

Understanding the mechanisms and the role that preharvest horticultural maturity, agronomic factors and growing conditions have on postharvest discolouration in celery.

Horticultural Development Company CP79

CRANFIELD HEALTH

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LITERATURE REVIEW

1.1 Celery (*Apium graveolens* L.)

Celery (*Apium graveolens* L.) originates from the Mediterranean region where it has been cultivated for the last 3000 years (Whitlock, 1979). Most likely, the wild plant was used for medicinal purposes hundreds of years before it was used as food. The first mention of the medicinal properties of celery leaves dates back to the 9th century B.C., when celery made an appearance in the Odyssey, the famous epic by the Hellenic poet, Homer.

Celery belongs to the *Umbelliferae* (or *Apiaceae*) family and the *graveolens* species (NCBI, 2012). The Umbelliferae family is named after the shape of its flowers, which are called umbels. Other members classified under the *Umbelliferae* family are carrots (*Daucus carota*), fennel (*Foeniculum vulgare*), parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*) and dill (*Anethum graveolens*) (Reinhold, 1989). The celery prospers in a stone free soil that is deep, loose and fertile. Also, consistent moisture is necessary for good yields (Choudhury, 1977).

Celery is in the order Apiales in the genus *Apium* (NCBI, 2012). Cultivated celery is a diploid with $2n=22$ chromosomes but there are reports of plants with $2n=18$ chromosomes (Sharma and Bhattacharyya, 1959). The celery genome has a large size compared to carrot and consists of 11 large chromosomes, nine submetacentric, one metacentric and one telocentric, described by Murata and Orton (1984).

Every 100 g of fresh celery gives 16 kcal and 95% water. Celery is a good source of Vitamin C (3.1 mg/100g), Vitamin K (29.3 mg/100g), Sodium (80 mg/100g) and Potassium (260 mg/100g) (USDA, 2012). In addition, it has a strong protective effect against colorectal cancer (Jinfu *et al.*, 1991). Celery can be eaten fresh or dried. The dried products are used as a spice; leaves and stalks, which are full of flavour, are used as garnish, in salads and in soups. Celery should be eaten as fresh as possible, but typically has a 1-2 week postharvest life at 5°C depending on the time of year (Gomez, 2004). In the market fresh-cut celery is a 'ready to eat product' due to the progress of technologically advanced packaging techniques.

1.2 Industrial landscape

According to the Agricultural Commissioner in California during 1999 the average harvested acreage was 11.300 while the price per carton was \$8.04 (Table 1). Figure 1 shows

the proportion of cost for each activity during celery production in the U.S.A. The proportion consists of 55% for harvest and postharvest cost, 30% for land preparation, planting and growing costs, 11% for cash overhead, 1% for interest on operating capital and 3% for non-cash overhead. These divisions though may not be proportional to the European market. Harvesting costs include the cost of the cartons, picking, packing, loading, hauling to the nearest cooling facility and selling. Land preparation, planting and growing costs include fuel, lube and machinery repairs as well as materials and labour for all production practices. Cash overhead costs include land rent, office expenses, liability insurance, supervisor, foremen wages, property taxes and insurance and investment repairs (Takele, 1999).

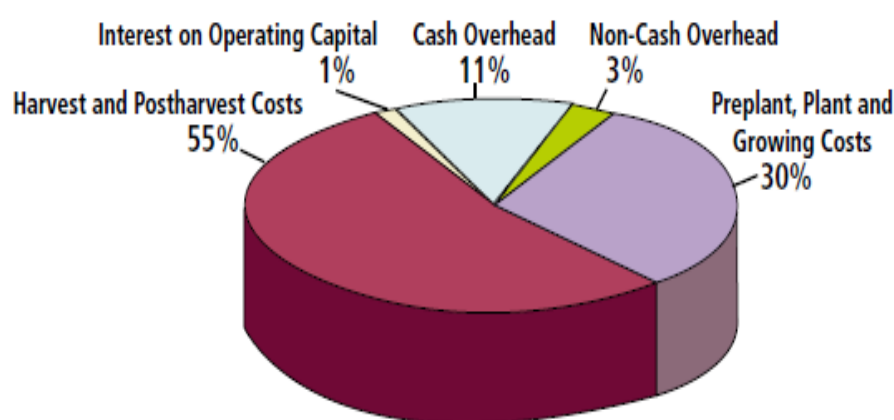


Figure 1 Proportion of production costs for celery in California (Takele, 1999)

Table 1 Harvested acreage, average yield and average prices for celery in California, U.S.A (Takele, 1999)

Year	Harvested acreage	Cartons per acre*	Price per carton (\$)
1995	10,341	1,172	11.57
1996	10,152	1,199	7.22
1997	9,232	1,158	8.83
1998	11,923	1,161	8.50
1999	11,300	1,247	8.04
Approximate average	10,590	1,190	8.80

*One carton equals 60 pounds.

During 2010, American consumers used an average of 6.2 pounds of fresh celery per person (USDA 2011) and the total value of celery was \$398.9 million (NASS 2011). Currently Canada remains the largest market for U.S.A. celery, receiving about 94,110 metric tons

(MT) of fresh-market celery (FAS 2010). The United States continues to be a net exporter of celery worldwide, shipping about 118,085 metric tons (MT) overseas in 2010, slightly more than in 2009 (FAS 2010).

Data on celery trade and production are not available on FAOSTAT regarding the UK, thus the data included are referring to the U.S.A production.

1.3 Life cycle

Germination and emergence

Celery is biennial, producing seeds in the second year, with succulent, well developed roots. The seeds are small (about 2.500 per g), irregular in shape and difficult to sow (Whitlock, 1979). They germinate slowly and young seedlings require special attention. Celery can be sown directly into the soil or transplanted, depending upon different climates (Reinhold, 1989). Usually seeds are sown in the nursery and then transplanted after 2 months (Raid, 2004). Strict hygiene conditions are essential to prevent *Pythium* and *Rhizoctonia* infections (Whitlock, 1979). The seed germinates best when it is left uncovered, when temperatures are close to 18.5 °C and humidity is kept high until the first leaf is visible. Later the temperature can be lowered to 13-15.5 °C and while the plants are growing steadily can be hardened off at 13 °C. Onwards, late batches of plants may be hardened off outdoors in late May/July (Whitlock, 1979).

Celery stems are angular branched and leaves are oblong, 7-18 cm long, pinnate or trifoliate. Celery grows to a height of 30 to 40 cm and is composed of leaf-topped stalks arranged in a conical shape and joined at a common base (Choudhury, 1977).

In the second year a flower head is produced with masses of fruits. The flowers are white or greenish white, very small on compound umbels. The fruit consists of two united carpels and each carpel produces a single seed (Reinhold, 1989). The influence of the position of the seed on the plant has an obvious effect on seed size and dormancy characteristics and performance. Effects of the environment, mineral nutrition, temperature and photoperiod during seed formation and maturity have a profound effect on germination and seedling emergence (Whitlock, 1979).

Dormancy

An important problem often apparent in the cultivation of celery is poor seed germination (Robinson, 1954). The complex dormancy mechanism of seeds often results in the complete inhibition of germination. In that case, the number of available transplants produced in the green house is decreased as well as the total yield in the field (Thomas *et al*, 1983). Growth regulators can break the celery seed thermo-dormancy. Celery seeds fail to germinate in the dark when the temperature rises above 18 °C but this dormancy can be broken with a mixture of the gibberellins A4 and A7 (GA4/7) and certain other growth regulators. According to Thomas (1986), the thermo-dormancy of celery seeds in the dark was partially broken by a 30 min light exposure on the third day of incubation at 20–22 °C, promoting 50 % seed germination after 20 days. It is suggested that light stimulates the biosynthesis of gibberellins which are essential for dormancy-break in celery seeds (Thomas, 1989). The presence of abscisic acid (ABA) and gibberellins during incubation in the dark completely overcame the inhibitory effects of homogenates. This indicates that thermo-inhibition of celery seeds is associated with the accumulation of a germination inhibitor which interacts with cytokinins (Thomas *et al.*, 1986). Also, Thomas (1989) found that celery seeds were less thermo-inhibited when dried back after a seed soak treatment with the gibberellins A4 and A7 (GA 4/7) plus ethephon (G+E) or an osmotic priming treatment in the light with polyethylene glycol. Pre-pelleting growth regulator treatments may be another possible method to enhance plant growth, since pelleting creates a similar type of dormancy in the seeds (Thomas *et al*, 1983).

1.4 Postharvest factors affecting celery quality and storage life

Harvest and sale

Celery may be hand harvested or machine harvested. In the open field, celery is harvested by hand using a specially shaped stainless steel knife at soil surface level (Whitlock, 1979). Harvesting mechanically does not deliver the same quality end product as hand harvesting. Due to uniform crop growth fields are cut once. Usually celery is cut, selected and packed in the field on small field houses called 'rigs'. The end product is then brought to the depot within 3 hours; kept there for a day in temperature controlled conditions, until it reaches the supermarket shelves (Whitlock, 1979). Harvesting occurs when stalks are size and before any pith has developed in the petioles. In some production areas, celery plants produce hearts (head centres) which are packaged 2 or 3 per package with the stalks. It is critical that harvested stalks be cooled as soon as possible. After harvest, celery is packed in

cartons. Depending on the producer company, each carton contains around 36-48 celery stalks and weights 27 kg which is then cooled and palletized at low temperatures (Takele, 1999).

Preparation and transportation

During the preparation of fresh-cut vegetables, wounding encourages the formation of enzymes involved in phenylpropanoid metabolism and the formation and accumulation of phenolic compounds which can result in the browning phenomenon (Ke and Saltveit, 1989; Brecht, 1995; Lopez-Galvez *et al.*, 1997; Saltveit, 1997).

Postharvest activities create browning of some fruits and vegetables that reduces quality and shortens shelf-life (Bolin and Huxsoll, 1991). This deterioration increases steadily during storage (Vina and Chaves, 2006; Ke and Saltveit, 1989; Brecht, 1995; Lopez-Galvez *et al.*, 1997; Saltveit, 1997). Studies have shown that for whole celery heads storage temperatures of 0 °C in combination with 92–95 % relative humidity maintains a shelf life of about 2 months (Vina and Chaves, 2003). During transport and commercial distribution it is difficult to maintain these recommended conditions therefore celery is subjected to variables like changing temperature and humidity, but also to mechanical damage and remarkable weight loss, sometimes higher than 30%, in which case it is not marketable (Tei *et al.*, 2000).

Storage temperature

After harvest plants are sized to the desired length with the use of sharp knives and then are stored at 0–5 °C and 90–95 % relative humidity (Gariépy *et al.*, 1986). Temperatures over 0 °C during storage may result in minor growth in celery along with the blanching of the stalks that reduces the product marketability (Whitlock, 1979). The optimum storage temperature is 0 °C as long as freezing is avoided (Hodges and Toivonen 2008; He and Luo 2007). According to Whitlock (1979) hydro-cooling is the most common method and temperatures should be near to 0-2 °C. Theoretically hydro-cooling is the ideal system because it does not remove water and it may even improve a slightly wilted crop by rehydrating it. However, vacuum cooling has been shown to be more effective with celery (Rogers, 2006; Kader, 1992). Vacuum cooling is widely used for celery packed in corrugated cartons for long-distance shipment. Air circulation can be maintained around crates by using

strips between the crates and leaving air channels between rows. Caution during shipment must be taken not to damage the celery cartons. Vacuum cooling is the ideal method for crops like celery that have a large surface-to-volume ratio (Whitlock, 1979; Kader, 1992). In addition precautions must be taken when celery is packed since it can absorb odours from other fruits and vegetables so therefore should not be stored close to other vegetables with strong odour (Daugovich *et al*, 2008). For better storage, celery should be cut with a small piece of root attached and harvested before the outer stalks become pithy.

Storage time

Storage time seemed to influence quality of minimally processed celery stored for protracted periods, as it resulted in an increase in total phenol concentrations at 10 °C, while a temperature of 0 °C would lessen such reaction (Vina and Chaves, 2006; Figure 2). Ke and Saltveit (1988) have observed a marked increase in the total soluble phenols content for iceberg lettuce exposed to several kinds of stress (attack of pathogens, ethylene treatment), under conditions causing no variations in the control samples. Furthermore, Amanatidou *et al.* (2000) found an eight-fold increase in total phenol concentrations in carrot discs stored in air for 12 days at 8 °C.

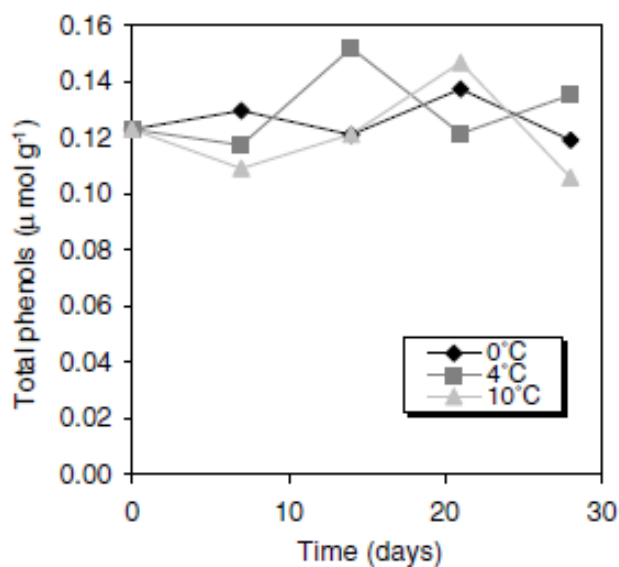


Figure 2 Total phenols content of minimally processed celery stored at 0, 4 or 10 °C for 28 days. Source: Vina and Chaves (2006)

Controlled atmospheres (CA)

Controlled atmospheres provide benefits during postharvest storage. Gariépy *et al.* (1986) reported that the best results for cv. Istar with 10 kPa O₂ and 2 kPa CO₂ while the celery cv. Utah could be stored for more than 2 months in a CA of 3 kPa O₂ and 5 or 10 kPa CO₂. Atmospheres containing 3 kPa O₂ and 5 kPa CO₂ minimised the loss of green colour and developed better texture and crispness. Another study showed that celery cannot withstand a CO₂-enriched environment over 10 kPa, developing off-odours, decay and internal leaf browning (Suslow and Cantwell, 2000). For celery stored at 4 °C CA with 2.7–11 kPa O₂ and 6 kPa CO₂ showed some advantages (Knee and Aggarwal, 2000). On the other hand, Saltveit (2003) recommended 5 kPa O₂ and 5 kPa CO₂ levels when duration of storage, temperature, relative humidity (RH) and ethylene concentration are constant parameters; any change in these factors would demand another combination of CO₂ and O₂ concentrations. It has been found that celery under air-stored circumstances has 30% losses after 29 days at 1.5 °C due to rotten leaves, stems or stem sections (Gariépy *et al.*, 1986). Reyes (1988) found that under high CO₂ levels bacterial disease developed. Also, it has been found that damage from high levels of CO₂ (>10 kPa) induced off-odours, off-flavours and internal leaf browning (Suslow and Cantwell, 2000). Thus, Saltveit (2003) suggested levels close to 5 kPa. Controlled atmospheres of 5 kPa O₂ and 15 kPa CO₂ offered the best overall quality of celery stalks, preventing butt end cut browning, decay and pithiness (Gomez and Artes, 2004), retarded chlorophyll loss (Kader, 1986) and kept a bright green colour in celery (Shewfelt and Henderson, 2003).

Effects of polymeric films packaging

Rizzo (2009) studied the influence of two polymeric films: polyolefin anti-fog (AF) and micro perforated (MP) on shelf life of whole celery. The AF non-perforated is a co-extruded polyethylene and polypropylene film, treated with an antifogging additive; while MP is a co-extruded polypropylene with a piercing density of 7 holes/cm² (Rizzo, 2009). Un-packed vegetables have an unacceptable condition after 20 days, while package in the AF and MP films delivers 31 days shelf life. The study showed that the polyolefin anti-fog (co-extruded polyethylene and polypropylene film) provided better results by increasing shelf life until 31 days, preventing weight loss and allowing for better hygiene and conservation. The reduced

total weight loss of celery in AF film was lower than 3%. However, this film is costly and should be considered carefully before being applied in industry (Rizzo, 2009).

Modified atmosphere packaging (MAP)

The combination of modified atmosphere packaging (MAP) refrigeration during storage and product distribution to the supermarket is a way to maintain freshness and quality of minimally-processed fruits and vegetables (Artes *et al.*, 2006). Some advantages of MAP are increased safety and a significant reduction of losses during handling, storage, transport, distribution and retail sale (Artes *et al.*, 2006). Disadvantages of MAP include slower cooling rate of the packaged product and the potential risk of water condensation within packages due to temperature fluctuations (Artes *et al.*, 2006). Also, under anaerobic conditions off-flavours and off-odours might develop because the volatile emissions and bioactive compounds may decrease. Robbs *et al.* (1996) found that fresh cut celery segments stored in sealed film bags at < 5°C developed water soak of the cut surfaces and moisture accumulation inside the bags. The segments softened, discoloured and in some cases disintegrated.

Recommended storage conditions of green celery sticks are 4 °C for 10 days, but there are no reports on optimal MAP conditions to preserve the stalks for longer. On the contrary of what the previous authors found, Gomez and Artes (2005) have found that MAP treatment improved the sensory quality, avoided the loss of green colour, decreased the development of pithiness and retarded the growth of microorganisms (Gomez and Artes, 2005). Green sticks of celery that were placed in hermetically sealed plastic bags (MAP) improved sensory quality, avoided discoloration and minimised the development of pithiness and microorganisms. After 15 days at 4°C, celery sticks within oriented polypropylene (OPP) bags showed the best quality, neither off-odours nor off-flavours were detected (Gomez and Artes, 2005).

Use of coatings and other treatments

Edible coatings have been used to coat fresh vegetables since the 1930s (Platenius, 1939), mainly for water loss control and to prevent decay. Loaiza-Velarde *et al.*, (2003) suggested the use of coatings on the cut surfaces of vegetables to avoid water loss that result in vascular tissue protuberance. The edible coatings film are made of different materials; lipid, resin, protein and carbohydrate compounds that are used separately or in compositions.

Resins are a group of acidic substances that are produced by plant cells in response to wound or damage. Synthetic resins are petroleum-based products such as paraffin wax, polyethylene wax and mineral oil (Baldwin, 2003). Moreover, the coating acts as a carrier of useful compounds that delivers the desired effects to the coated vegetable. Such materials can be plasticizers that increase the strength and flexibility of coatings (Baldwin, 2003). In addition to moisture losses avoidance, edible coatings (e.g. pectin with glycerol, both 7% aqueous) are used to deliver coating antioxidant to inhibit PPO and PAL activity (He and Luo 2007). The most common additives are ascorbic and citric acids. An optimal edible coating consisting of 1.5% Alanate-310 (a calcium caseinate) and 1.5% Myvacet-5-07 (an acetylated monoglyceride) produced a 75% reduction in moisture loss from celery sticks (Avena-Bustillos *et al.*, 2006).

An alternative method to minimise browning is the application of anti-microbial agents. He and Luo (2007) found that sodium chlorite conferred anti-microbial protection and inhibited PPO. Garcia and Barrett (2000) suggested cysteine applications to avoid browning, but the levels required affect the celery taste, resulting in bitterness. Moreover, Prakash *et al.*, (2000) examined the use of gamma irradiation as an anti-microbial factor to measure maturity discolorations. The authors found that gamma irradiation at 0.5-1.0 KGy extended shelf-life by almost a month while maintaining freshness parameters such as texture, aroma and colour. Another similar study, showed that with 1 KGy gamma radiation in fresh-cut celery the PPO and respiratory rate were much inhibited than the untreated celery. The number of bacteria and fungi were also decreased by the order of 10^2 and 10^1 respectively (Lu *et al.*, 2005).

The application of ozonated water on PPO activity of fresh-cut celery was examined as another treatment to inhibit browning on celery. The PPO activity was inhibited by ozonated water application and the efficacy of inhibition to PPO activity increased as the increasing ozone concentration in water. The treatment of 0.18 ppm ozonated water was beneficial for preservation of fresh-cut celery during storage at 4 °C (Zhang *et al.*, 2005). Generally ozonated water application was effective to reduce the population of microorganisms and retard physiological metabolism, thus assuring microbiological safety and value of fresh-cut celery (Zhang *et al.*, 2005).

Methyl Jasmonate (MJ)

Methyl jasmonate (MJ) is a volatile organic compound derived from jasmonic acid and the reaction is catalyzed by S-adenosyl-L-methionine: jasmonic acid carboxyl

methyltransferase (Rotem *et al.*, 1984). Plants produce jasmonic acid and methyl jasmonate in response to wounding, which is build up in the damaged parts of the plant. Methyl jasmonate can retard deterioration of celery sticks. A vapour from a source of 10^{-4} or 10^{-5} mol contained in one litre retarded deterioration for 2 weeks at 10 °C. The number of bacterial colonies was reduced to 1/1000 of control after 1 week of storage and the appearance of soft rot was retarded by the jasmonate treatment (Buta and Harold, 1998). The same effect was observed for pepper sticks. Less than 1×10^{-6} mol of MJ vapour was necessary to cause the biochemical changes in the stored vegetables resulting in extended storage life and decreased microbial growth (Buta and Harold, 1998). In Table 2 it can be seen that MJ vapour of 1×10^{-4} mol inhibited browning of celery stalks during the first week of storage, but the inhibitory effect was more intensive during the second week (Buta and Harold, 1998).

Table 2 Inhibition of browning on cut surfaces of celery by Methyl Jasmonide

Source: Buta and Harold (1998)

	Treatment	Treatment	Treatment
Time(weeks) in 10 °C	control	MJ 10^{-4} mol	MJ 10^{-5} mol
1	0.59 ^a	0.26	0.30
2	2.15	0.75	1.46

^a: Visual observations of browning on the celery cut surfaces were made on 0-5 scale, where 0=no browning, 1=very slight discoloration, 2=slight browning, 3=light to moderate browning, 4=moderate browning and 5=dark browning. Average values are shown.

1.5 Postharvest physiological and biochemical changes in celery during storage

After harvest, biochemical changes occur in celery heads for instance altered carbohydrate levels, and phenols. These factors are influenced by celery physiology such as changes in respiration rate and weight loss which affect the quality and therefore the marketability of celery. It is important that these changes are minimised to maintain high quality celery while maintaining flavour and health benefiting properties.

Respiration rate

Respiration rates of fresh-cuts vegetables are generally higher than those of intact products. For whole celery plants (petioles and leaves), respiratory rates were reported by Ryall and Lipton (1979) to be 3.7 ml CO₂ kg⁻¹h⁻¹ at 0 °C and 14.2 ml CO₂ kg⁻¹h⁻¹ at 10 °C. The results obtained in Vina and Chaves (2007) experiment in pre-cut celery petioles are comparable to those published, since at 0 °C, a value of 3.16 ml CO₂ kg⁻¹ h⁻¹ was found. In contrast, cut petioles at 10 °C showed a respiratory rate of 7.44 ml CO₂ kg⁻¹h⁻¹ about half the value reported for the whole plant at this temperature. The respiration rate for celery has been estimated at around 75% of its value under controlled atmosphere (CA) compared to air (Gariépy *et al.*, 1986), but Gomez and Artes (2004) found that the effect of CA (30% of decrease, gross average) in their study was more important. This activity could effectively design controlled atmosphere facilities and modified packaging for optimum selection of polymeric films in celery.

Blackheart

Blackheart may appear when celery is at the growth stage. The loss might be at the total field level or affected plants may be scattered. Infection symptoms start at the youngest leaflets that are folded in the crown. The skin turns black and necrosis expands rapidly to the entire heart. Secondary bacteria in the dead tissue result in a slimy decay. Chlorosis of the nearly mature leaves may precede a sudden appearance of the black tissues. No other symptoms appear and the heart seems to be surrounded with quite normal leaf stalks (Whitlock, 1979).

The disorder is associated with soil moisture instability. Flooding after a dry period in the field brings a black heart. Thus the disease does not appear in a crop that has been under uniform moisture conditions or low moisture levels (Whitlock, 1979). Where nitrogen and soluble salts are excessive, plants are susceptible to black heart when the weather is cloudy. Chemical analysis in infected heart tissues has shown that the calcium levels in soil were low. In addition studies have shown that high calcium content in heart can prevent black heart disease. Besides preventing the disorder, increased yield of up to 0.2 kg calcium solution per plant have been reported (Whitlock, 1979).

Cracked stem

‘Transverse cracking of the epidermis above the vascular bundles occurs sometimes over the entire outer surface of maturing fleshy petioles’ (Whitlock, 1979). After that the epidermis turns black and the immediately adjacent tissue turns brown. This symptom happens together with mottling of the leaves, browning and dying of the roots, brittleness and some brown streaking of the fresh petioles (Whitlock, 1979). The disorder is induced by inadequate boron levels in soil.

Pithiness

There are two types of pithiness: one in which the entire plant is pithy even when it is very young and the other in which the outer petioles become pithy when the plant approaches maturity. Studies on this have shown that the former type is inherited and it depends on a dominant gene. It has been suggested that by removing the pithy plants from the seed crop, the inherited type of pithiness could be eliminated in a short time period. Moreover, there is no direct correlation between climate and pith occurrence (Whitlock, 1979).

Weight loss

Weight loss of celery increased with the progress of storage at 0 °C and 10 °C temperatures. At 0 °C the weight loss reached 1.6%, while at 10 °C it was higher, 6.2%. At all sampling times, weight losses were higher at 10 °C than at 0 °C. For instance, the weight loss after 27 days at 0 °C was equivalent to that determined after 7 days at 10 °C. The maximum water loss acceptable for white celery has been established as 10% (Vina and Chaves, 2003).

Lengthening of the inner petioles

At harvest plants are sized to the desired length with the use of sharp knives and then are stored at 0–5 °C and 90–95 % relative humidity. During storage at temperatures over 0 °C minor growth occurs such as the lengthening of the inner petioles. This lengthening can reduce sales as the product is rejected by the customer (Gariépy *et al.*, 1986). Controlled atmosphere storage under high CO₂ levels can have a major influence on celery stalk elongation, the higher the CO₂ level the higher the growth inhibition (Shewfelt and Henderson, 2003).

Browning

Colour and celery petioles firmness are the most evident parameter by which consumers judge celery quality (Rizzo, 2009). Celery of high quality are plants that have tight stalks, thick petioles that have been minimally processed, crisp texture and bright green colour that gives a fresh appearance (Gómez, 2004). Gomez and Artes (2005) noticed that the initial signs of quality loss for celery are discoloration and onset of pithiness. Browning of both the cut petioles and the butt end is one of the major problems that the celery industry deals with annually. The browning disorder is more intensive during May-June and August-September depending on the climate.

Ethylene

Exposure to ethylene concentrations that could conceivably be encountered during processing and marketing, e.g., up to 20 $\mu\text{l}\cdot\text{liter}^{-1}$, had no significant effects on density loss of celery during storage for 2 week at 5 °C. However, there appeared to be a progressive decline in density as ethylene concentrations increased from 20 to 200 $\mu\text{l}\cdot\text{liter}^{-1}$, with a significant reduction in density occurring at 200 $\mu\text{l}\cdot\text{litre}^{-1}$. Tissue exposed to 200 $\mu\text{l}\cdot\text{liter}^{-1}$ ethylene in air were rapidly senescing, and it is difficult to conclude if the ethylene effect was induced through senescence or aerenchyma formation (Saltveit and Mangrich, 1996).

Antioxidants

Celery is a vegetable with high flavonoid content. Flavones (a class of flavonoids) in their native form are conjugated to sugars, simple acids (acetyl and malonyl), and cinnamic acids (Figure 3) rather than existing as aglycones. *In vitro* studies demonstrated that the flavone apigenin and luteolin offer positive biological benefits such as reduced risk of cancer and cardiovascular disease (Robards *et al.*, 1999; Heim *et al.*, 2003; Astley, 2003; Block *et al.*, 1992; Gutteridge 1993; Hertog and Hollman, 1996; Kinsella *et al.*, 1993; Trewavas and Stewart, 2003). Studies on celery have identified the flavonoids 7-O-apiosylglucosides and 7-O-glucosides of luteolin, apigenin and chryseoriol (Galensa and Hermann 1979; Leung and Foster 1996; Lin *et al.*, 2007). In addition the biological activity of flavones and their

function as antimicrobial factors are well recognised, as are their antiallergenic and anti-inflammatory means and antimutagenic action (Rice-Evans *et al.*, 1996).

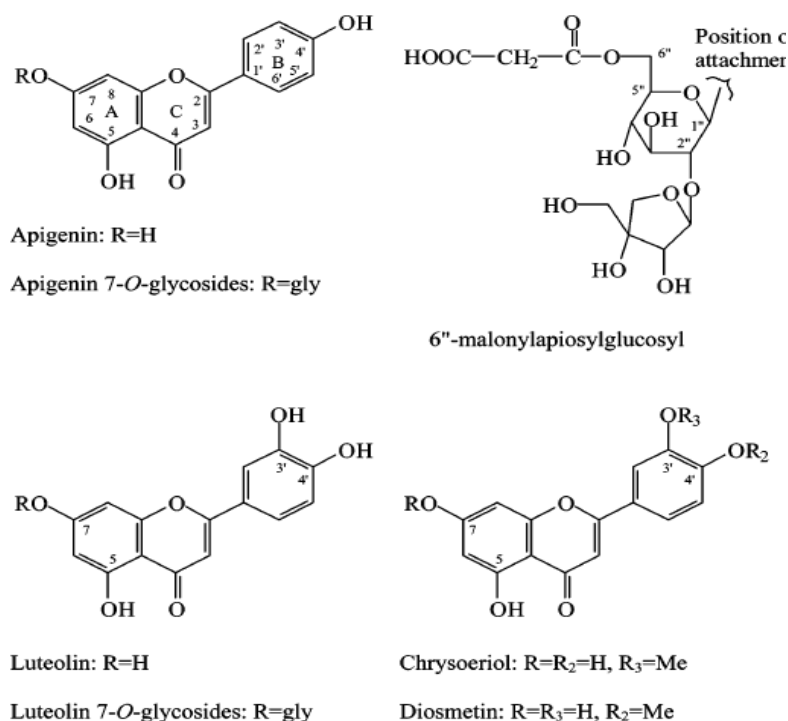


Figure 3 Structures of the flavonoids in celery

Source: Lin *et al.*, 2007

According to Vina and Chaves (2006) minimally processed celery retained its initial antioxidant capacity for a period of 21 days at 0°C, showing the lowest levels of browning potential at this temperature (Figure 4). During refrigerated storage of minimally processed celery, chlorogenic acid decreased and the deterioration was more intense at higher storage temperatures (Vina and Chaves, 2006).

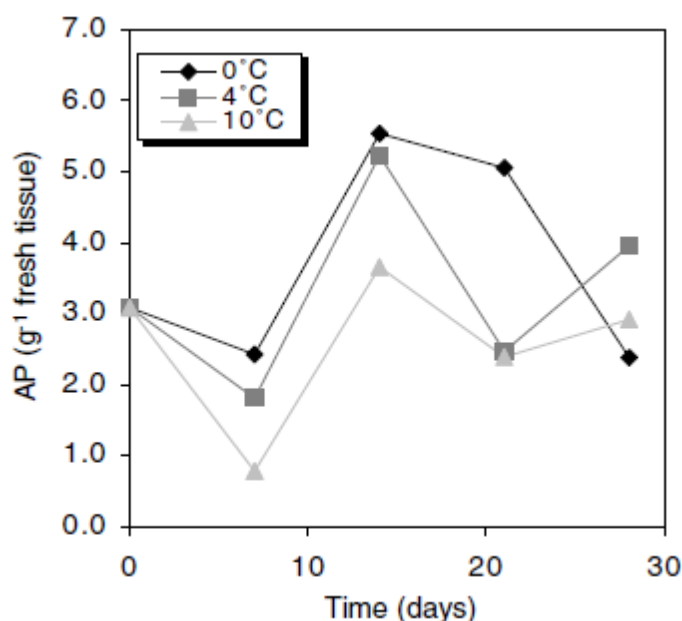


Figure 4 Antioxidant power of minimally processed celery stored at 0, 4 or 10 °C for 28 days (EC50: effective mean concentration) Source: Vina and Chaves, 2006.

Non-structural carbohydrates

Celery contains fructose, glucose, sucrose and mannitol. The content of free sugars was analysed for fructose, glucose and mannitol in several studies. In a study by Vina and Chaves (2003), the sucrose content was found to be below the detection threshold. Sugar content at harvest was 7.08, 5.81 and 5.26 mg/g fresh tissue for fructose, glucose and mannitol, respectively. These initial fructose and glucose contents are in agreement with values measured by Kwon et al, (1998) who also found very low sucrose content.

Keller (1991) suggested mannitol was the main carbohydrate present in celery plants, representing about 60% of the total soluble carbohydrates, whereas Vina and Chaves (2003) found the initial value for mannitol was very close to that of glucose. The proportions of sugars present are highly influenced by variety and growing conditions. The mannitol content at 0 °C remained constant during the first week of storage, then decreased between the second and third week and subsequently stabilised again by the end of the experiment (Vina and Chaves, 2003). The total mannitol loss was slightly above 50% of the initial value. At 10 °C, levels fell after 7 days in storage, remained constant during the following two weeks and then decreased again by day 27. Total consumption was greater than 70% of the initial value. Thus the consumption of mannitol was found to be higher than that of fructose and glucose at both 0 °C and 10 °C (Vina and Chaves, 2003).

EXPERIMENTAL DATA

2.1 Plant material

Celery plants from the Monterey cultivar of the late Spanish season with high susceptibility to browning were chosen for this study. All plant material was provided by G's Ltd. from the Barranquillo farm in Spain. Celery was planted on 7 March 2011 according to the spring crop season and was harvested on 5 June 2011. All samples were harvested from the same field and were processed (harvesting, removal of field heat, transport, transit time etc.) in the same way across the season. Celery was sent to the Plant Science Laboratory, Cranfield University within 2 days of harvest.

2.2 General Harvest conditions and postharvest cycle

Harvesting started at 6am and lasted for 8-12 h. The product was cut at the soil height and was packed into bags and corrugated boxes (60x40 cm) in a rig in the field. Whole sticks of celery were trimmed to approximately 30 cm length, the root was also trimmed and outer petioles were removed. Approximate weight per stick was 450 g to 600 g. Each celery head was packed in an OPP bag (oriented polypropylene plastic bag) and the bags were packed into boxes.

Each box contained 24 celery heads, cut to supermarket specification and packed in the same type of individual celery bag to mirror the commercial shipment. Boxes were tightly packed with the product to minimize excess air around the packs. Pallets took between 20 min to 40 min to be completed and vacuum cooled to 4 °C. At the cooling site, the top box of each pallet was quality check and transferred to the vacuum chamber. The time from leaving the field to entering the vacuum was 2 h. Cooling of the product down to 5 °C in the chamber took from 20 min to 1 h depending on the ambient air temperature. Once the celery was removed from the vacuum cooling chamber it was stored at 5 °C for 12–24 h.

2.3 Sample preparation

Over a two month period, celery (Figure 5) packaged in carton boxes were sent from Spain to the Plant Science Laboratory, Cranfield Health. Each box contained 24 celery heads stored in bags. The content of the box was divided in two parts; 12 celery batches were

processed the day of arrival (day 0) and the other 12 batches were stored at 18°C and were processed after 6 days (day 6). There were nine boxes of celery sent every week, each representing a different stage of maturity.



Figure 5 Images of degree of browning on samples supplied by G's Marketing

The processing involved cutting 1 cm portions from 5 different sections; butt end, middle outer stalk, middle inner stalk, outer petioles and inner petioles (Figure 6). Each section was bagged separately and immediately snap frozen in liquid nitrogen. Samples were then stored at -40 °C for a week and then the frozen tissues were lyophilised using an Alpha 1-4 Christ LDC-1 freeze-dryer (Christ, Lower Saxony, Germany) for a further week. All of the samples were finely powdered prior to extraction with a mechanised pestle and mortar.

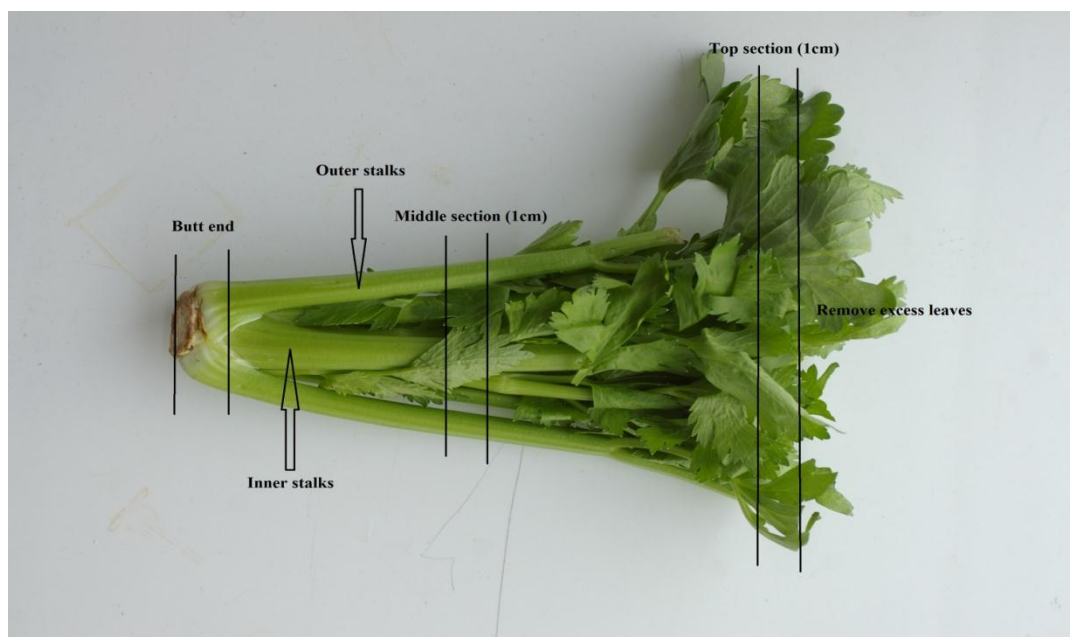


Figure 6 Celery sample (first week, day 0) and the areas that were cut for the experiment

2.4 Colour measurement

Objective colour was assessed using a Minolta CR-400 colorimeter and DP-400 data processor (Minolta Co. Ltd., Japan), and was expressed in terms of chroma (C^*), hue angle (H°) and lightness (L^*). Colorimeter data was taken from frozen tissue. Each objective colour value was the mean of three measurements taken from around the equator of the cut butt celery ends and petioles. All biochemical analysis was carried out on frozen material. Due to a limited number of plant material of the fifth maturity week, the colorimeter results were excluded from the statistical analysis. Pictures of the frozen cut butt ends and petioles were taken to assess colour score data of the different maturity weeks and storage times.

2.5 Extraction of sugars

Sugars were extracted and measured according to Downes and Terry (2010) with slight modifications due to differences in column length. The sugars were extracted from 50mg of dried celery powder using 1ml of 62.5:37.5 HPLC grade methanol: water (v/v). The vials were incubated in a shaking water bath at 55 °C and agitated every 15 min for 20 sec to avoid layering at the bottom of the vials. Cooled samples were filtered through a 0.2 μ m Millex GV filter unit and stored at -40 °C.

2.6 Quantification of sugars

The extract (10 μL) was injected into a Prevail Carbohydrate ES column of 250 mm x 4.6 mm diameter, 5 μm particle size (Alltech, UK; Part no. 35101) with a Prevail Carbohydrate ES guard cartridge of 7.5 mm x 4.6 mm diameter (Alltech, UK; Part no. 96435). The mobile phase consisted of HPLC grade water (A) and EtOH (B). The gradient involved a linear increase/decrease of solvent B; 85-65%, 9 min; 65-85%, 3 min; 85% 8 min at a flow rate of 0.5 mL min^{-1} and column temperature was set at 40°C. An evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the system via a UCI-50 universal chromatography interface detected the eluted carbohydrates. Carbohydrate concentrations were calculated against calibration standards.

2.7 Data analysis

Statistical analyses were conducted using Genstat for Windows Version 10.1.0.147 (VSN International Ltd., Herts., UK). Analysis of variance (ANOVA) was used to identify the main effects of storage time, maturity week, celery sections and the interaction between these factors to a significance of $P < 0.05$ unless otherwise stated. Colorimeter results of the butt cut ends and the petioles were also analysed to identify significant differences between the celery sections from different maturity weeks and storage time. Correlations between sugar concentrations and colour were carried out using Pearson's Product Moment Correlation.

RESULTS

3.1 Colour

The celery butt cut ends (Figure 7) were significantly more vivid (C^*) and had a higher L^* compared to the celery petioles (Figure 8-9). The butt ends were significantly lighter in the third week of maturity. In the petioles, C^* was higher in the youngest celery heads (first maturity week) and overall higher after six days of storage at 18 °C indicating a more vivid colour. Storage time affected H^o of the petioles and was higher than in the butt cut ends. That said, H^o was significantly higher in the petioles for the different storage times and different maturity weeks. Overall the differences in the C^* values for both the butt cut ends and the petioles were between the different maturity weeks yet not affected by the different storage time (Table 3). C^* was higher in the young celery and the older celery compared with celery from maturity weeks 3 and 5.



Figure 7 Photos of celery cut butt ends of the first week (top) and the ninth week (bottom) at the first day (left) and after six days (right) of cold storage at 18 °C.



Figure 8 Photos of celery petioles of the first week at the first day (left) and after six days (right) of cold storage at 18 °C.



Figure 9 Photos of celery petioles of the ninth week at the first day (left) and after six days (right) of cold storage at 18 °C.

Table 3 Chroma (C), hue (H°) and lightness (L*) of celery butt cut ends and petioles from different maturity weeks in ambient temperature (day 0) and after six days (day 6) of cold storage at 18 °C. Butt Grand Means: C=22.99, L=70.9 and H°=83.3. Petioles Grand Means: C=21.43, L=54.04 and H°=101.63. L.S.D (*P*=0.05)

	BUTT											
	Chroma (C)				Hue angle (H°)				Lightness (L*)			
	week 1	week 3	week 7	week 9	week 1	week 3	week 7	week 9	week 1	week 3	week 7	week 9
day 0	25.01	20.6	21.35	23.05	86.86	86.21	84.81	84.88	60.5	82.1	74.4	71.9
day 6	24.23	27.47	19.23	22.97	77.61	82.41	84.39	79.63	65	74.5	68.9	69.5
	PETIOLES											
	Chroma (C)				Hue angle (H°)				Lightness (L*)			
	week 1	week 3	week 7	week 9	week 1	week 3	week 7	week 9	week 1	week 3	week 7	week 9
day 0	24.26	19.81	18.31	20.19	113.3	112.46	105.93	102.51	41.6	48.46	70.21	71.16
day 6	24.85	21.02	22.33	20.68	103.72	104.96	86.32	83.83	36.87	46.05	57.31	60.65

3.2 Sugars

The concentration of fructose, glucose and mannitol was significantly higher in the middle inner stalks than in the other celery sections while sucrose concentration was significantly higher in the cut butt end (Table 4). Storage for 6 days at 18°C significantly affected the fructose concentration while had no effect on the concentrations of the other sugars (Table 5).

Among the different celery sections, the concentrations of fructose and glucose (Table 6) were significantly higher in the middle inner stalks and middle outer stalks. The levels of mannitol were significantly higher in all celery sections compared with the other sugars and were highest in the middle inner stalks (Table 4). In contrast, sucrose concentration was significantly lower in the petioles and significantly higher in the cut butt area.

There were no significant correlations between the colour of the celery and the sugar concentrations in the butt end (Table 7). This said, in the petiole, there was a weak yet significant negative correlation between mannitol and H° (Table 8).

Table 4 Sugars concentrations in different celery sections.

Sections	Fructose (mg/g DW)	Mannitol (mg/g DW)	Glucose (mg/g DW)	Sucrose (mg/g DW)
M.I	46.75470319	125.784866	22.05206246	6.93891
M.O	34.45977846	117.103711	16.70262026	8.0252
P.I	26.08149149	103.066498	13.07954726	3.28845
P.O	22.97576486	118.39369	11.90214298	5.29565
BUTT	15.22241069	112.850816	14.77879659	14.4679
mean	32.5679345	115.439916	15.70303391	7.60322
l.s.d	3.977	14.22	2.832	3.651

M.I= middle inner stalks, M.O= middle outer stalks, P.I= inner petioles, P.O= outer petioles, BUTT= cut butt area, DW= dry weight L.S.D ($P=0.05$)

Table 5 Fructose concentrations (mg mL⁻¹ DW) in different celery sections coming from different maturity weeks and times of storage M.I= middle inner stalks, M.O= middle outer stalks, P.I= inner petioles, P.O= outer petioles, BUTT= cut butt area, DW= dry weight L.S.D=12.575 (*P*=0.05)

FRUCTOSE						
Celery section	Storage day	week 1	week 3	week 5	week 7	week 9
BUTT	0	28.84	16.31	8.52	6.37	14.2
	6	32.54	13.35	9.75	4.69	5.48
M.I	0	54.91	47.91	48.72	45.07	45.22
	6	45.31	46.87	47.43	47.95	38.17
M.O	0	37.7	35.4	36.03	28.05	48.03
	6	31.41	25.97	31.82	41.25	28.95
P.I	0	8.28	27.7	32.17	32.53	32.28
	6	10.8	17.51	32.51	42.68	24.36
P.O	0	9.14	24.73	29.02	28.46	27.82
	6	14.28	15.81	25.95	29.9	24.65

Table 6 Glucose concentrations (mg mL⁻¹ DW) in different celery sections coming from different maturity weeks and times of storage M.I= middle inner stalks, M.O= middle outer stalks, P.I= inner petioles, P.O= outer petioles, BUTT= cut butt area, DW= dry weight L.S.D= 8.955 (*P*=0.05)

GLUCOSE						
Celery section	Storage day	week 1	week 3	week 5	week 7	week 9
BUTT	0	12.15	10.01	5.72	19.19	8.51
	6	17.15	12.13	5.34	28.97	10.13
M.I	0	22.68	18.49	22.79	25.32	20.36
	6	20.37	14.44	28.43	26.34	21.3
M.O	0	17.33	12.31	17.36	13.42	27.68
	6	16.09	11.5	17.64	19.93	13.76
P.I	0	3.59	12.07	16.06	15.88	16.04
	6	6.54	10.75	17.51	20.21	12.14
P.O	0	4.24	10.37	14.45	17.03	18.89
	6	7.92	9.09	12.29	11.93	12.8

Table 7 Correlation matrix comparing sugar concentrations (mg/ml DW) of celery butt cut ends and colorimeter results C*=chroma, L*=lightness, H°= hue angle

*probability $P < 0.05$

BUTT CUT ENDS								
C*	1	-						
L*	2	-0.3517	-					
H°	3	-0.3804	0.8237*	-				
Mannitol	4	-0.1756	-0.1167	-0.3036	-			
Sucrose	5	0.2909	-0.064	-0.2912	-0.0598	-		
Fructose	6	0.5503*	-0.238	-0.1362	-0.292	0.3565*	-	
Glucose	7	-0.259	-0.1379	0.0416	0.2381	-0.3498	-0.1842	-
		1	2	3	4	5	6	7

Table 8 Correlation matrix comparing sugar concentrations (mg/ml DW) of celery petioles and colorimeter results C*=chroma, L*=lightness, H° = hue angle

*probability $P < 0.05$

PETIOLES								
C*	1	-						
L*	2	-0.3879	-					
H°	3	-0.4132	-0.1597	-				
Fructose	4	0.0722	0.5158	-0.3901	-			
Glucose	5	-0.0672	0.6827*	-0.2329	0.8823*	-		
Mannitol	6	-0.0602	0.3768	-0.4929*	0.7644*	0.6684*	-	
Sucrose	7	-0.1921	0.4501*	0.0096	0.5657*	0.7285*	0.6518*	-
		1	2	3	4	5	6	7

DISCUSSION

Mannitol increased steadily during the different maturity weeks most likely due to its accumulation. Mannitol is transferred to the sink tissues through the phloem by the mannitol transporter-1 (MAT-1) and it is catabolised in the sink tissues upon the action of the mannitol dehydrogenase enzyme (MTD) (Noiraud *et al.*, 2001). MTD provides the initial steps by which translocated mannitol enters the citric acid cycle for use as carbon and energy (Pharr *et al.*, 1995). Expression of MTD is repressed by both hexoses (Everald *et al.*, 1993; Prata *et al.*, 1997). Sugar repression allows mannitol to be stored in large quantities and function as a reserve carbohydrate. Fructose and glucose may have repressed the activity of MTD and therefore mannitol was stored as a reserve carbohydrate that increased during the maturity weeks. At the same time fructose and glucose depleted during the different maturity weeks as they may have been used for energy.

Sucrose invertase activity is high in many tissues which need sucrose. For example, young leaves, which have not developed the ability to fix carbon through photosynthesis, rely on imported sucrose to grow (Foyer *et al.*, 1997). Invertase activity falls as the leaf matures and this may explain the increase in sucrose concentrations after the seventh maturity week. Sucrose concentration was higher at the fifth maturity week, after that invertase may have hydrolysed sucrose to hexoses and as a result sucrose began to decline until the seventh maturity week. Furthermore, L^* was significantly higher during the fifth maturity week indicating that increased sucrose may be connected to browning.

A relationship between fructose and sucrose was found in the butt cut ends using Pearson's Product Moment Correlation. In the petioles, mannitol concentration was correlated with the hexose concentrations and sucrose concentration. This correlation is possibly due to the suppression that hexoses have on mannitol catabolism and therefore hexoses were used as a source of energy while mannitol was stored in the plant.

Most importantly, mannitol was negatively correlated with hue angle in the petioles. Hue angle is a colour indicator and in the range of 60° to 90° plant tissues go from brown/orange to green colour. This correlation suggests the higher the mannitol concentration, the browner the plants. Mannitol is a stress factor and thus high amounts of mannitol occur when the plant is stressed and the plant tissues acquire a browner colour. Also, mannitol increased during the maturity weeks, suggesting increased stress as the celery matured.

There is a lack of previous studies which have quantified sugar concentrations in different celery tissue hence discrepancies were found between the data herein and previous findings. In previous studies, the whole celery head was used or celery plant was trimmed and then processed. In the current study the sugar concentrations were measured for five different celery sections and the sugar amounts ranged between these sections. In addition, in this study, extraction was done using methanol and the results were measured in mg/g of dry weight, while in Keller and Matile (1989) study the chryosap method of Tomos *et al.* (1984) was used for the extraction of sugars in the celery petioles.

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