Project title:	Understanding the underlying mechanisms and the role that pre-harvest horticultural maturity, agronomic factors and growing conditions have on postharvest discolouration in celery
Project number:	CP 079
Project leader:	Prof. Leon A. Terry Vincent Building (B52a), Department of Agrifood, School of Energy, Environment and Agrifood (SEEA), Cranfield University, Cranfield, Bedfordshire, MK43 0AL
Report:	2nd Annual Report 2015
Previous report:	1st Annual Report 2014
Location of project:	Plant Science Laboratory (PSL), Vincent Building (52a), Department of Agrifood, School of Energy, Environment and Agrifood (SEEA), Cranfield University, Cranfield, Bedfordshire, MK43 0AL
Industry Representative:	Emma Garfield (G's Fresh Ltd)
Date project commenced:	16th September 2013
Date project completed (or expected completion date):	16th September 2016

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

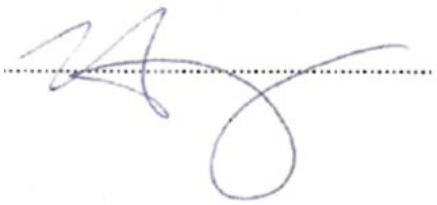
We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Leon A. Terry

Head of Agrifood

Cranfield University, SEEA

Signature



Date 24/03/2016

Report authorised by:

Emma Garfield

Head of Technical

G's Fresh Ltd (The Shropshire Group)

Signature ..... Date .....

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GROWER SUMMARY

Headline

- Celery cut-end browning increased with water stress applied to celery plants.

Background

Celery (*Apium graveolens* L.) is a minor crop which belongs to the *Apiaceae* (or *Umbelliferae*) botanical family. It is commonly eaten for its long and thick petioles which are sold on the market in various retail formats. Celery is appreciated by consumers for its freshness and bright green colour. These two main parameters are visually evaluated on supermarket shelves. One of the main problems affecting celery is postharvest browning, a physiological "disorder" occurring in many fresh vegetables which manifests as black/brown stains occurring on cut surfaces. Although the browning is not thought to be due to microorganism activity, it has a serious impact on consumers who tend to view affected celery as decaying and/or rotten, leading to purchase decreases. This has negative consequences on the profitability of fresh produce production. Postharvest browning is thought to be due to the activity of two enzymes: polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PALwhich act together to produce the brown pigments which are responsible for the black/brown colour on cut surfaces.

In celery and related crops, there are some studies in the literature which have investigated the incidence of postharvest browning in relation to postharvest factors. Even though this is a starting point, the detailed underlying physiology and biochemistry involved in this "disorder" still remains unknown. In addition, it is still unclear how pre-harvest factors and growing practices may affect celery browning. Thus, further investigation is required to better understand the role of the pre-harvest-postharvest continuum in determining cut-end browning in celery.

Summary

During the 1st year, two experiments were carried out to investigate the role that crop developmental stage and the plant hormone ethylene have on cut-end browning in celery.

Work in year 2 aims were to understand the underlying mechanism involved in the "disorder", particularly paying attention to the effect of deficit irrigation on browning and the changes in the profile/diversity of phenolic compounds.

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The experiment, which started on 7th July 2015 and terminated on 20th November 2015, was conducted in a garden polytunnel at Cranfield University, Bedfordshire, UK. All material (transplants and growing substrate) and agronomic advice were provided by G's Fresh Ltd. Three irrigation treatments were applied in total: 800 mL (control), 600 mL (light stress irrigation) and 400 mL (heavy stress irrigation) every alternate day. Water was administered by hand with a graduated plastic container. Soil moisture was maintained at or near field capacity from planting date to commencement of water treatments (5 weeks) to properly develop plants and strengthen roots before applying drought stress. Soil moisture content was periodically measured with a Thetaprobe meter to confirm water treatments and to record soil moisture variation throughout the growing period. Since the different irrigation regimes affected crop growth, plants were harvested at different dates to obtain the same developmental stage. After harvest, samples were transferred to the Plant Science Laboratory for visual and physiological attributes at regular time intervals over storage at 20 °C temperature and 55.5% relative humidity. Samples were assessed immediately after harvest, after 3, 6 and 10 days of storage (baseline, sampling day 1, sampling day 2 and sampling day 3 respectively). After each assessment, samples were cut with a knife, snap-frozen in liquid nitrogen and stored at -80 °C freezers for further analysis.

Extraction and detection of phenolic compounds was carried out on celery dry material of 1st year experiments. High Performance Liquid Chromatography was utilized to detect the key molecules according to plant tissue type and browning severity. Preliminary results showed spatial and temporal changes in the profile of phenolic compounds of celery as affected by various degrees of browning. Considering that the detected phenolic compounds have not been identified yet in literature, more detailed biochemical analyses need to be performed using the appropriate equipment.

Regarding the deficit irrigation trial, soil moisture content results confirmed the different water treatments although periods of hot and cold weather affected the evapotranspiration rate. On the other hand, results from visual assessments and physiological analysis showed that respiration rate and cut edge browning were more pronounced in the less irrigated plants.

Financial Benefits

None at this time.

Action Points

Results arising from this part of the project suggest that irrigating celery slightly under its optimum condition can result in reduced postharvest browning.

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SCIENCE SECTION

Introduction

Current literature reports scarce research about the influence of pre-harvest factors on cut-end browning in celery. Guerra *et al*., (2010) reported that browning potential and total quinone content were higher in over-mature plants of the self-blanching Golden Clause cv. Lettuce is another crop which suffers from postharvest discolouration, and has been extensively studied. For instance, Luna *et al*., (2013) found that cut-edge browning was significantly reduced by applying deficit irrigation (-35% of the standard irrigation dose) in Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) despite the accumulation of phenolic compounds in affected midrib tissues. Another study conducted by the same authors reported that over-irrigated (*Lactuca sativa* L. var. *capitata*) plants of Iceberg lettuce showed more browning than the under-irrigated ones despite the accumulation of phenolic compounds (Luna *et al*., 2012). The results revealed in these two studies lay the foundations for further investigations aimed to find relationships between irrigation management and cut-end discolouration in celery with associated molecules.

According to current literature, postharvest browning is an enzymatic process which consumes phenolic compounds. Despite this evidence, the two studies above mentioned seem to contrast with his statement as they both showed a positive correlation between phenolic content and browning appearance in lettuce cut tissues. This contradiction demonstrates how little is still known about the biochemical mechanisms involved in this “disorder”. In addition, there still remains a knowledge gap regarding how the profile and concentration of specific phenolic compounds varies depending on browning level in celery. The most detailed study was conducted by Yao *et al*., (2010) who identified 6 different phenolic molecules in 11 celery varieties. Although the author did not correlate the phenols with postharvest discolouration and considering that the paper is lacking in some hard detail, it however represents an initiation point for beginning further investigation.

Materials and methods

Plant material and growing conditions

The experiment was conducted in collaboration with G’s Fresh Ltd which provided all plant material. A 5m-wide, 8m-long and 2.5m-high polythene polytunnel (Haygrove Ltd, Ledbury, Herefordshire, UK) (**Figure 1**) located in Cranfield University, Bedfordshire, UK, was used to carry out the trial, which started on 07th July 2015 and finished on 20th November 2015. Celery

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plants cv. Imperial were grown in 12 L capacity plastic pots (diameter: 30cm, height: 23cm) filled with Fenland peat directly collected from Dimmocks Cote Farm (Stretham, Ely, Cambridgeshire) fields (coordinates: 52°19'57.7"N, 0°15'58.3"E). Once received, soil was sieved to remove any pebbles, weeds and other non-soil material. A ground cover fabric was placed at the base of the polytunnel (Phormisol 100g/m^2 Ground Cover, LBS Horticulture Ltd) to suppress weeds. Celery seeds were sown on 4th June 2015 in peat block (mixture of black, brown turf and fertilizer), covered with sand and transferred into germination rooms for 6 days at 18 °C. Then, they were moved to a greenhouse at a minimum temperature set up at 16 °C for 27 days. After this period, transplants were transported to Cranfield University at ambient temperature for 1.5 h and transplanted in the polytunnel on 7th July 2015. Plants were sprayed with Aphox® (pirimicarb) (2.5g in 5 L of water) and Movento® (spirotetramat) (1 mL in 1 L of water) to prevent incidence of the willow-carrot aphid (*Cavariella aegopodii* Scop.) and were fertilized with a NPK 10-10-30 + 3.3MgO + trace elements water soluble fertilizer (Universol Violet, LBS Horticulture Ltd). Polytunnel temperature was monitored daily with a tube thermometer. Lateral plastic covers were partially removed to allow ventilation in case of excessive hot weather, which could be detrimental to the crop. Optimum harvest date was determined by taking into account two parameters: stalk length (about 55 cm) and the expected harvest date indicated in reference schedule tables prepared by G's Fresh Ltd every growing season.

Figure 1 - Garden polytunnel (Haygrove Ltd) used to conduct the deficit irrigation trial.

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Experimental design and sampling

A total of 135 celery plants were grown in this experiment, 108 for laboratory analyses and 27 additional spares in case of any loss of plant material occurring during the trial (**Figure 2**). Three irrigation treatments were applied: 800 (control irrigation), 600 (light water stress) and 400 (heavy water stress) mL every alternate day. Control irrigation treatment was decided according to the standard celery growing practise adopted by G's Fresh Ltd. Plants were divided into 9 blocks, 3 for each water treatment. Each block was composed by 15 plants (12 for postharvest analyses and 3 spare). Plots were arranged in a completely randomized block design as shown in **Figure 3**. In order to appropriately establish roots in the pots, plants were maintained at or near field capacity for five weeks between planting date and commencement of water treatments. Harvest was completely randomized and was performed by cutting plants at the soil line with a knife and trimming the apical part to remove leaves as per standard commercial practice. Harvest dates of 800, 600 and 400 mL per alternate day irrigated plants were 16th October, 3rd November and 20th November 2015 respectively. After harvest, samples were immediately transferred to the Plant Science Laboratory (PSL) and stored at 20 °C constant temperature and 55.5% relative humidity (RH). Visual and physiological attributes were performed at regular time intervals over storage. In more detail, samples were assessed: immediately after harvest, after 3, 6 and 10 days of storage (baseline, sampling day 1, sampling day 2 and sampling day 3 respectively) (**Figure 4**).

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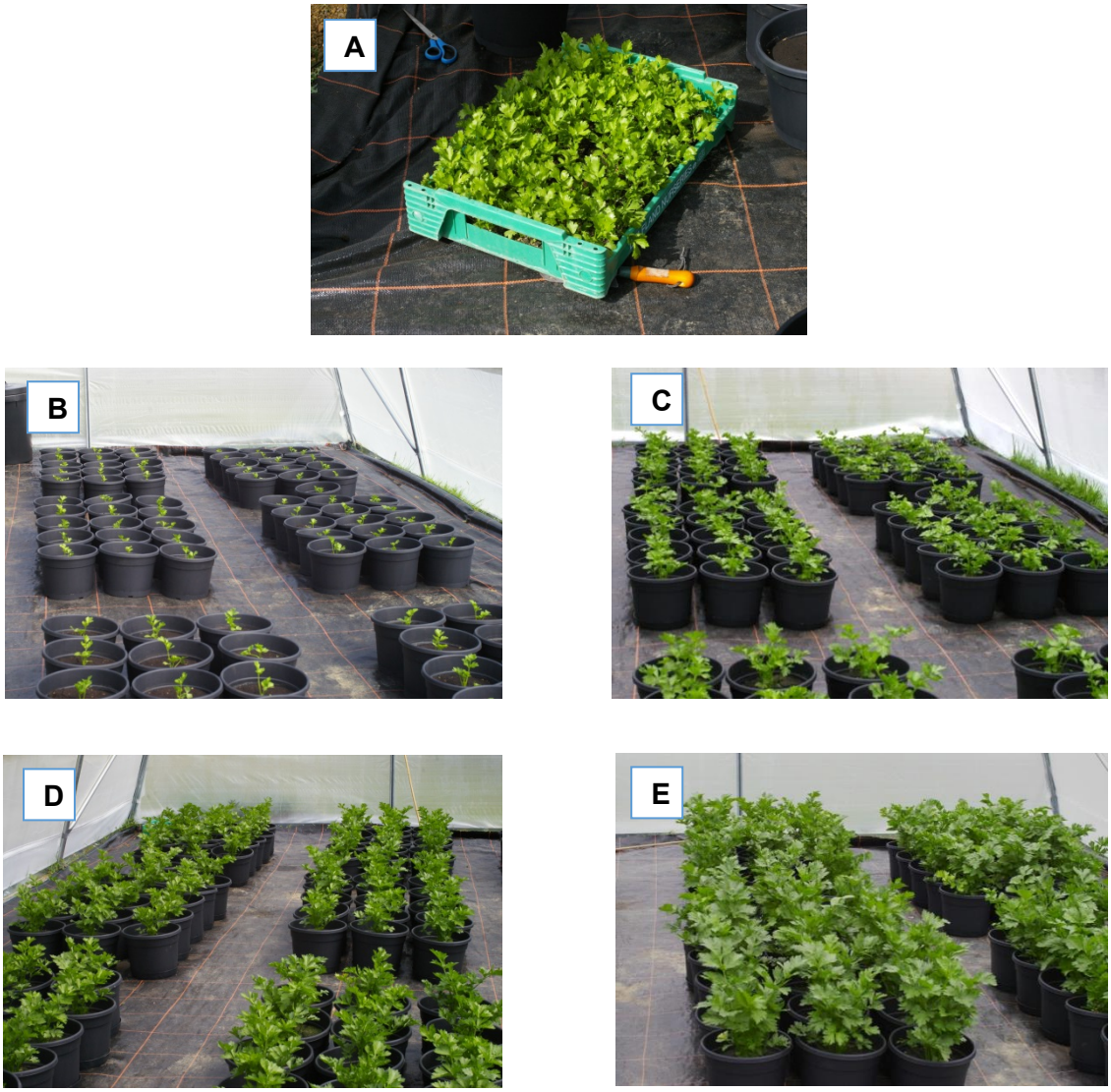

Figure 2 - Growing stages of celery before commencement of water treatments expressed in days after planting (DAP): Planting (A); 5 DAP (B); 16 DAP (C); 27 DAP (D); 38 DAP (E).

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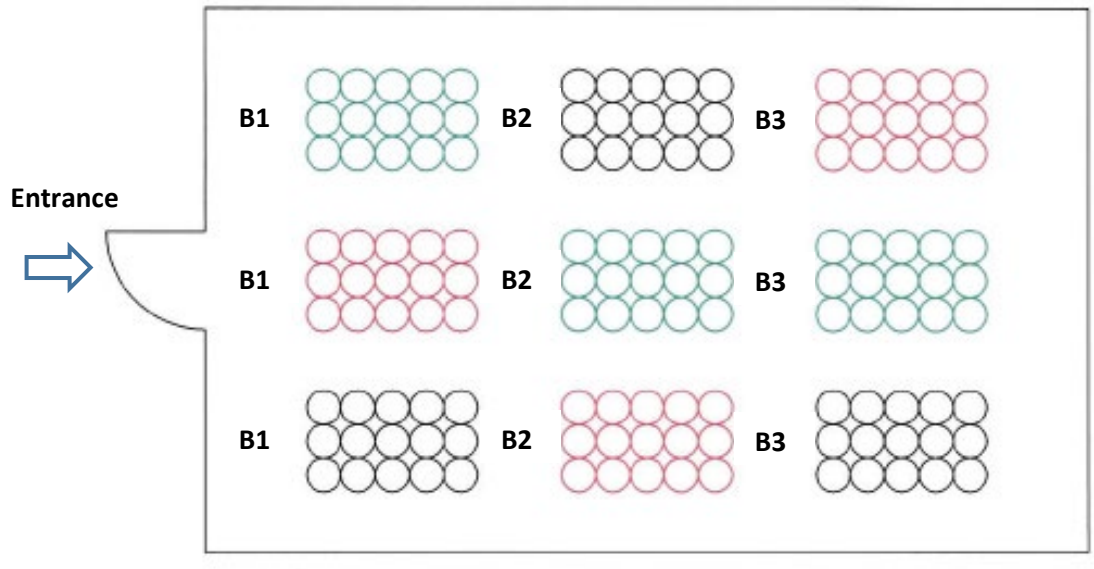

Figure 3 - Disposition of pots (circles) and blocks (B) in the experimental polytunnel. Colours correspond to the application of different water treatments (black: 800; green: 600; red: 400 ml every alternate day).

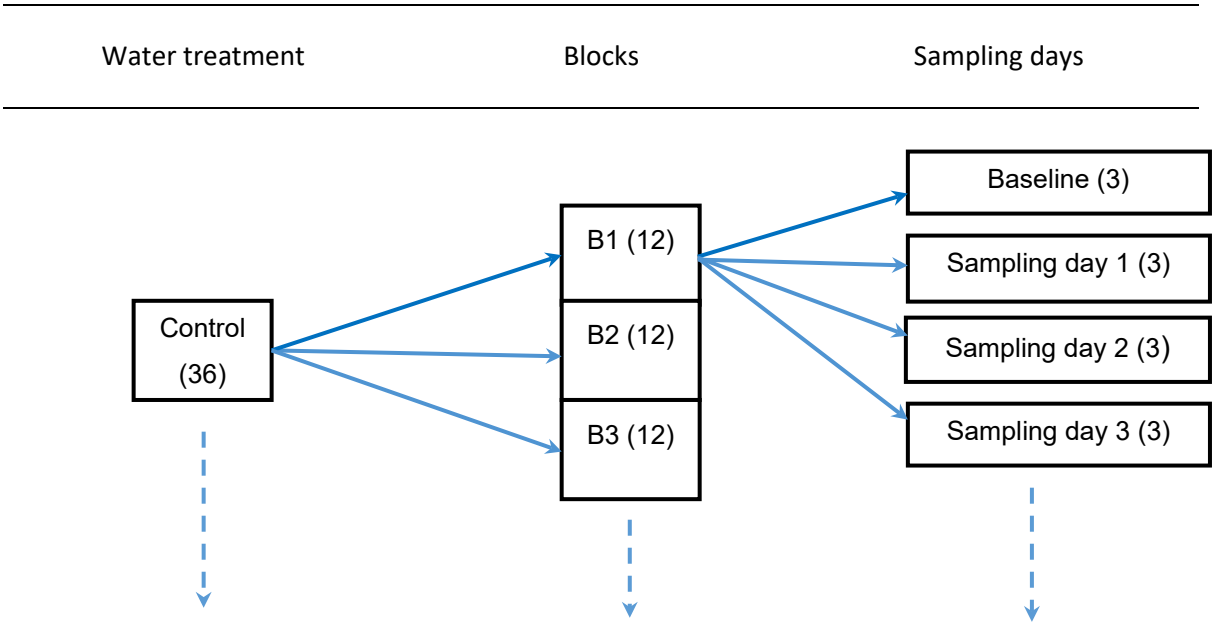

Figure 4 - Celery irrigation experiment layout (the number of samples are indicated in brackets).

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Soil moisture measurement

In order to confirm irrigation treatments, soil moisture content was measured periodically by impedance using a Thetaprobe meter (ThetaKit type ML2x, Delta-T Devices, Cambridge, UK) (**Figure 5**). Readings were taken after completely inserting the rods in the soil and were expressed in terms of relative water content (m^3 water per m^3 soil or %vol.). Water retention characteristics of the growing substrate were determined by collecting soil in metal rings and placing them in suction tables at different suction pressures as shown in **Figure 6**.

Figure 5 - Thetaprobe meter used to ascertain soil moisture (ThetaKit type ML2x).

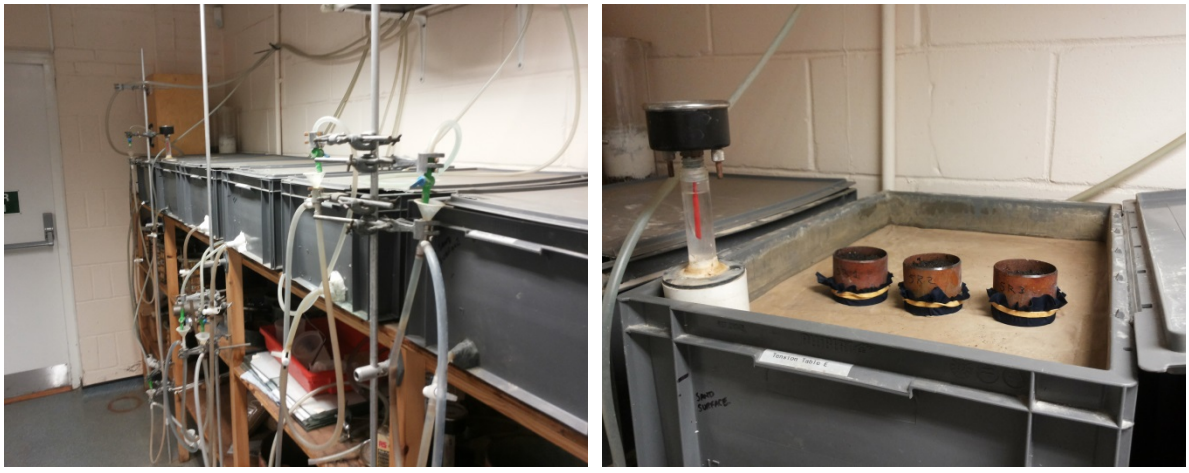

Figure 6 - Suction tables (on the left) with soil sample rings (on the right) placed inside.

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Visual and physiological attributes

Respiration rate

The respiration rate of celery heads was measured as reported by Collings *et al*., (2013) with slight modifications. A Sable Respirometry System (**Figure 7**) (Model 1.3.8 Pro, Sable Systems International, NV, USA) was used. Samples were incubated in air-tight plastic boxes (**Figure 8**) in continuous air flushed by an 80 HP pump (Hiblow, Techno Takatsuki Ltd., Philippines) to avoid modified atmosphere build up. Boxes were connected through Nalgene PVC tubes of 3 mm internal diameter (Thermo Scientific, Rochester, NY) to CA-10 carbon dioxide detector (Firmware version 1.05) and FC-10 oxygen detector (Firmware version 3.0) of the Sable System in order to detect O_2 consumption / CO_2 emission in real time for 10 minutes over 2 cycles. In addition, a water vapour pressure detector (RH-300) was used to measure relative humidity in the outflowing air. Due to the fact that air flow requires some time to stabilize in the incubating boxes, measurements were taken at the third cycle. Data were analysed with ExpeData software (Release 1.3.8, PRO Version) and reported in mL CO_2 h^{-1}. Subsequently, respiration rate data were converted in mL CO_2 Kg^{-1} h^{-1}. Measurements were taken at the constant temperature of 20 °C.

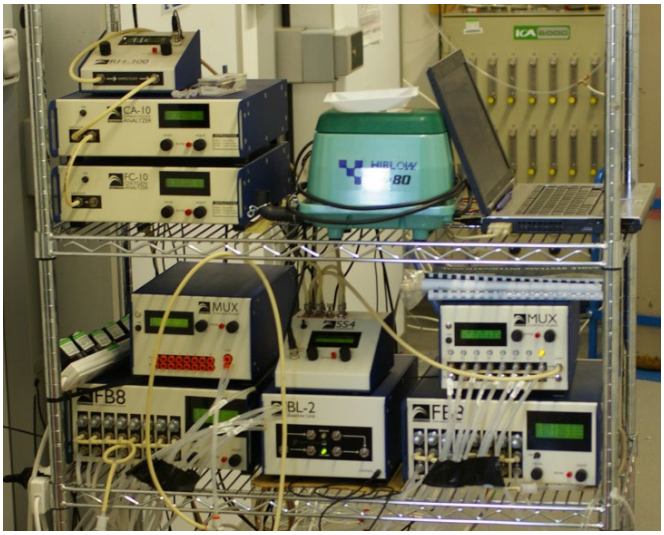

Figure 7 - Sable Respirometry System

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Figure 8 - Air tight plastic boxes connected to the Sable Respirometry System filled with celery samples.

Subjective colour

Samples were visually assessed for browning level at both cut petiole and butt ends. A 1 to 4 visual scale was used in order to assign the proper score (**Figures 9 and 10**). Photos of celery samples were taken at both cut petiole and butt ends in order to create a collection of file pictures to be used as references.

Level 1

Level 2

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Level 3 Level 4

Figure 9 - Comparative visual scale for assessing browning at cut petiole ends (1 = no browning, 2 = slight browning, 3= evident browning, 4= severe browning).

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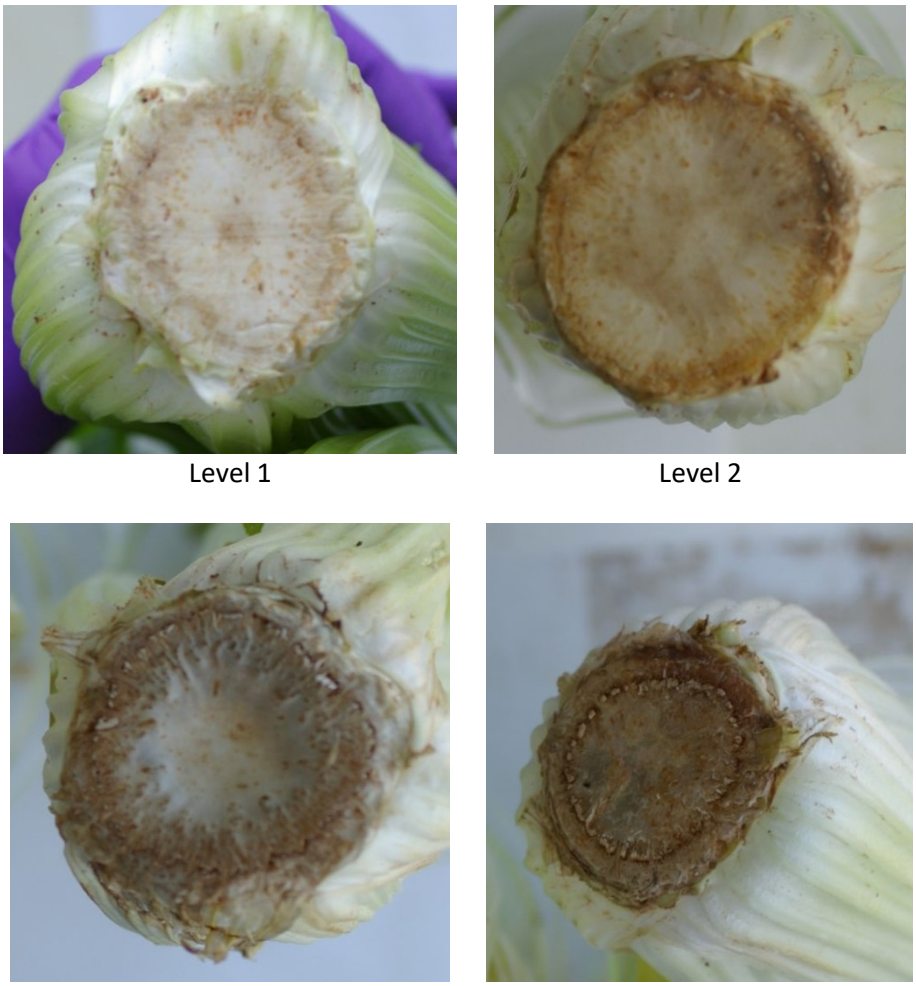

Figure 10 - Comparative visual scale for assessing browning at butt ends (1 = no browning, 2 = slight browning, 3= evident browning, 4= severe browning).

Pithiness

As well as subjective colour, pithiness level was visually assessed at both cut petiole and butt ends by using a 1 to 4 visual scale (**Figure 11**).

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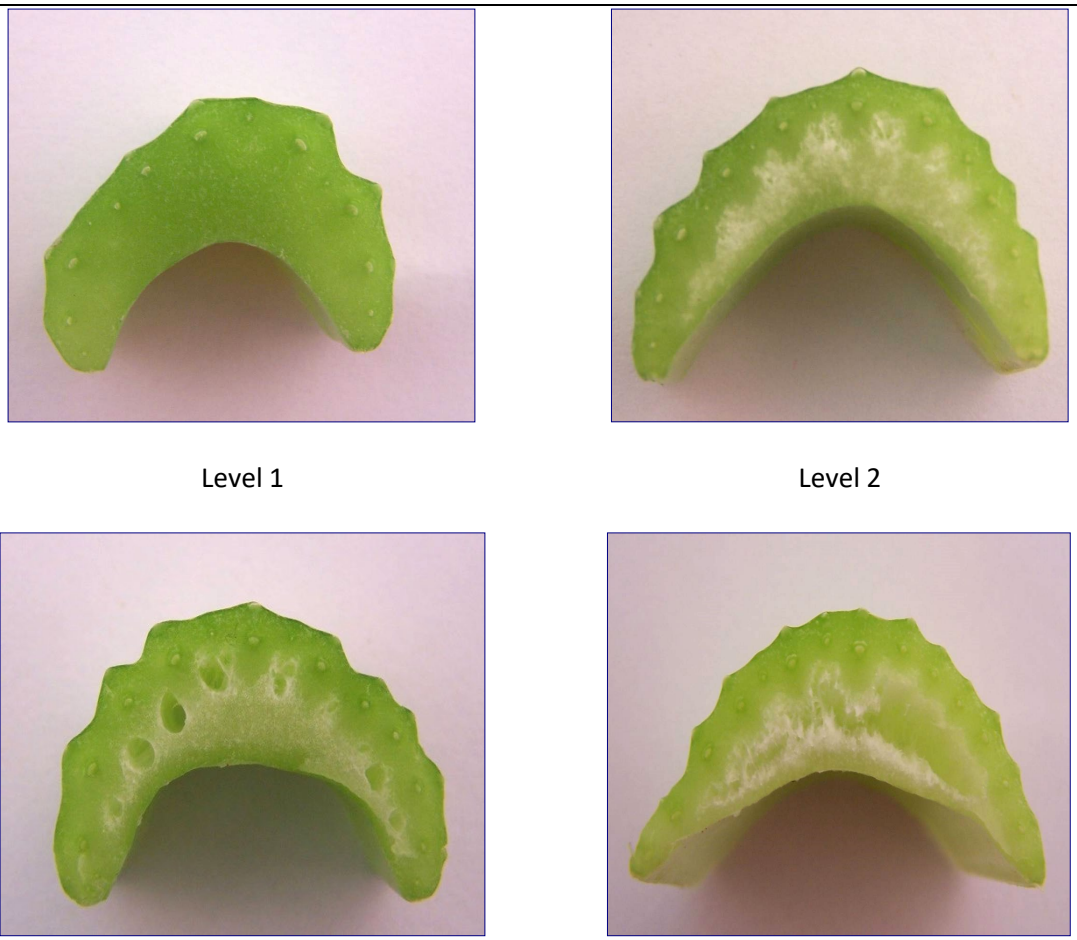

Figure 11 - Comparative visual scale for assessing pithiness (1= no pithiness, 2= slight pithiness, 3= evident pithiness, 4= severe pithiness) (photos provided by G's Fresh Ltd).

Bolting

A bolting assessment was conducted before snap-freezing the celery samples. Each sample was cut longitudinally and looked at the basal part of the plant in order to observe the core length. A visual scale, showed in **Figure 12**, was used as reference for evaluating the bolting level.

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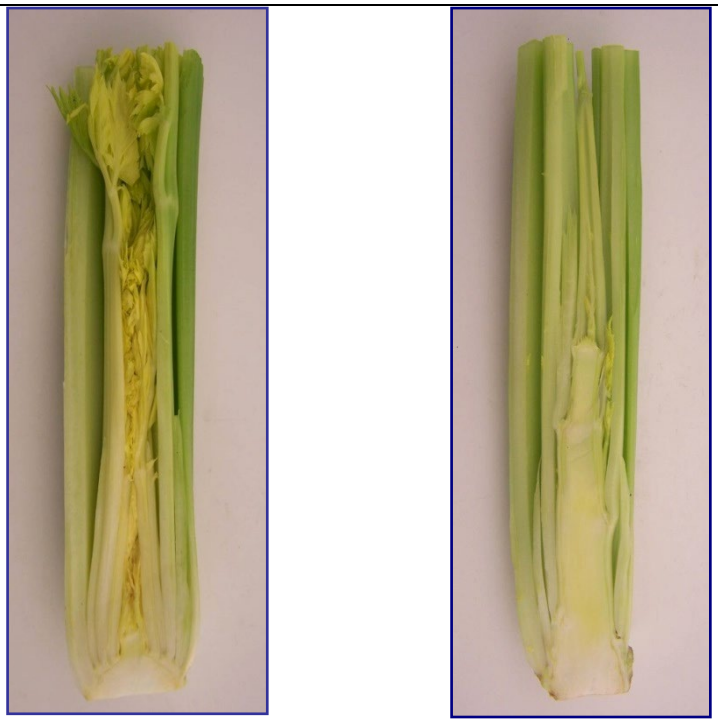

Level 1 Level 2

Figure 12 - Comparative visual scale for assessing bolting (1= no bolting, 2= bolting) (photos provided by G's Fresh Ltd).

Objective colour

Objective colour was measured with a CR-400 Minolta colorimeter (**Figure 13**) and DP-400 data processor at both cut petiole and butt ends of each celery head. Each individual measurement was the mean of four measurements randomly taken on both cut surfaces. Due to the relatively small surface on cut petiole ends, stalks were clustered together in order to create as continuous as possible (**Figure 14**) to be accurately processed with the colorimeter. Data were expressed in terms of Chroma (C*), hue angle (H°) and lightness (L*).

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Figure 13 - CR-400 Minolta colorimeter.

Figure 14 - Cut petiole ends clustering for objective colour measurements.

Biochemical analyses

Sample preparation

After performing visual and physiological attributes, each celery sample was cut with a knife into 5 different 1-cm long portions: butt ends, outer middle petioles, outer apical petioles, inner middle petioles and inner apical petioles (**Figure 15**). Thereafter, cut portions were separately sealed in plastic bags and immediately snap-frozen in liquid nitrogen to stop biochemical reactions, then were stored in -40 °C freezers until being lyophilized. Lyophilisation was done in a Coolsafe 55-9 Scanvac freeze-drier (Scanlaf A/S, Lynge, Denmark) (**Figure 16**) equipped with a vacuum pump (Vacuumbrand® RZ 2, Wertheim, Germany) for 7 days at -50 °C. Lyophilized samples were powdered with a mechanical pestle, weighed and placed into -40 °C freezers until required for extractions. All biochemical analyses were performed on celery dry material from crop maturity experiments (1st year trials).

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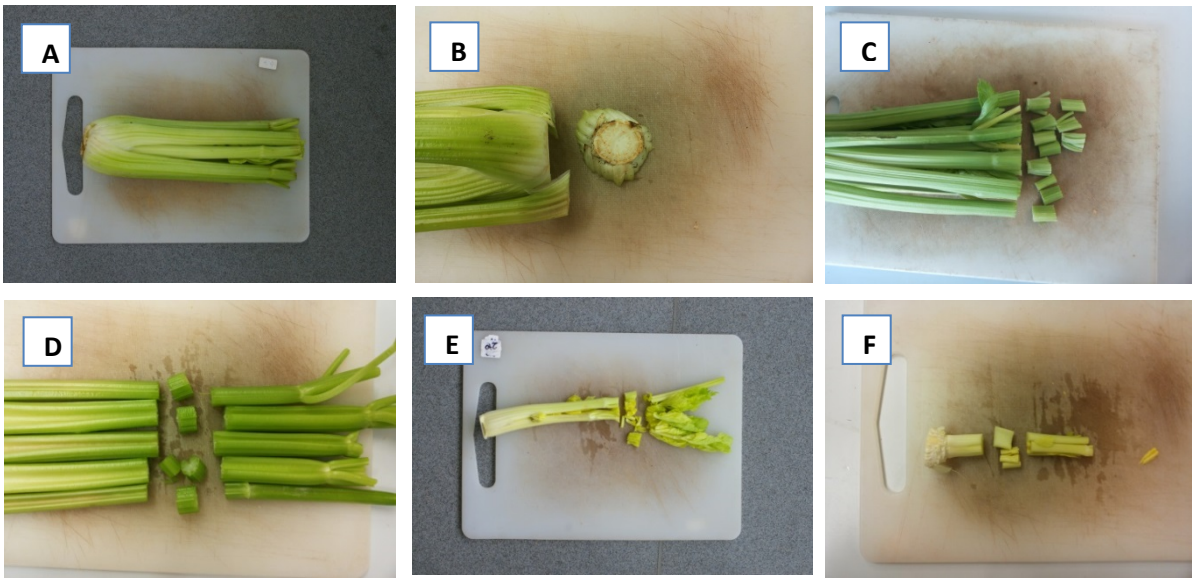

Figure 15 - Celery excised in different portions to snap-freeze in liquid nitrogen: celery head (A); butt end (B); apical outer petiole end (C); middle outer petiole end (D); apical inner petiole (E); middle inner petiole (F).

Figure 16 - Freeze-drier used to lyophilize fresh celery samples (Coolsafe 55-9 Scanvac).

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Extraction and identification of phenolic compounds

Extraction of phenolic compounds was performed as previously described by Downes *et al* (2010) with slight modifications. From each freeze-dried sample bag, 50 mg of celery powder was taken and subsequently mixed with 1 mL of extracting solution of 70:29.5:0.5 HPLC-grade methanol:water:formic acid solution (v/v/v) to make a concentration of 50 mg/mL. The mixture was then incubated in a shaking water bath for 1.5 h at 35 °C and vortexed for 20 sec every 15 min to avoid layering. Thereafter, samples were cooled at ambient temperature, put in a 3 mL plastic syringe and filtered with a 0.2 µm polytetrafluoroethylene (PTFE) filter (Jaytee Biosciences Ltd, Kent, UK). Filtered extracts were put in 1.5 mL amber glass vials and then stored in a -40 °C freezer until analysis. Identification of phenolic compounds was performed using an Agilent 1200 series HPLC (High Performance Liquid Chromatography) system (Agilent Technologies, West Berkshire, UK) (**Figure 17**) embedded with a DAD (Diode Array Detector) (model no. G1315D), quaternary pump (model no. G1311A), degasser (model no. G1322A) and fraction collector (Analyt FC, model no. G1364C). The stationary phase was formed by an Agilent Zorbax Eclipse XDB-C18 column (4.6 mm x 150 mm) (part no. 993967-902) fitted with a C18 Opti-guard column (part no. 820950-938). The mobile phase was constituted by two solutions: 100% acetonitrile (A) and a 95:5 HPLC-grade water:formic acid (v/v) solution (B). The program involved a linear increase/decrease of solvent B: 0-10%, 30 min; 10-16.4%, 30-50 min; 16.4%, 50-70 min; 16.4-100%, 70-71 min; 100%, 71-73 min; 100-0%, 73-74 min; 0%, 74-75 min at a flow rate of 1.0 mL min^{-1} and a column temperature of 35 °C. The Agilent ChemStation Rev. B.02.01 software was used to present data.

Figure 17 - HPLC-DAD system used to identify and quantify phenolic compounds.

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Extraction and quantification of sugars

Sugars were extracted from freeze-dried celery powder using a solution constituted by 62.5:37.5 HPLC-grade methanol:water (v/v) as previously described by Terry *et al*, (2007). From each freeze-dried sample bag, 50 mg of powder was taken and mixed with 1 ml of the extracting solution above mentioned to make a concentration of 50 mg/mL. The mixture was subsequently placed in a shaking water bath for 30 min at 55 °C and vortexed every 5 min for 20 sec to avoid stratification. Samples were then cooled at ambient temperature, put in a 3 mL plastic syringe and filtered with 2 µm PTFE filter (Jaytee Biosciences Ltd, Kent, UK). Filtered extracts were put in 1.5 mL clear glass vials and afterwards stored in a -40 °C freezer until biochemical analyses. Sugar content in the extracts was analysed using a HPLC (Agilent Technologies 1260 series, Berkshire, UK) system equipped with ELSD (Evaporative Light Scattering Detector) (**Figure 18**). The stationary phase was constituted by a 5 µm Prevail carbohydrate Es 250 mm x 4.6 mm (part no. 35101). The mobile phase was composed of two solutions: HPLC-grade water (solvent A) and HPLC-grade acetonitrile (solvent B). The program involved a linear increase/decrease of solvent B: 80-50%, 15 min; 50-80%, 5 min; 80%, 5 min at a flow rate of 1.0 mL min^{-1} and column temperature of 30 °C. Eluted sugars were identified and subsequently quantified by comparison with the respective calibration standards.

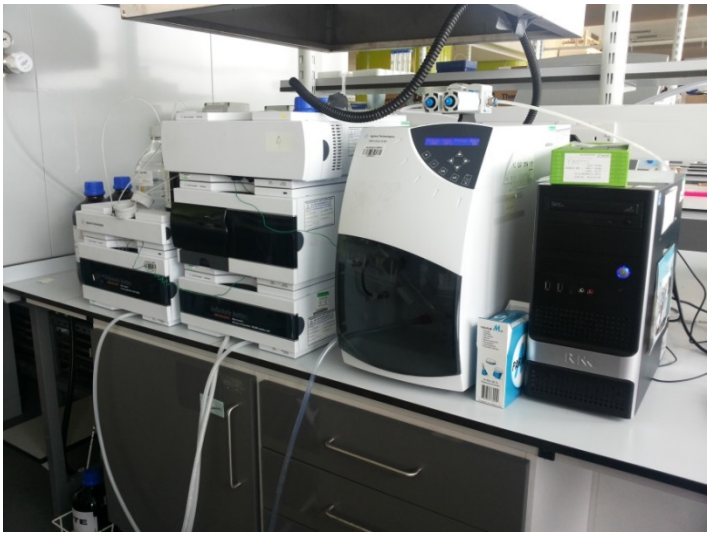

Figure 18 - HPLC-ELSD system used to identify and quantify non-structural carbohydrates.

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Statistical analysis

Data analysis was performed with the software Statistica 64, file version 12.0.1133.2. (StatSoft, Inc. UK). Means were firstly checked for their residuals and secondly submitted to Analysis of variance (ANOVA) in order to identify the main effects of the factors and the interactions between the factors to a probability of 5% ($P < 0.05$) unless otherwise stated. The statistical design included water treatment and storage time as factors. On the other hand, respiration rate, browning, pithiness, bolting, chroma, hue angle and lightness were included in the design as variables, taking into account the blocks as statistical units. Least Significant Differences (LSDs; $P= 0.05$) were calculated from each analysis to compare the means.

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Results and discussion

Identification of phenolic compounds of celery (Year 1)

Significant levels of phenolic compounds were found in affected tissues of celery. Conversely, middle outer, apical inner and middle inner petiole tissues showed lower levels of the phenolics (**Figure 19**).

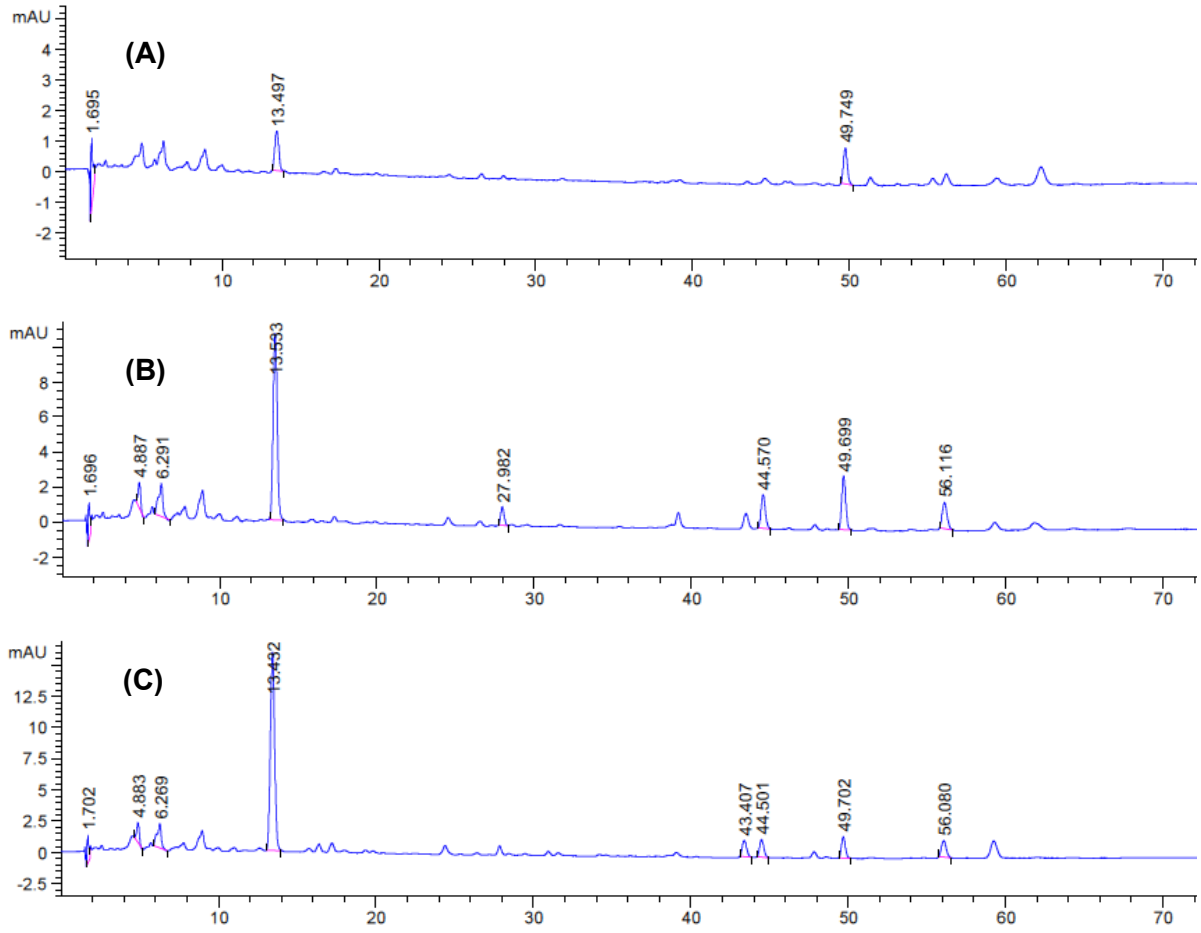

Figure 19 - Chromatographs showing the phenolic compounds profile in celery tissues at 320 nm wavelength DAD signal: middle outer petioles (A), apical inner petioles (B) and middle inner petioles (C).

Yao *et al*., (2011) identified 6 phenolic compounds in different celery varieties. In more detail, the authors reported the presence of luteolin, apigenin and kaempferol, which belong to the class of flavonoids, and caffeic, ferulic and *p*-coumaric acid, which belong to the class of phenolic acids (**Figure 20**).

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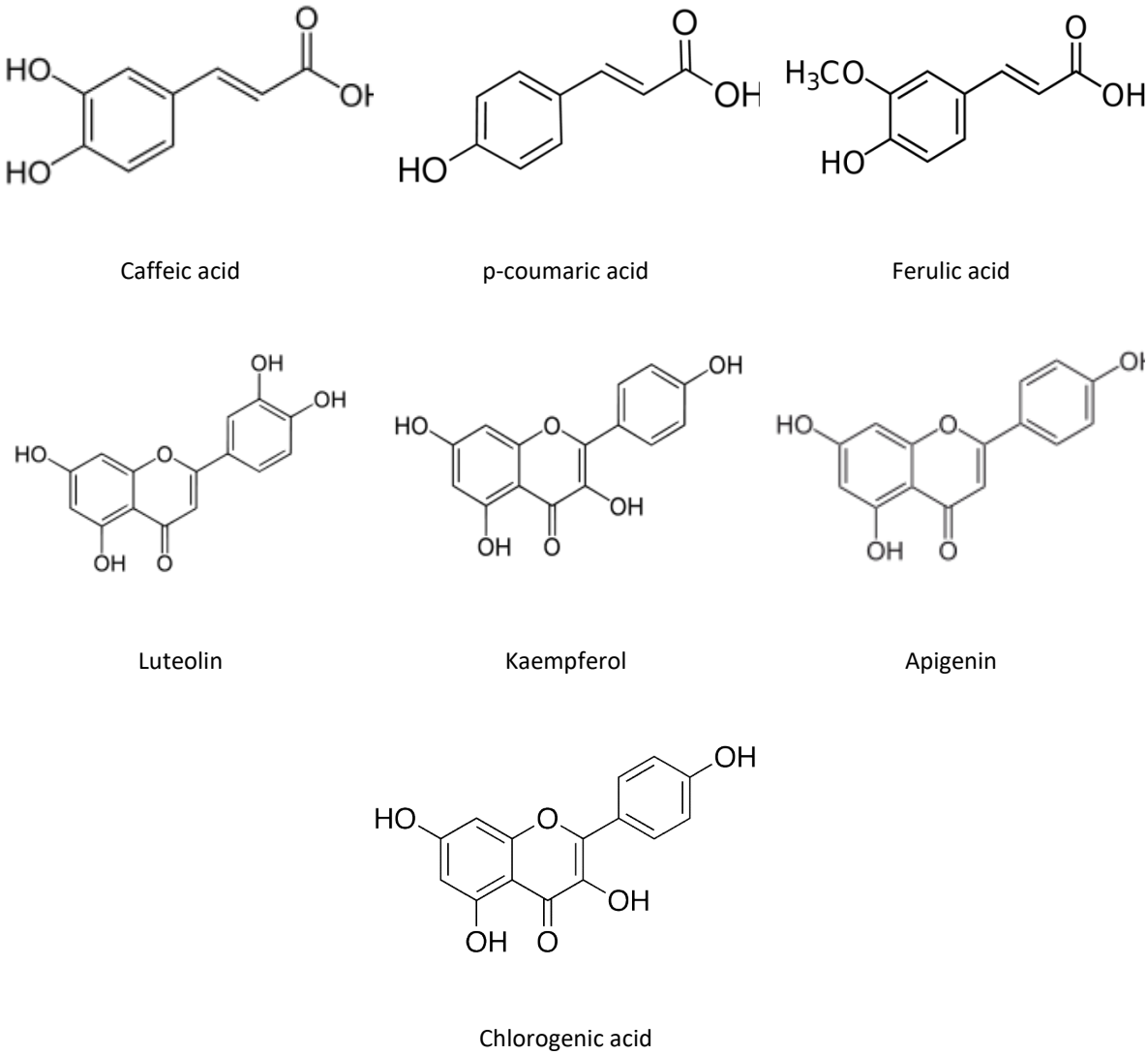

Figure 20 - Chemical structures of the phenolic compounds found in celery by Yao *et al*., (2011).

According to these findings, standards of these compounds were purchased and subsequently analysed with HPLC-DAD to detect the respective retention times (**Figure 21**). Results showed two main points: firstly, all molecules identified by Yao *et al*., (2011) were completely absent in Monterey celery cv. analysed tissues. Secondly, several unknown phenolic compounds were isolated. The most important according to the peak areas (mAU*sec) are displayed in **Figures 22, 23, 24 and 25**.

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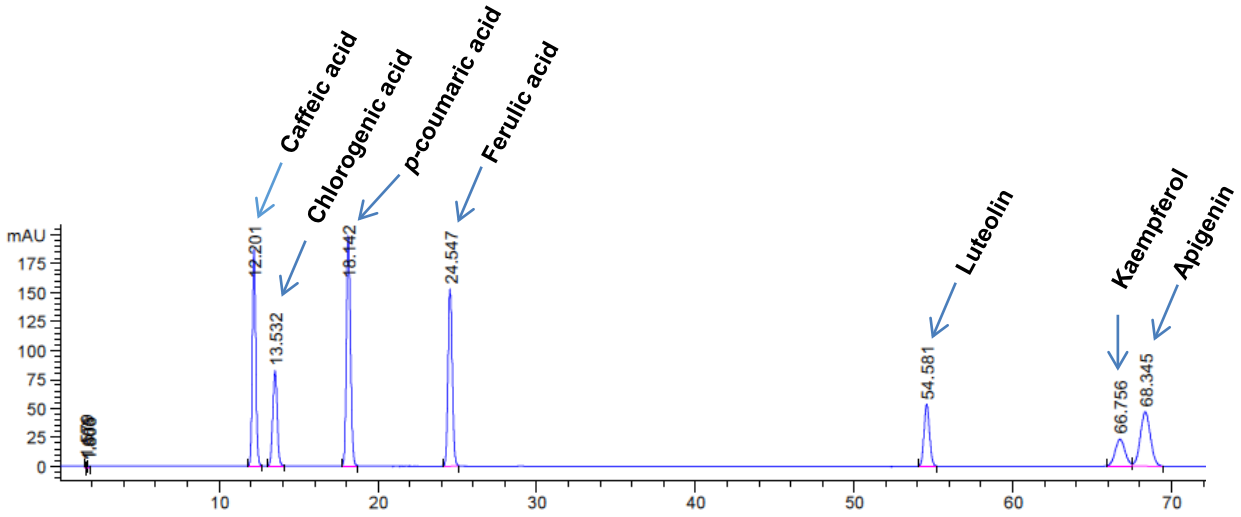

Figure 21 - Chromatographs showing the retention times (minutes) of the all the phenolic standards in celery tissues at 320 nm wavelength DAD signal according to Yao *et al*., (2011).

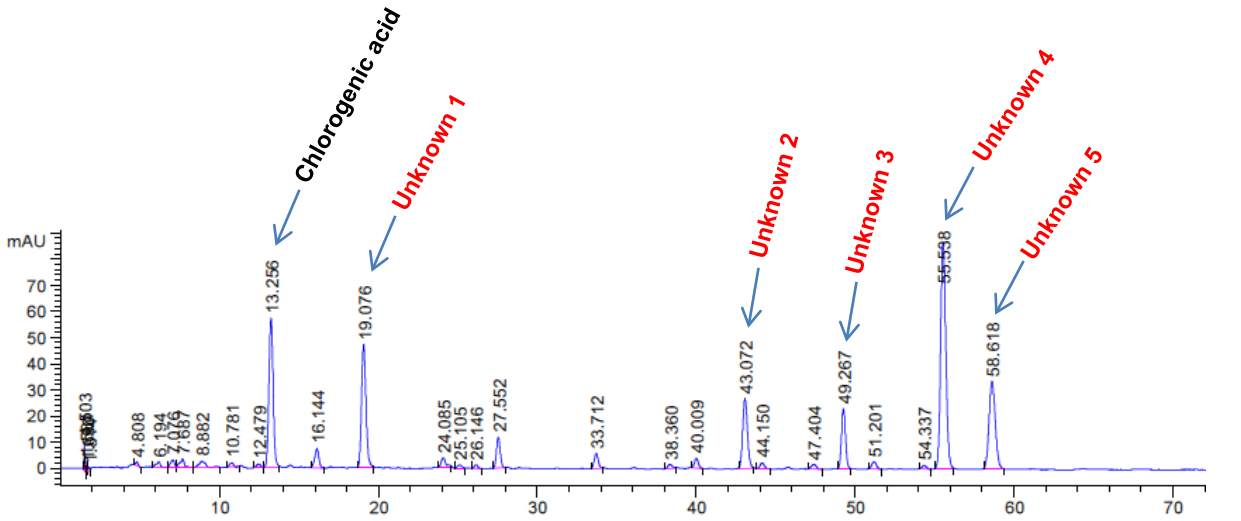

Figure 22 - Chromatographs showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery butt ends with no browning at 320 nm wavelength DAD signal.

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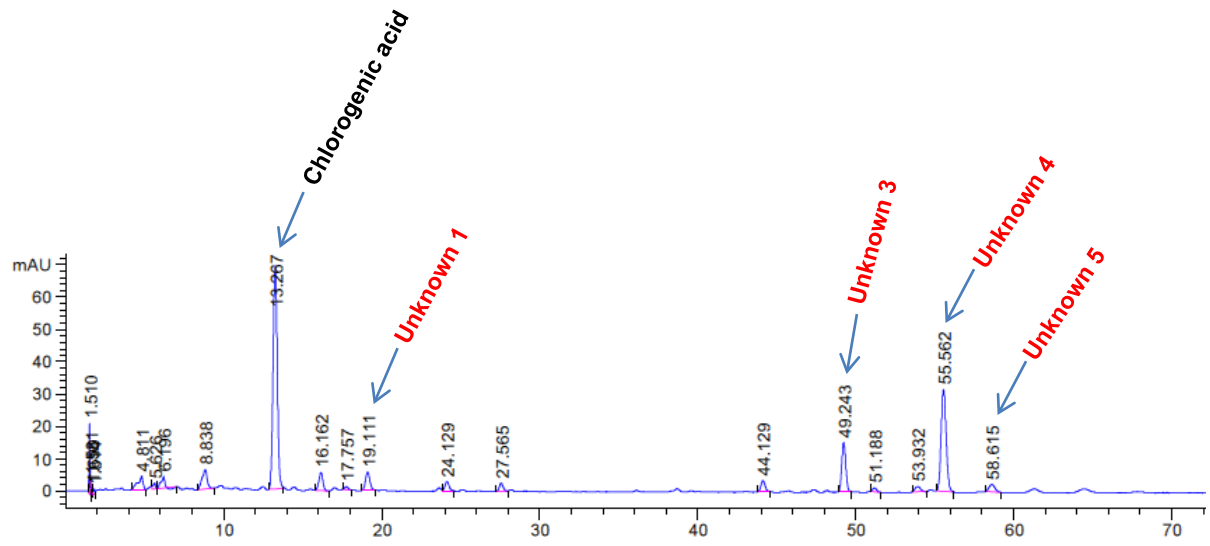

Figure 23 - Chromatographs showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery apical cut petiole ends with no browning at 320 nm wavelength DAD signal.

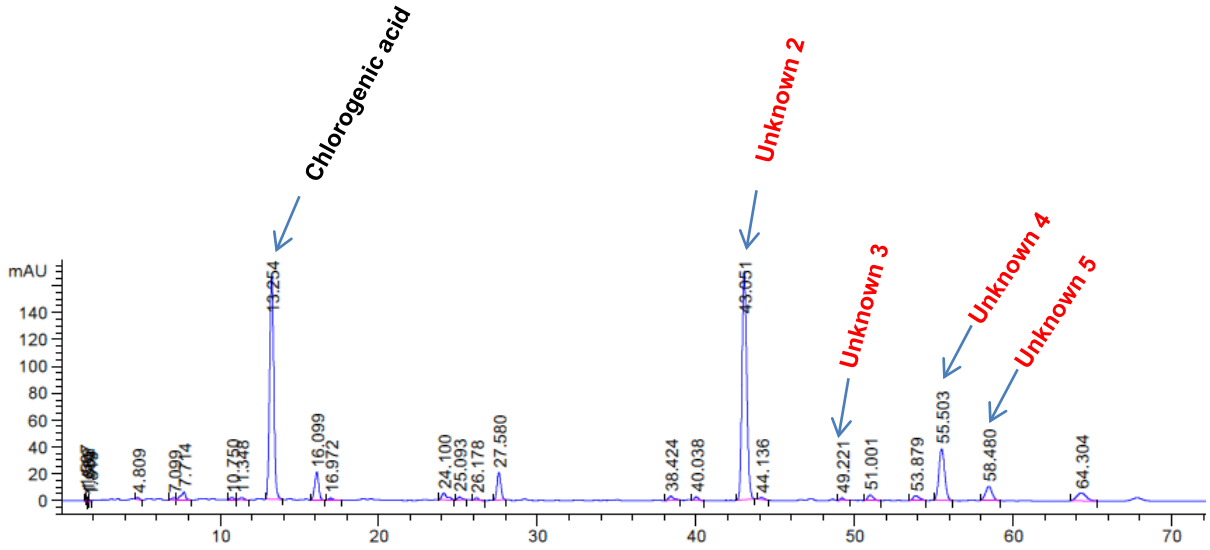

Figure 24 - Chromatographs showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery butt ends with severe browning at 320 nm wavelength DAD signal.

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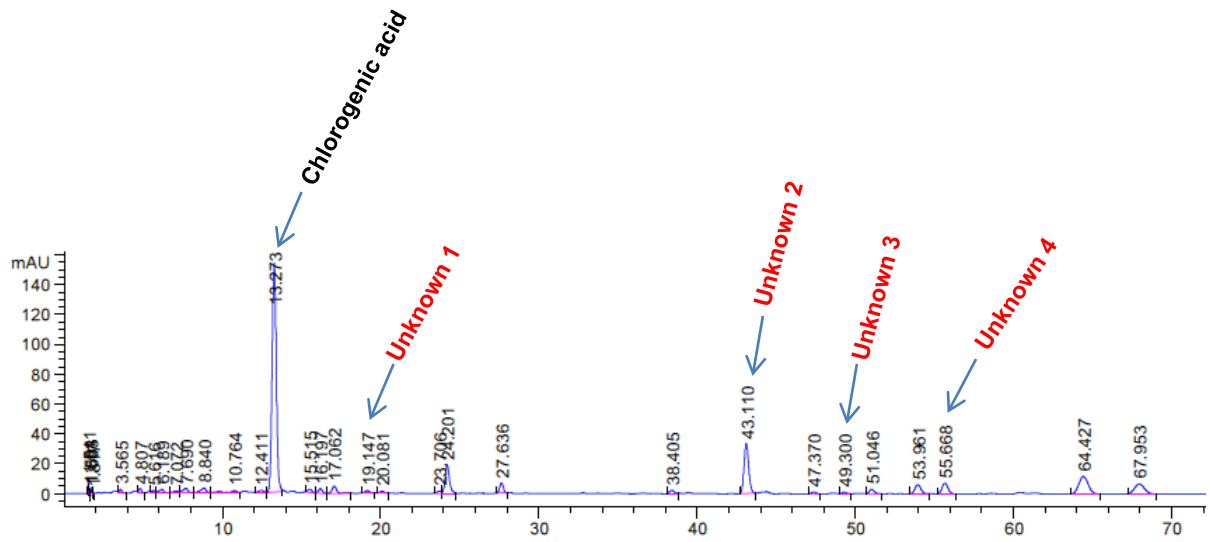

Figure 25 - Chromatographs showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery apical cut petiole ends with severe browning at 320 nm wavelength DAD signal.

Chlorogenic acid is present in large quantities at both butt ends and apical cut petiole ends, regardless of browning level. On the other hand, the unknown phenolic compounds clearly change in their concentration depending on tissue type and browning level. For instance, unknown n.1 was present in butt ends tissues with no browning yet it was completely absent in those with severe browning. Unknown n.2 level evidently increased from white to dark tissues while unknown n.3, 4 and 5 showed the opposite trend. Regarding apical cut petiole end tissues, unknown n.1 remained unchanged in its quantity, whilst unknown n.3, 4 and 5 showed almost the same trend of butt ends (i.e. decreased). Unknown n.5 completely disappeared in severely discoloured tissues. All these patterns clearly showed a potential involvement of unknown analytes in postharvest browning. It is expected that appearance, disappearance and change in concentration of phenolic compounds according to different tissues and browning levels could further elucidate the role that these compounds have on cut-end browning in celery. In order to do this, identification of all unknown analytes shown in the chromatographs above should be performed.

Soil water status (Year 2)

Volumetric water content of the growing medium (Fenland peat) was intensively affected by irrigation treatments (**Figure 26**) with values ranging from 13.5 to 47.0 $m^3 m^{-3}$ of soil. Values clearly fluctuated throughout the examined growing period (from commencement of water

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treatments to harvest of control irrigated samples) with fluctuation being more pronounced in the control irrigated plants. This is likely attributable to the characteristics of peat, which tended to lose most of the water in its surface layers (where the soil meter records the moisture) as peat absorbs high quantities of radiation in this zone. Despite the different fluctuation intensities of the water treatment curves, trends are generally similar depending on the trial stage. For instance, soil moisture was low during the middle stage and scored high during the final stage, due to differences in the amount of radiation and temperature the crop received.

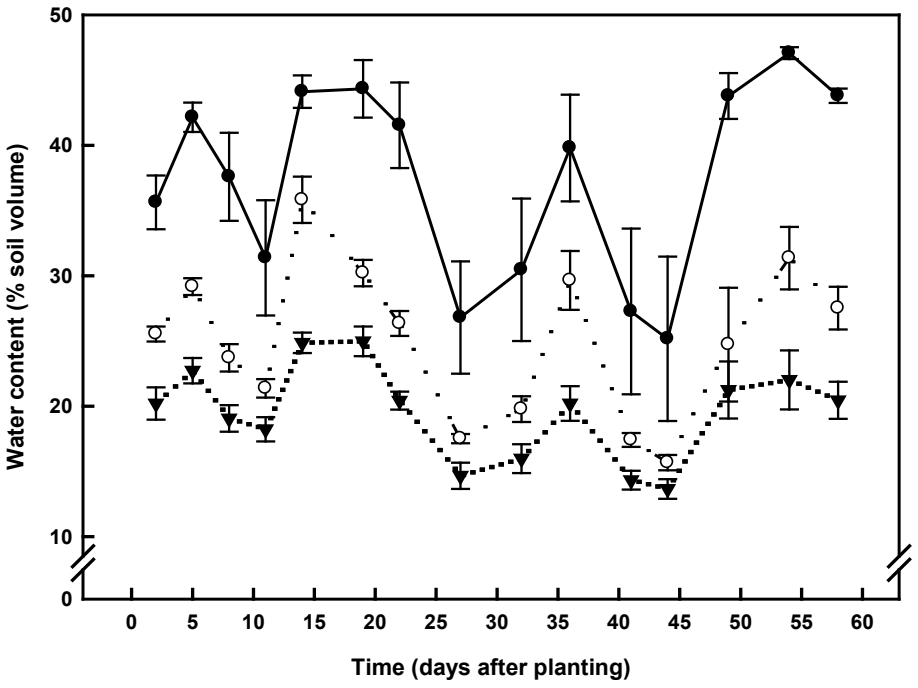

Figure 26 - Water volume of the growing substrate used in the celery irrigation trial. Different symbols correspond to each water treatment: 800 ml (●), 600 ml (○) and 400 ml (▼) every alternate day. Values represent the means every alternate day of soil moisture content in the pots. Standard error bars of the means are shown.

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Respiration rate (Year 2)

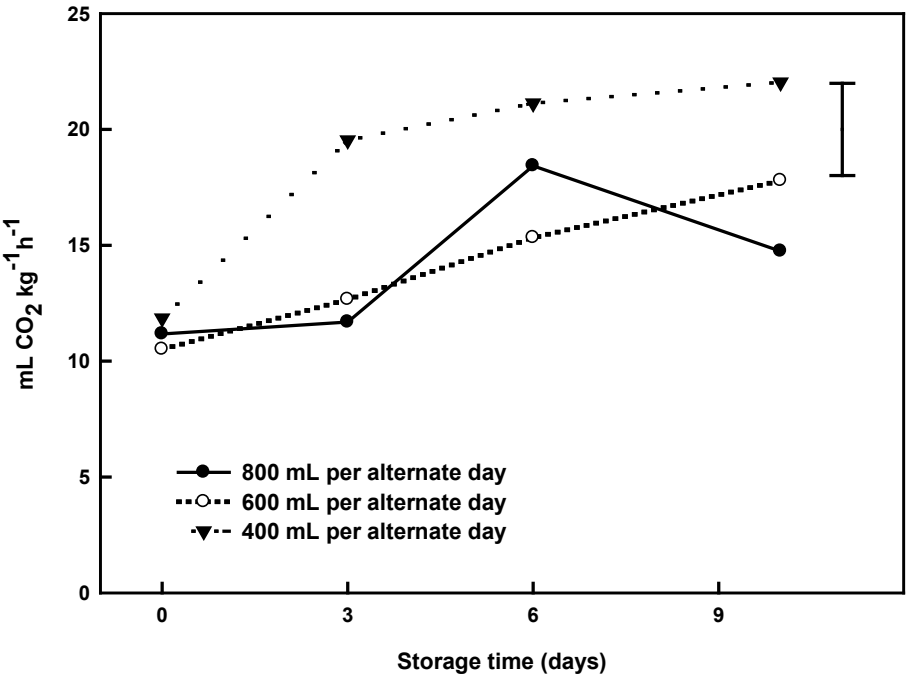

Figure 27 - Respiration rate of celery treated with three different irrigation regimes. LSD bar is shown.

Overall, respiration rate ranged from 10.5 to 22.0 mL CO_2 kg^{-1} h^{-1} and was influenced by water treatments and time of storage (**Figure 27**). The less watered plants showed the highest level of respiration rate, with an evident increase in the first 3 days of storage. On the other hand, the 600 and 400 mL per alternate day treated plants showed similar values yet with two different trends. In effect, the former treatment caused respiration rate to be steadily augmenting over storage, whilst the latter fluctuating.

According to these findings, the rise in metabolic activity of celery plants is likely attributable to the water stress applied during the trial. Luna *et al.*, (2013) found similar results, with fresh-cut Romaine lettuce showing the highest respiration rate when treated with the lowest irrigation dose.

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Subjective colour (Year 2)

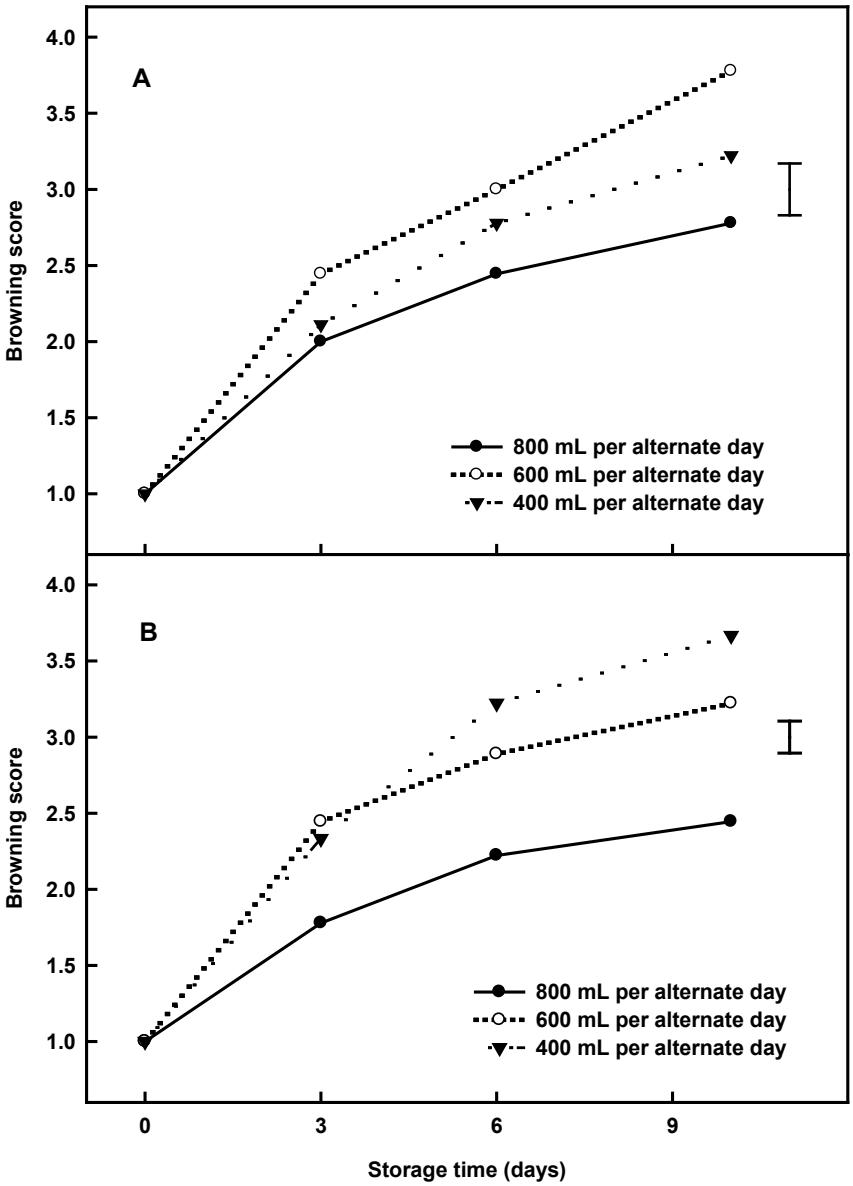

Figure 28 - Subjective browning assessment of celery butt ends (A) and cut petiole ends (B) treated with three different irrigation regimes. LSD bars are shown.

The two above graphs show a clear pattern. Celery plants developed browning over storage time and with increasing levels of water stress (**Figure 28**). In more detail, 800 mL per alternate day irrigated samples showed the lowest level of browning over time. Overall, most of the discolouration developed during the initial phase of storage.

The patterns shown in the above two graphs seem to evidence that an increasing level of stress induced by irrigation treatments reflected in an increasing level of cut-end browning in

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celery. These results are in accordance with a previous experimental work carried out by Luna *et al*., (2013) on fresh-cut Romaine lettuce. In effect, the authors reported that the less irrigated midrib tissues showed reduced cut edge browning over storage in air.

Objective colour (Year 2)

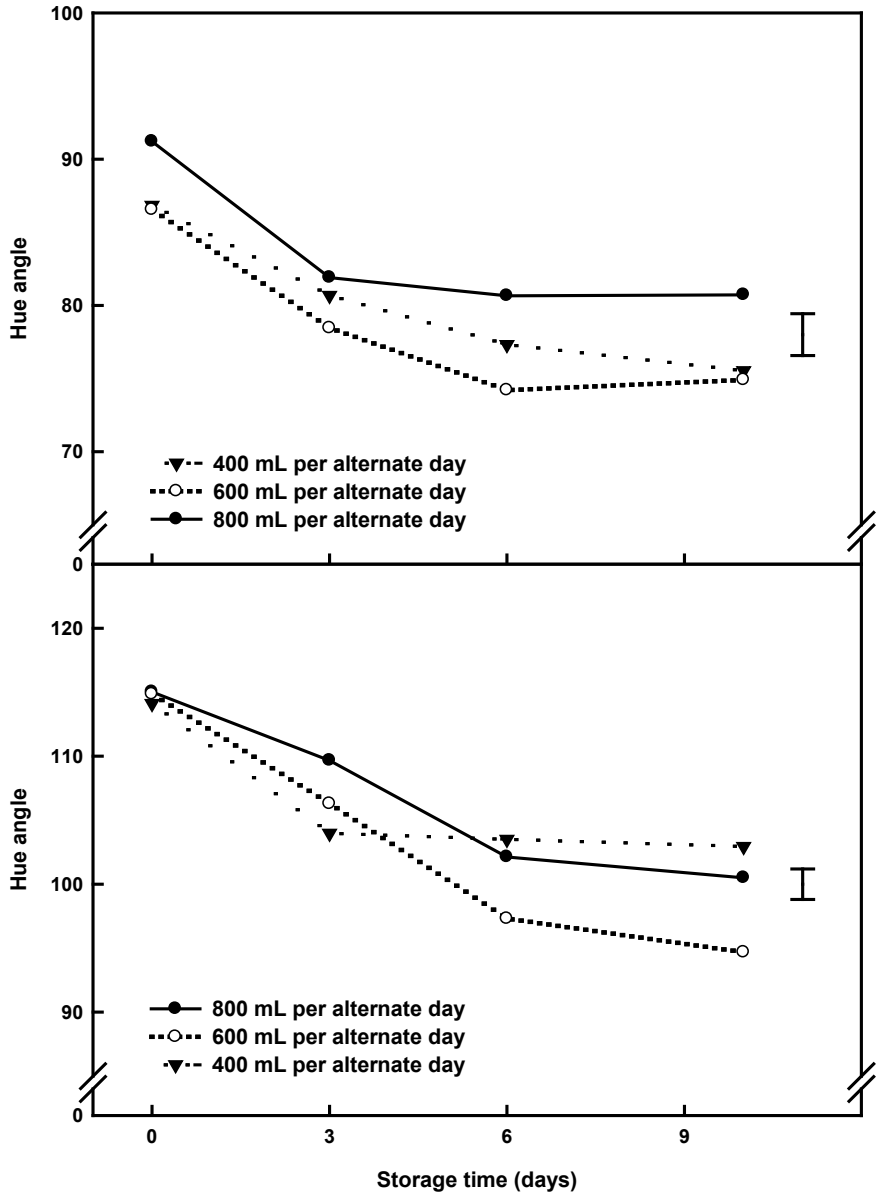

Figure 29 - Hue angle of celery butt ends (A) and cut petiole ends (B) treated with three different irrigation regimes. LSD bars are shown.

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Hue angle decreased over storage time and water stress treatment applied to plants (**Figure 29**), confirming the subjective visual assessments reported in **Figure 28**.

Pithiness (Year 2)

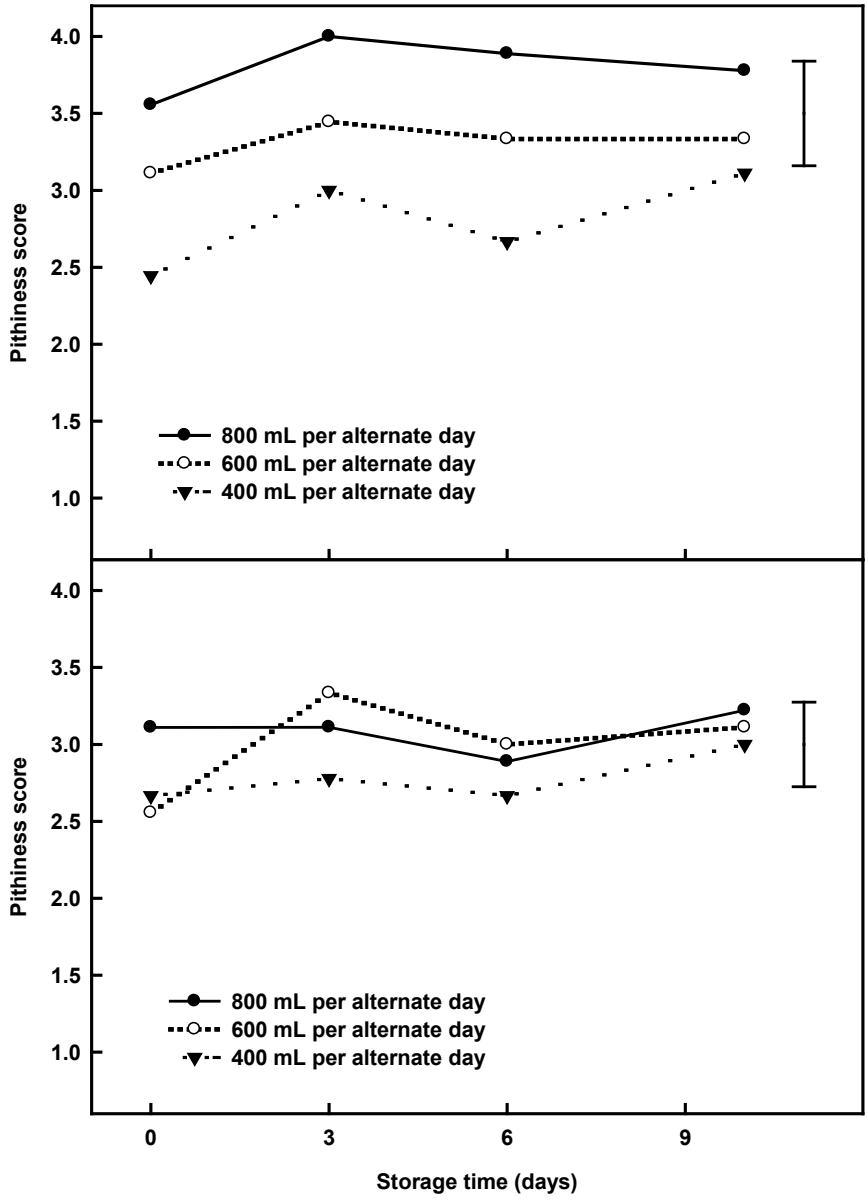

Figure 30 - Pithiness assessment of celery butt ends (A) and cut petiole ends (B) treated with three different irrigation regimes. LSD bars are shown.

Pithiness was generally high at both butt and cut petiole ends of celery in plants which had received the higher rate of irrigation, with scores ranging from 2.5 to 4.0 (**Figure 30**). This is

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probably the result of the infrequent irrigation applied throughout the trial, which was administered every alternate day. This statement founds confirmation in a previous study conducted by Breschini and Hartz (2002) on celery grown in the open field with drip irrigation system. Celery prefers soils with constant soil moisture. Thus, it is likely that the lack of this condition may have triggered the stress response in plants, manifested with the collapse of parenchyma tissue and the development of pithiness which is less evident in the plants which received a higher level of irrigation.

Conclusions

Extraction and detection of compounds believed to be related to postharvest browning has been performed on dry material of celery collected during the 1st year trials which focused on crop maturity. Preliminary results showed spatial and temporal changes in the profile and composition of phenolic compounds depending on cut-browning level. None of the phenolic compound standards reported in previous literature have been detected in the celery used to conduct the 1st year experiments. Several unknown molecules were isolated, yet only the most relevant were collected according to their quantity. Appropriate detailed analysis should be performed to reveal their identity in order to better understand the underlying biochemistry of cut-browning in celery. Further details are reported in the following section.

An experiment involving deficit irrigation treatments in protected environment was set up, carried out and completed. Results from visual assessments and physiological analysis showed that respiration rate and cut edge browning was higher in the less irrigated plants. These two findings seem to indicate a probable involvement of the plant stress response in the cut edge browning in celery. In order to better understand the key mechanisms of this physiological “disorder”, additional biochemical analysis should be performed. Further details are reported in the following report.

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