313.

Mahlow, S., Hejazi, M., Kuhnert, F., Garz, A., Brust, H., Baumann, O. and Fettke, J. 2014. Phosphorylation of transitory starch by α-glucan, water dikinase during starch turnover affects the surface properties and morphology of starch granules. *New Phytologist,* 203(2)**,** pp 495-507.

Matsuura-endo, c., Kobayashi, a., Noda, t. (2004) Changes in sugar content and activity of vacuolar acid invertase during low-temperature storage of potato tubers from six Japanese cultivars J Plant Res 117: 131. https://doi.org/10.1007/s10265-003-0137-z

Orzechowski, S., Grabowska, A., Sitnicka, D., Siminska, J., Felus, M., Dudkiewicz, M., Fudali, S. and Sobczak, M. (2013). Analysis of the expression, subcellular and tissue localisation of phosphoglucan, water dikinase (PWD/GWD3) in Solanum tuberosum L.: a bioinformatics approach for the comparative analysis of two α-glucan, water dikinases (GWDs) from Solanum tuberosum L. *Acta Physiologiae Plantarum,* 35(2)**,** pp 483-500.

Plieth, C. (2001). Plant calcium signaling and monitoring: pros and cons and recent experimental approaches. *Protoplasma* 218, 1–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11732314 [Accessed October 3, 2017].

R Core Team. 2016. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Ritte, G., Heydenreich, M., Mahlow, S., Haebel, S., Kotting, O. and Steup, M. 2006.Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. *Febs Letters,* 580(20)**,** pp 4872-4876.

Rosen, C.J. & Bierman, P.M. (2008) Potato Yield and Tuber Set as Affected by Phosphorus Fertilization. *Am. J. Pot Res* 85: 110. https://doi.org/10.1007/s12230-008-9001-y
Rosen, C.J., Kelling, K.A., Stark, J.C. (2014). Optimizing Phosphorus Fertilizer Management in Potato Production. Am. J. Potato Res. 91: 145. https://doi.org/10.1007/s12230-014-9371-2

Rughani, G., Chandra, J., Chandrakar, V. and Keshavkant, S. (2015). Lipid Peroxidation and In-Situ Localization of ROS in Assorted Seeds Exposed to Salinity and Artificial Ageing. *App. Sci. Rep.,* 12(123-127).

Smith, A. M., Zeeman, S. C. and Smith, S. M. (2005). Starch degradation. *Annual Review of Plant Biology.*

Spychalla, J. P. and Desborough, S. L. (1990). Superoxide-dismutase, catalase, and alpha-tocopherol content of stored potato-tubers. *Plant Physiology,* 94(3)**,** pp 1214-1218.

Vincent, J. F. V., Jeronimidis, G., Khan, A. A. & Luyten, H. (1991). The wedge fracture test a new method for measurement of food texture. *Journal of Texture Studies,* 22(1)**,** pp 45-57.

Ji, X., Van den Ende, W., Van Laere, A., Cheng, C. and Bennett, J. (2005). Structure, evolution, and expression of the two invertase gene families of rice. *J Mol Evol* (2005) 60: 615. https://doi.org/10.1007/s00239-004-0242-1

Liu, X., Zhang, C., Ou, Y. et al. (2011) Systematic analysis of potato acid invertase genes reveals that a cold-responsive member, *StvacINV1*, regulates cold-induced sweetening of tubers. *Mol Genet Genomics* 286: 109. https://doi.org/10.1007/s00438-011-0632-1
White, P. J. (2000). Calcium channels in higher plants. *Biochim. Biophys. Acta - Biomembr.* 1465, 171–189. doi:10.1016/S0005-2736(00)00137-1.

Wills, R., McGlasson, B., Graham, D., Joyce, D., Wills, R., McGlasson, B., Graham, D. and Joyce, D. 2007. *Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals*, 5th edition: CABI.

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