CRANFIELD UNIVERSITY

SIMONE ROSSI

UNDERSTANDING THE UNDERLYING MECHANISMS AND THE ROLE THAT PRE-HARVEST HORTICULTURAL MATURITY, AGRONOMIC FACTORS AND GROWING CONDITIONS HAVE ON POSTHARVEST DISCOLOURATION IN CELERY

SCHOOL OF WATER, ENERGY AND ENVIRONMENT (SWEE)

PhD THESIS
Academic Years: 2013- 2017

Supervisor: Prof. Leon A. Terry
March, 2017

CRANFIELD UNIVERSITY

SCHOOL OF WATER, ENERGY AND ENVIRONMENT (SWEE)

PhD Thesis

Academic Years 2013- 2017

SIMONE ROSSI

UNDERSTANDING THE UNDERLYING MECHANISMS AND THE ROLE THAT PRE-HARVEST HORTICULTURAL MATURITY, AGRONOMIC FACTORS AND GROWING CONDITIONS HAVE ON POSTHARVEST DISCOLOURATION IN CELERY

Supervisor: Prof. Leon A. Terry
March, 2017

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Celery is a foliage crop which is commonly consumed for its fresh stalks, which are sold on the market in various retail formats. Celery is appreciated by consumers for its freshness, bright green colour, crisp texture, low calorific content and health promoting properties. An important problem affecting this crop after harvest is browning at cut ends, which is a physiological "disorder" manifesting with brown/black stains on cut or damaged surfaces. It manifests during handling and processing of vegetables, which is the phase where most of the postharvest damage occurs. Cut-end browning is believed to be due to the activity of two enzymes; polyphenol-oxidase (PPO) and phenylalanine ammonia-lyase (PAL). These two enzymes synergistically act to produce melanins, which are the pigments responsible for the black/brown appearance. This is a relevant economic issue as discoloured celery tends to be rejected by retailers, with negative implications for the growers and the UK fresh produce industry. Postharvest browning is not thought to be due to the activity of fungi or bacteria, yet the blackening of surfaces suggests to customers that the product is decaying.

Research has been conducted to understand which postharvest factors can influence browning in celery. It has been shown that the use of controlled atmosphere (Gomez and Artes, 2004), heat shock treatments (Loaiza-Velarde *et al*., 2003) and postharvest application of high intensity light (Zhan *et al*., 2013) were effective in reducing incidence of the problem. On the other hand, research on pre-harvest factors is still scarce. The aim of the present work was to: 1-) investigate which agronomic factors can affect the severity of postharvest browning in celery; 2-) provide a better understanding of the underlying physiological and biochemical mechanisms involved in this "disorder".

Results showed that agronomic practices can affect postharvest browning in celery. Harvesting at late maturity stages and irrigating below the normal requirements increased postharvest discolouration in this crop. Additionally, the browning mechanism does not seem to be mediated by ethylene in celery. Further biochemical analysis on phenolic compounds and enzymatic activity (PPO and PAL) need to be performed to provide a better elucidation of the browning reaction in this crop.

ACKNOWLEDGEMENTS

I am grateful to my Supervisor, Prof. Leon Terry, who gave me the opportunity to study for a PhD programme in the Plant Science Laboratory at Cranfield University. In particular, I acknowledge his professional advice and guidance to shape my experimental works and, in general, my research. I also wish to acknowledge my committee members, Prof. Leon Williams, Prof. Andrew J. Thompson and Dr. Paul Burgess for their helpful feedback and comments during my annual progress reviews.

I would like to express the deepest appreciation to all the technical staff, students and research fellows of the Plant Science Laboratory, especially Dr. Katherine Cools, Dr. Maria Anastasiadi, Dr. Maria del Carmen Alamar Gavidia, Dr. Emma Collings, and Antonio Bermejo for training me with all laboratory equipment and experimental protocols. I would like also to recognize G's Fresh professional staff, especially Emma Garfield, Dan Jolly, Hamish Mellor and Pedro Montoro de Antonio for supplying me all the plant material to conduct my experiments, giving technical support and providing agronomic advice on the celery crop. Other thanks are due to AHDB Horticulture for financially supporting my research project.

In the end, I am infinitely grateful to my brother, Damiano Rossi, for his endless moral support during my PhD studies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

β	beta
°C	degree Celsius
%	percent
1-MCP	1-methylcyclopropene
µm	micrometre
µL	microlitre
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	analysis of variance
ATP	adenosine triphosphate
AU	artificial unit
b*	yellowness
B	boron
BOC	British Oxygen Company
BP	browning potential
C*	chroma
C_2H_4	ethylene
CA	controlled atmosphere
Ca	calcium
Cl	chloride
cm	centimetre
CO_2	carbon dioxide
Cu	copper

cv.	cultivar
cwt	hundredweight
DAD	photodiode array detector
DAP	days after planting
DPA	dihydrophaseic acid
DW	dry weight
EIN	ethylene insensitive
ELSD	evaporative light scattering detector
ER	endoplasmic reticulum
ERS	Ethylene response sensor
Ethephon	2-chloroethylphosphonic acid
ETR	ethylene resistant
EU	European Union
FID	flame ionisation detector
FW	fresh weight
g	gram
GA	gibberellin
GC	gas chromatograph
GAF	cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA
GRAS	Generally regarded as safe
g/m^2	grams per square metre
h	hour
H°	hue angle
H_2O	water
H_2O_2	hydrogen peroxide

HD	harvest date
HM	harvest maturity
HPLC	high performance liquid chromatography
I.D.	internal diametre
IU	International Unit
K	potassium
kcal	kilocalories
kg	kilogram
kGy	kilogray
kJ	kilojoule
K_m	Michaelis-Menten constant
kPa	kilopascal
L	litre
L*	lightness
L-Dopa	L-3,4-dihydroxyphenylalanine
LDPE	low density polyethylene
LSD	least significant difference
Ltd	limited
m	metre
MAP	modified atmosphere packaging
mbar	millibar
meq	milliequivalent
Mg	magnesium
mg	milligram
MgO	magnesium oxide

min	minute
mL	millilitre
mM	millimolar
mm	millimetre
MeOH	methanol
Mn	manganese
Mo	molybdenum
MP	microperforated polypropylene
MS	mass spectrometer
MTA	methylthioadenosine
n	number of replicates
N_2	nitrogen
n.a.	not available
$NaClO_2$	sodium chlorite
nm	nanometre
no.	number
O_2	oxygen
O.D.	outside diameter
P	phosphorus
PA	phaseic acid
PP	polypropylene
PAL	phenylalanine-ammonia lyase
PGI	Protected Geographical Indication
PGRs	plant growth regulators
pH	$- \log_{10} [H^+]$

ppm	parts per million
PPO	polyphenol oxidase
POD	peroxidase
PSL	Plant Science Laboratory
PTFE	polytetrafluoroethylene
R^2	coefficient of determination
RH	relative humidity
ROS	reactive oxygen species
RTE	Reversion-to-ethylene-sensitivity gene
sec	second
SIR	standard irrigation regime
S.M.D.	soil moisture deficit
SNS	soil nitrogen supply
SO_4	sulphates
t/ha	tonnes per hectare
TQ	total quinone content
UK	United Kingdom
UPLC	ultra performance liquid chromatography
USA	United States of America
USDA	United States Department of Agriculture
UV	ultraviolet
var.	variety
V_{max}	maximal velocity (enzyme reaction)
v/v	ratio by volume
Zn	zinc

1. INTRODUCTION

1.1 Project background

Celery (*Apium graveolens* L.) is a foliage crop which is grown for its long and thick petioles. Although belonging to the minor crops category, celery is considered important because it has several characteristics that render it appreciated by consumers. Some of these characteristics include: freshness, crisp texture, low calorific content and presence of several health-promoting compounds (especially anti-cancer and anti-inflammatory) (Winston, 1999; Craig 1999). Celery is quickly marketed after harvest to preserve its typical freshness and palatability so that it can be better appreciated by consumers. Yet, since it contains high water percentage (approximately 95%), it is highly perishable, thus, susceptible to losing its quality rapidly after harvest. One of the most relevant problems affecting celery is postharvest browning at cut surfaces; a physiological "disorder" that commonly occurs in many fresh fruits and vegetables. The phenomenon basically appears on cut or damaged surfaces manifesting itself with black/brown stains of varying severity. Browning represents a serious problem for growers and the fresh produce industry, as affected celery is perceived by consumers to lose its freshness and tends to be considered as a decayed or diseased product in all market formats. This results in considerable economic losses as sales decrease because the product does not meet supermarket specifications. Gómez and Artés (2004) reported that stalks with bright green colour and fresh appearance are the two main parameters by which consumer determine good celery quality.

According to current literature, postharvest browning is an enzymatic process which is mainly thought to be due to the activity of polyphenol oxidases (PPOs) and phenylalanine-ammonia lyase (PAL). These two enzymes work synergistically to produce melanins; the pigments responsible for the undesirable black/brown colour on cut surfaces of fresh products (He and Luo, 2007). Also, another enzyme, peroxidase (POD), is thought to contribute to the browning reaction, yet its role is believed to be minor (Landi *et al*., 2013).

Scientific research has attempted to understand which factors can influence postharvest browning in celery. Loiaza-Velarde *et al*., (2003) reported that the application of heat

shock treatments significantly reduced browning potential and the wound induced PAL activity in excised petioles. Gómez and Artés (2004) showed that celery stalks stored in controlled atmosphere (CA) with high CO_2 concentration maintained a greener colour compared to the ones stored at normal CO_2 levels without producing any undesirable off-odours and off-flavours. Another study conducted by Zhan *et al*., (2013) demonstrated that continuous exposure to high intensity light (2000 lux) resulted in reduced activity of PPO, POD and browning index in celery.

Despite the studies mentioned above, there still remains a knowledge gap regarding the underlying physiological and biochemical mechanisms involved in postharvest browning in celery. In addition, the influence that pre-harvest factors have on this disorder is still unknown. It is important to investigate the role of agronomic factors because, if correlated with cut-end discolouration, they can be modified by growers without any considerable issues around management and costs. Agronomic factors are all physical, chemical and biological factors that can influence crop production and quality (i.e. growing period, water quantity, harvest date, fertilization, crop protection practices and soil management).

In summary, a better understanding of the causes of postharvest browning in celery will potentially help growers to counteract the problem. It is expected that agronomic and commercial strategies will be set up to reduce the incidence of this unwanted "disorder" at both butt and cut petiole ends of celery. As a consequence, customers will increase their confidence in the product and suppliers will be able to meet supermarket specifications with positive implications on the economic profitability of fresh produce sector.

1.2 Aims and objectives

1.2.1 Project aim

The overall aim of the thesis was to understand the influence that pre-harvest factors have on postharvest discolouration in celery. Also, to provide a better elucidation of the underlying physiology and biochemistry involved in the mechanism of browning after harvest.

1.2.2 Specific research objectives

- To study the influence of crop maturity stage on the incidence and severity of postharvest browning in celery.
- To investigate the effects of exogenous ethylene supplementation and 1-MCP on the visual quality and physiology of celery. In particular, focusing on cut-end browning, pithiness, bolting, respiration rate, spatial and temporal changes in the levels of sugars and phenolic compounds over storage.
- To study the role that water stress induced by deficit irrigation has on the degree of postharvest browning in celery.

1.3 Thesis structure

The present work is divided into seven chapters. Chapter One describes the main aims and objectives of the research.

Chapter Two reports a literature review covering various aspects of the celery crop and of the related postharvest browning problem. In more detail, the aspects relating to the crop include: botany, biology, morphology, agronomy, postharvest handling, main marketing formats, economic importance, production statistics and nutritional value. On the other hand, the aspects concerning postharvest browning include: general description of the problem, overview of the underlying physiological and biochemical factors related to it, description of the main pre-harvest and postharvest factors influencing its incidence. Considering that research on celery browning is scarce, lettuce; another crop which similarly suffers from a form of cut end discolouration (named “pinking”); has been also reported in the literature review. Finally, the chapter reports a description of the plant hormone ethylene and its ethylene inhibitor 1-MCP and the effects that these two compounds have on postharvest visual quality in celery.

Chapter Three describes the general Materials and Methods used in this study. These include all the instrumentation and laboratory equipment used for performing physiological measurements, biochemical assessments, extraction protocols, detection and quantification of non-structural carbohydrates, phenolic compounds and plant growth regulators. Lastly, an explanation of the statistical methods used to analyse collected data is included.

Chapter Four shows and discusses the results regarding the effects that crop maturity stage has on the visual quality, physiology and biochemistry of fresh-cut celery. Prior to that, the chapter includes a brief introduction on the related background studies in literature, specific objectives, plant material, experimental design and sampling techniques. A general conclusion is then drawn to summarize the main points arising from the chapter.

In Chapter Five, the effects that continuous ethylene and 1-MCP treatments have on the visual quality, physiology and biochemistry of fresh-cut celery are presented. As well as chapter four, a brief introduction, specific objectives, plant material, experimental design, sampling methods and a general conclusion are included in the chapter.

Chapter Six describes and discusses the findings regarding the effects that irrigation management has on the visual quality, physiology and biochemistry of fresh-cut celery. A short introduction on the related background studies in literature is included, as well as specific objectives, plant material, experimental design and sampling methods. A general conclusion is finally drawn to summarize the main points arising from the chapter.

Chapter Seven presents the general conclusions of the project and suggestions for future work. The chapter also reports a list of the literature cited in this study followed by a list of appendices containing abstracts of oral and poster presentation at conferences attended during the PhD programme.

1.4 Declaration

I declare that the present work was carried out using facilities at the Plant Science Laboratory, Cranfield University, UK. All the visual assessments, physiological attributes and biochemical analysis were done by the author. All plant material and information about the agronomic practices of celery was provided by G's Fresh Ltd. Extraction of plant growth regulators was done by the author under the supervision of Dr. Maria Anastasiadi. Identification and quantification of phytohormones was done entirely by Dr. Maria Anastasiadi.

2. LITERATURE REVIEW

2.1 The celery crop

2.1.1 Introduction

Celery (*Apium graveolens* L. var. *dulce* (Mill.) Pwea) is a herbaceous plant which belongs to the *Apiaceae* (or *Umbelliferae*) family (Rubatzky *et al*., 1999). Other well-known cultivated plants belonging to the same family include: fennel (*Foeniculum vulgare* Mill.), carrot (*Daucus carota* L.), dill (*Anethum graveolens* L.), parsley (*Petroselinum crispum* (Mill.) Fuss), coriander (*Coriandrum sativum* L.), parsnip (*Pastinaca sativa* L.) and cumin (*Cuminum cyminum* L.) (Reinhold, 1989).

Celery is native to the British Isles and was probably cultivated as a medicinal plant in the Mediterranean region by the ancient Greeks and Romans. In the following centuries, the gradual process of breeding and selection has conferred celery the typical characteristics of the edible plant it is known today. From a botanical point of view, celery is a biennial plant, but it is harvested as an annual crop as only its vegetative portions are eaten. Such portions are the long, thick and succulent petioles (DEFA, 2005).

In addition to celery, two other botanical varieties are presently cultivated: smallage (*Apium graveolens* L. var *secalinum* Alef.) which is utilized as a foliage crop, and celeriac (*Apium graveolens* L. var. *rapaceum* (Mill.) Gaudin.), which is consumed as a root crop. Smallage is the botanical variety having the widest climatic adaptation. This is probably due to its broad distribution in the world, especially in Asia. On the other hand, celery is commonly produced in Europe and North America, especially in temperate zones (Rubatzky *et al*., 1999).

2.1.2 Morphology and biology

The celery root system consists of a taproot and a series of lateral roots expanding in the soil. The plant (sometimes called “head”) is composed of a variable number of solid and thick petioles, attached to the basal compressed stem (**Figure 2.1**). The petioles develop during the first vegetative phase and are composed of a solid collenchyma, parenchyma and vascular bundles under the epidermis (**Figure 2.2**).

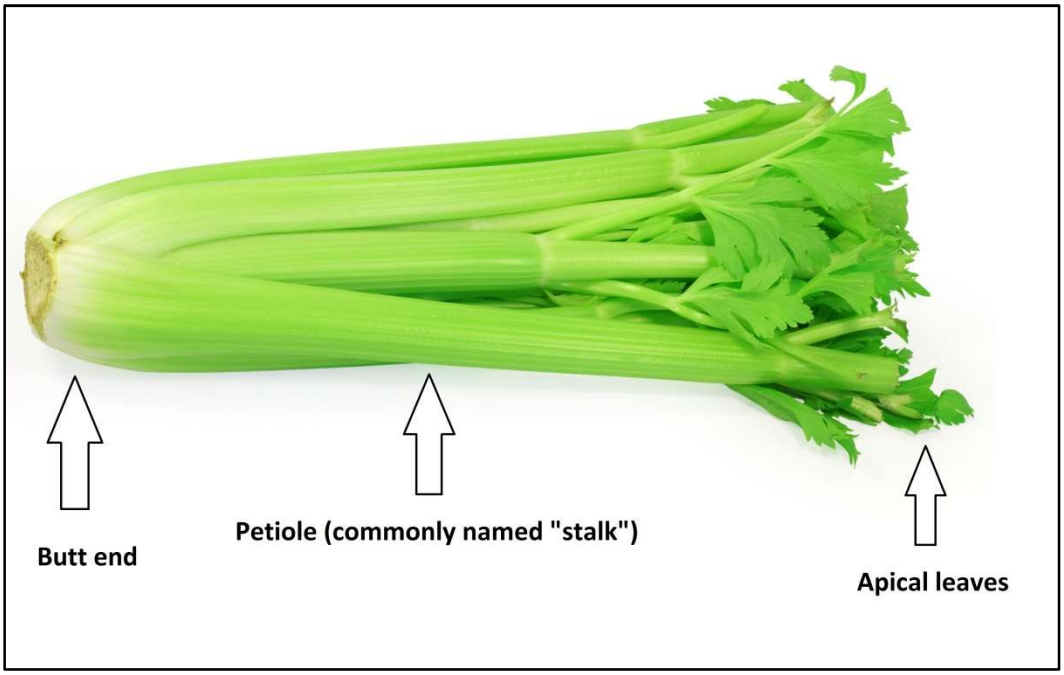


Figure 2.1 - Descriptive picture of the celery "head".
Source: http://producemadesimple.ca/celery.

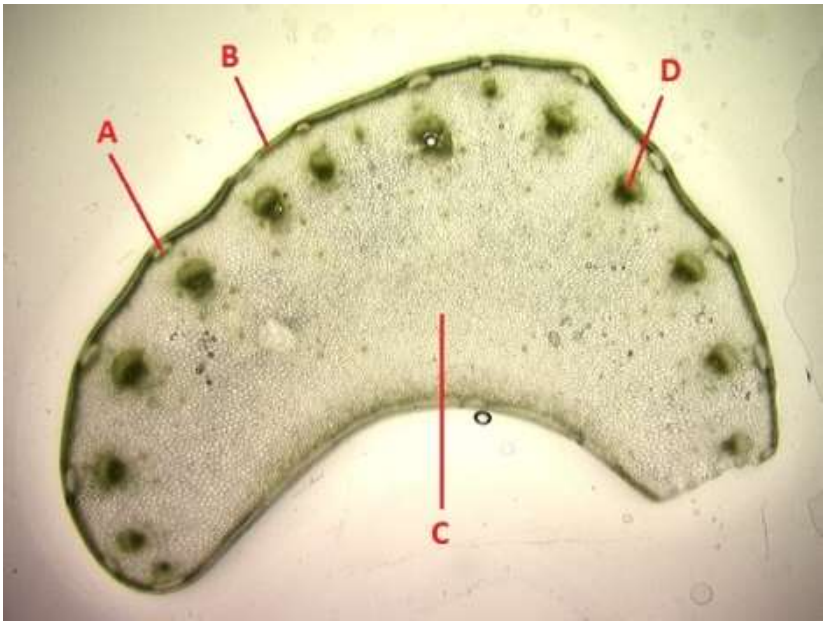


Figure 2.2 - Cross section of a celery stalk: (A): collenchyma, (B): epidermis, (C): parenchymal tissue, (D): vascular bundles.
Source: https://www.ccber.ucsb.edu/collections/botanical-collections-plant-anatomy.

In the reproductive phase, the plant develops an elongated seed stalk, which produces the apical inflorescence arranged in various umbels (that give the botanical family name) each containing many hermaphrodite and protandrous flowers. The fruit is a schizocarp, containing very small seeds with abundant endosperm (Hayward, 1938).

Celery is an allogamous crop and its pollination is guaranteed by insects and wind. These are two factors that assure out-crossing, which is important for avoiding detrimental inbreeding depression. When the stigma becomes receptive the stamens fall and the stigmata are positioned upright, ready to receive external pollen (Honma, 1959).

2.1.3 Crop growth and development

Celery seed germination, like in the majority of *Umbelliferae* plant species, is slow and variable to an extent that it can take several weeks for seedlings to emerge. Even if this is probably an evolutionary mechanism that helps the plant to survive adverse climatic conditions (known as "phenological escape"), growers tend to view it as an undesirable characteristic because it can have negative effects on crop uniformity. In order to overcome this issue, various treatments are utilized, like pre-germination in water at low temperatures (cold stratification). Such practice has shown to clearly improve the uniformity of germination and germination percentage (Finch-Savage, 1984). Another effective treatment is continually exposing celery seeds to direct light as this has been found to stimulate the synthesis of gibberellins (GA), which are fundamental hormones that promote the germination process (Thomas, 1989). Growers prefer to use transplants to improve commercial uniformity, thus, crop forecasting.

The yield of celery depends on the production of leaves with long developed petioles. In this regard, both temperature and photoperiod play a key role. Low and high temperatures discourage leaf growth and diminish petiole length, while long days reduce leaf number (Roelofse *et al*., 1990). Treatments with gibberellins can reverse these negative effects (Pressman and Negbi, 1987).

Bolting and flowering of celery depend on the influence of genotype, quality of light, temperature and photoperiod. The stem starts to elongate only if plants undergo vernalization. In this regard, the most satisfactory vernalizing temperatures range from 5 to 10 °C, but for other cultivars, higher temperatures could be sufficient to trigger the

physiological response. The effects of chilling temperatures and cold exposure period are cumulative.

2.1.4 Climatic and soil conditions

Celery benefits from temperatures ranging from 15 to 21 °C. Vernalization starts from temperatures less than 10 °C and, if prolonged, can cause early bolting. Celery can either directly be sown or transplanted. In the former case, the crop is harvested after about 5-6 months while in the latter case, the crop is harvested after about 3-4 months (Rubatzky *et al*., 1999). Like many other crops belonging to the *Umbelliferae* family, celery seeds germinate better and more rapidly when exposed to light and high temperatures. The light necessity can be replaced by daily alternated temperatures during germination (Pressman *et al*., 1977).

The most suitable soils for growing celery are those having good drainage, high organic matter and high water retention capacity. Soils with a high water-table are preferable because the plants can uptake water throughout the whole growing season. A 60-75 cm water-table during the UK summer months is recommended. If weather and moisture conditions are favorable and the agronomic technique is well performed, mineral and sandy loam soils will produce a satisfactory crop yield, even though sandy loam soils with high organic matter content are the most suitable. Heavy clay soils should be avoided as water can stagnate over. Acidic soils are preferable for celery, and they should range from pH 5.5 to 6.7 (Whitlock, 1979). **Table 2.1** shows the nutrient requirements of self-blanching celery grown in UK.

Table 2.1 - Self-blanching celery nutrients requirements (RB209, 2010).

	SNS, P, K or Mg Index						
	0	**1**	**2**	**3**	**4**	**5**	**6**
	kg/ha						
Nitrogen (N) - all soil types							
- seedbed	75	75	75	75	0[a]	0[a]	0[a]
- top-dressing[b]	See note below table						

Phosphate (P_2O_5)	250	200	150	100	50	0	0
Potash (K_2O)	450	400	350(2-) 300(2+)	210	50	0	0
Magnesium (MgO)	150	100	0	0	0	0	0

[a]: a small amount of nitrogen may be needed if soil nitrogen levels are low in the 0-30 cm of soil.

[b]: a top-dressing of 75-150 kg N/ha will be required 4-6 weeks after planting.

Celery requires high amounts of sodium, thus, appropriate supply of this element is recommended in all soils, except for peaty and some Fen silt soils, which generally contain adequate levels of sodium. Sodium can be applied as agricultural salt at 375 kg/ha. The application will not have any adverse effect on soil structure, even on soils of low structural stability. Additionally, it is recommended to ensure that the phosphate and potash offtake is balanced by application on Index 2 soils and verify that the soil is maintained at Index 2 by soil sampling every 3-5 years.

Celery also requires boron (1.2 - 2.4 kg/ha) and its deficiency can cause transverse cracking of stalks. Boron fertilization is normally applied as foliar spray. Excessive potassium availability can induce a potassium-boron imbalance, which may also result in stem cracking. Calcium imbalance or deficiency can cause black heart. This most frequently occurs during period of drought stress and rapid growth during period with high temperature. Agricultural salts like calcium chloride and calcium nitrate can be used for application in the soil (Barker and Pilbeam, 2015).

2.1.5 Agronomic technique

In order to facilitate mechanical planting operations and assure planting at a uniform depth, soil should be as smooth and even as possible. On the other hand, to prevent bolting, celery should not be planted in the eastern countries before late April. Planting the crop in July could result in a dangerous exposure to early frost during September and October.

Under field conditions, the most appropriate crop spacing is 30 x 30 cm as it results in a large finished plant with large heads to be put on the market. Given that celery is a shallow-rooted crop that originates from marshes, it requires an abundant and frequent supply of water. For this reason, irrigation is necessary and should be provided throughout the entire crop cycle. It would be ideal if the soil could be kept close to field capacity. In peat areas, irrigation should not be saline as this can increase the soil pH, which is not appropriate for the crop (Whitlock, 1979). The most critical period for celery production is the last month before harvest, in which the growth rate is high. In this phase, the water uptake is the maximum in the crop cycle. For this reason, soil moisture tension should be maintained at about 0.03 mbar to assure good yield.

According to ADAS Horticulture (2012), the annual water requirement of celery in UK is 200 mm. Pre-sowing or planting irrigation may be needed in June, and administered throughout crop cycle. The critical response period is from June to August. The irrigation plan is 18 mm at 20 mm S.M.D. for class A soils (with low water availability, hence, not more than 60 mm of water per 500 mm of depth; e.g. coarse sand, loamy coarse sand and coarse loamy sand). On the other hand, irrigation plan is 20 mm at 25 mm S.M.D. for class B soils (with medium water availability, hence, between 60 mm - 100 mm of water per 500 mm of depth; e.g. loamy sand, loam, silty clay loam and clay) and class C soils (with high water availability, thus, above 100 mm per 500 mm of depth; e.g. very fine sand, peat and peaty loam). Irrigation is absolutely essential to maintain soil moisture for quality and succulence. Also quality of water is important: celery is moderately sensitive to chloride levels in irrigation water. Safe levels are up to 200 mg/L and from 200 to 300 mg/L for limited use. Risk of foliar damage occurs if chloride levels are over 300 mg/L.

Celery is sensitive to water stress; petioles pithiness can occur even in short-term water stress in drip irrigated fields. No more than 20-30% of available water in the primary root zone should be depleted between irrigations to maximize growth rate and obtain good quality celery. Since drip irrigation does not wet the entire soil volume, irrigation must be applied more frequently than with sprinklers (Hartz, 1999).

2.1.6 Harvesting and marketing

Celery plants are traditionally hand-cut at soil surface level with a long knife and the outer petioles are removed. Then, heads are trimmed off on the apical part and packed directly in the field in covered harvesting units called “rigs”. The final product is then brought to the depot and kept there in temperature controlled conditions until it reaches the supermarket shelves. Plants are harvested when stalks reach the marketable size and before any pithiness develops in the petioles (Whitlock, 1979). Celery growth is generally uniform and for this reason the crop is harvested only once.

Celery is mainly marketed through three different product formats: Hearts (inner petioles), pre-pack (heads without apical leaves) and stick-pack (individual petioles), which are shown in Error! Reference source not found..

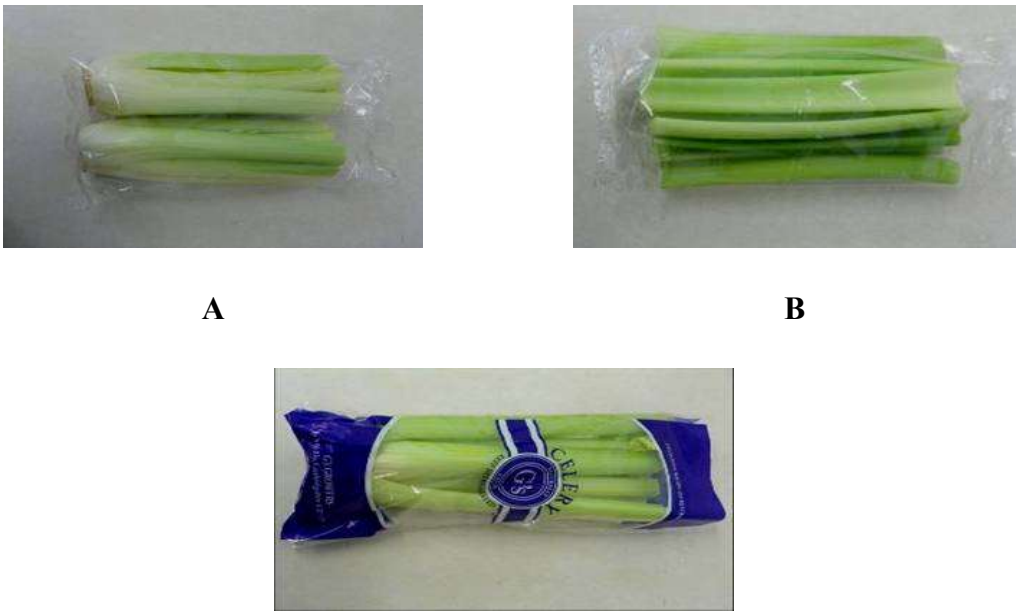


Figure 2.3 - Main celery market formats: (A): hearts, (B): stick-pack, (C): pre-pack (photos provided by G’s Fresh Ltd).

2.2 Economic importance and production statistics

Celery is commonly consumed fresh and only in minimal quantities dried, canned or frozen to make soups and other stew-like dishes. Sometimes the leaves are cut and used as a garnish. Celery oil, extracted from the seeds, can be used as a medicine to heal

arthritis, rheumatics and regulate blood pressure. Celery cultivars with fleshy stems are preferred in both USA and the UK, while in Europe celeriac cultivars are more popular. In Asia, particularly in China, smallage is the most popular, and it is commonly eaten as salad.

Table 2.2 - Area of production and harvested production of the major EU celery producers in three different years (European Commission, 2013).

Country	Area of production (ha)			Harvested production (t)		
	2003	**2007**	**2011**	**2003**	**2007**	**2011**
Belgium	500	400	400	28,500	19,200	22,700
Cyprus	100	100	100	700	700	500
Germany	/	200	300	/	7,700	11,500
Greece	/	800	500	/	17,000	13,700
Spain	1,800	1,600	1,400	87,900	84,400	68,500
France	700	600	500	24,000	22,900	18,000
Italy	4,000	4,100	3,300	107,700	109,600	97,700
UK	800	900	1,000	39,400	45,200	51,000

The most significant celery producing country in the European Union is Italy, followed by Spain and United Kingdom (**Table 2.2**). In Italy and Spain, the area of production and the harvested production is decreasing, while in United Kingdom the trend is opposite, although slight.

According to a report produced by USDA (2014), celery planted area corresponded to 1.7% of the total United States fresh market vegetable area in 2013. On the other hand, the harvested area corresponded to 1.8% (**Table 2.3**). Regarding the single States, the

most important U.S. celery producers are California and Michigan, with the former accounting for about 94% of the total production (**Table 2.4**).

Table 2.3 - Celery and total fresh market vegetable area planted, harvested, production and value - United States: 2011-2013 (USDA, 2014). (cwt: Hundredweight, which corresponds to one hundred pounds).

Crop	Area planted (acres)			Area harvested (acres)		
	2011	2012	2013	2011	2012	2013
Celery	28,700	29,600	29,400	28,200	29,000	28,800
Total	1,761,750	1,743,400	1,701,730	1,667,420	1,668,780	1,631,860
Crop	**Production (1,000 cwt)**			**Value of production (1,000 cwt)**		
	2011	**2012**	**2013**	**2011**	**2012**	**2013**
Celery	19,362	19,752	18,003	381,780	358,988	457,765
Total	432,297	435,465	414,714	10,724,426	9,975,198	11,383,945

Table 2.4 - Celery for fresh market and processing area planted and harvested, yield, production, price and value - United States: 2011-2013 (USDA, 2014).

State	Area planted (acres)			Area harvested (acres)		
	2011	2012	2013	2011	2012	2013
California	26,700	27,500	27,500	26,400	27,000	27,000
Michigan	2,000	2,100	1,900	1,800	2,000	1,800
U.S.	28,700	29,600	29,400	28,200	29,000	28,800
State	**Yield per acre (cwt)**			**Production (1,000 cwt)**		

	2011	2012	2013	2011	2012	2013
California	700	690	630	18,480	18,622	16,968
Michigan	490	565	575	882	1,130	1,035
U.S.	687	681	625	19,362	19,752	18,003

State	Price per cwt (dollars)			Value of production (1,000 dollars)		
	2011	2012	2013	2011	2012	2013
California	20.00	18.10	25.80	368,822	336,608	437,406
Michigan	14.70	19.80	19.70	12,958	22,380	20,359
U.S.	19.70	18.20	25.40	381,780	358,988	457,765

Celery is one of the British products which have earned Protected Geographical Indication (PGI) status from the European Commission (**Figure 2.4**). It is the first English vegetable to receive the award as the result of the unique nature of the soil and climate where it is grown, (Cambridgeshire, Suffolk and Norfolk), and the method of production. This recognition clearly shows how celery is important as a UK crop. Hence, it is currently better protected from global competition, imposters and retailer bargaining power. Fenland celery grows in the deep peat type soils, which contain high organic matter, deriving from undecayed dead vegetation that grew in the Fen and bog. This type of soil is naturally very fertile and contains all the nutrients necessary for a successful celery production. The nature of the black peat means that the celery plant benefits from extra warmth, due to its colour and consistency. Fenland celery typically starts life as a seed. If used on a commercial basis, this seed is sent to a propagation greenhouse between May and June where it germinates and spends three weeks. From the greenhouse, the young plant is sent to the Fenland-base farm where it is planted in the deep peat soil within one day. In the field, plants are grown in wide rows separated with deep trenches. Rows must be at least 90 cm apart. As it grows, the trenched soil is

banked up around the plant to prevent the sunlight turning it green. During growth, Fenland celery can be sprayed to combat diseases and pests. Harvest takes place between September and December and it is made by hand using a knife. The roots may be trimmed completely or left with a small amount. Celery is then washed, cut and packed into boxes directly in the field (DEFRA, 2006).

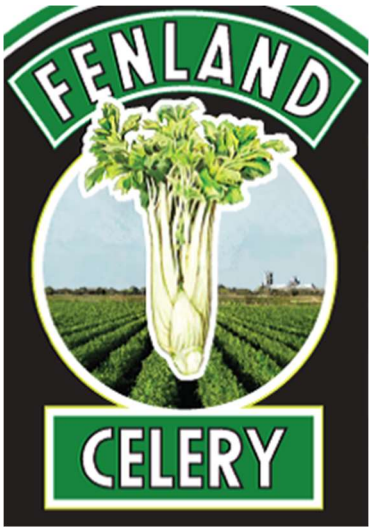


Figure 2.4 - Fenland celery logo. Source: http://www.fenlandcelery.com.

2.3 Nutritional characteristics

Celery, like the vast majority of fresh vegetables, is mainly constituted by water (95%). Its low energy content, due to the low levels of carbohydrates, lipids and proteins, renders this product particularly suitable for diets. Celery also contains significant quantities of vitamins and minerals (

Table 2.5). Although eaten for its long and thick petioles, and appreciated for the crisp texture, the most evident characteristic of this vegetable is the aromatic smell and taste, which is primarily due to 3-butylphtalid. This compound has been found to combat high blood pressure (Welbaum, 2015). Celery is also important for its health-promoting properties as it contains several bioactive compounds related to cancer, inflammations and heart disease prevention. These molecules include flavonoids, phenolic acids, carotenoids and coumarins.

Table 2.5 - Characteristics of raw celery (USDA, 2013)

Nutrient	Unit	Value per 100 g
Main Constituents		
Water	g	95
Energy	kcal	16
Proteins	g	0.7
Lipids	g	0.17
Carbohydrates	g	2.97
- sugars	g	1.83
- fiber	g	1.6
Minerals		
Calcium (Ca)	mg	40
Iron (Fe)	mg	0.20
Magnesium (Mg)	mg	11
Phosphorous (P)	mg	24
Potassium (K)	mg	260
Sodium (Na)	mg	80
Zinc (Zn)	mg	0.13
Vitamins		
Vitamin C	mg	3.1
Thiamin	mg	0.021
Riboflavin	mg	0.057
Niacin	mg	0.320
Vitamin B-6	mg	0.074
Vitamin A	IU	449
Vitamin E	mg	0.27

Vitamin K	mg	0.029

2.4 The fresh-cut produce industry

The fresh-cut produce industry adds value to raw agricultural products by preparing and processing them for direct consumption. Market possibilities of this type of industry are currently having a positive trend in Europe and North America. The final recipients are retail consumers, restaurants, hotels and other food service establishments. The typical characteristics of fresh-cut products make them attractive for consumers since they are convenient, uniform in size, fully usable and quick to prepare (UC DAVIS, 2012). These characteristics led to increasing success of this industry sector. According to a research report by Cook (2008), US fresh-cut produce industry sales increased from 3.3 billion dollars in 1994 to 15.9 billion dollars in 2007 taking into account all marketing channels. Around 60% was estimated to be sold via food service channels.

According to a technical report by WRAP (2011), household waste of fruits and vegetables was around 3 million tonnes per year in UK, resulting in concerns about the environmental and economic impacts of food waste. A detailed summary of waste and percentage loss for 11 different fruits and vegetables is reported in **Table 2.6**. Based on reported data, loss and waste in the supply chain is typically less than 10%, although it can approach 25% for some products. The results showed that the level of waste at any single stage of the supply chain varied yet was between 1% and 3% overall. For certain products, loss is more significant at different points in the supply chain.

Table 2.6 - Summary of percentage loss and waste for 11 different fruits and vegetables through the UK supply chain (WRAP, 2011).

Product	Field loss	Grading loss	Storage loss	Packing loss	Retail waste
Strawberry	2-3%	1%	0.5%	2-3%	2-4%
Raspberry	2%	n.a.	n.a.	2-3%	2-3%
Lettuce	5-10%	n.a.	0.5-2%	1%	2%
Tomato	5%	7%	n.a.	3-5%	2.5-3%
Apple	5-25%	5-25%	3-4%	3-8%	2-3%

Onion	3-5%	9-20%	3-10%	2-3%	0.5-1%
Potato	1-2%	3-13%	3-5%	20-25%	1.5-3%
Broccoli	10%	3%	0%	0%	1.5-3%
Avocado	n.a.	30%	5%	3%	2.5-5%
Citrus	n.a.	3%	n.a.	0.1-0.5%	2-2.5%
Banana	n.a.	3%	n.a.	0-3%	2%

2.4.1 Generalities of fresh-cut produce

Fresh-cut produce is defined as any fresh vegetable and/or fruit which has been subjected to physical alteration from its original form, but remaining still in a fresh state (Varzakas and Manolopolou, 2017). Examples of alteration processes include trimming, cutting, shredding, slicing, peeling and washing. Fresh-cut products are convenient and conserve their nutritional valuable as a consequence of the minimal processing operations incurred between harvesting and marketing (Barrett *et al*., 1998). Fresh-cut fruits and vegetable differ in their physiology and biochemistry from intact products. For instance, fresh-cut vegetables are more susceptible to deterioration than intact ones, as a result of the operations they have undergone after harvest, which negatively affect the quality of final products (Saltveit, 1997). Deterioration symptoms include increased flaccidity, weight loss, increased respiration rate, water loss and microbial contamination (Brecht, 1995; Varoquaux and Wiley, 1994; Klein, 1987). Effects on the retention of vitamin, minerals and other nutrients during handling and storage remain still unclear.

The main physical alteration occurring during processing of fresh products is wounding, which stimulates a series of physiological and biochemical changes in close and distant tissues (Ke and Saltveit, 1989). These alterations include increased ethylene production rate and respiration rate (Brecht, 1995). Ethylene is a plant hormone which stimulates senescent processes like abscission, chlorophyll loss, production of unwanted flavours and other undesirable changes in plant tissues leading to quality loss. Wounding also renders tissues susceptible to attacks by pathogens like fungi and bacteria. Pathogen attacks render products inedible but also dangerous to customers in case of

contamination by food-poisoning microorganisms (mainly *Bacillus*, *Lysteria*, *Salmonella* and *Clostrydium*).

2.4.2 Postharvest processing, handling and storage of celery

Celery is harvested when plants reach marketable size (when the petioles from the soil line to the first node are at least 6 inches long) and before any pithiness develops in the outer petioles. Plants are trimmed at the apical part to remove the leaves and harvested by cutting at the soil line. The "heads" should have sufficient compactness without excessive open space in the centre of the stalk. During machine harvesting, some mechanical damage may occur, resulting in negative side-effects like water loss and increased diseases. For this reason, hand harvesting is still the best option, even though labour costs are becoming higher. Once harvested, plants are packed directly in the field and delivered to the depot within 3 hours where they will be maintained at controlled temperature before being dispatched to the supermarket. After harvest, celery should be precooled to remove field heat and refrigerated as soon as possible in order to maintain quality. The most common ways commercial growers precool are vacuum cooling and hydro-cooling at temperatures close to 0 °C. Hydro-cooling is theoretically the most appropriate system because it maintains water on the samples, preventing them from wilting. Yet, this system is not effective in maintaining the water clean and free of microorganisms. Many horticultural companies use vacuum cooling as it has been shown to be more effective in case of crops having a large surface-to-volume ratio like celery. The only problem with this system is the high cost. For this reason, large growers or co-operatives tend to use it (Whitlock, 1979). According to Gómes and Artés, (2004), the main parameters through which consumers judge high quality celery are: stalks having a good tight formation, thick and minimally twisted petioles, crispness and bright green appearance. Since celery contains high water content, it is highly perishable, thus, susceptible to lose its quality during storage. Major indicators of quality loss include cut-end browning and pithiness.

2.4.3 Main physiological disorders of celery

Blackheart: Consists of the tissue turning black because of cell necrosis (**Figure 2.5**). It can cause complete field loss. It is thought to be due to sudden variations of soil moisture during the growing season. Evidence suggests that excessive presence of

nitrogen salts and low amounts of calcium in the soil appear to cause the same problem (Whitlock, 1979).

Figure 2.5 - Celery blackheart. Source: http://vegetablemdonline.ppath.cornell.edu-PhotoPages/Impt_Diseases/Celery/Celery_Black.htm.

Cracked stems: Can occur when the plants produce fleshy and mature petioles, resulting in rapid decay of tissues (**Figure 2.6**). This disorder is induced by boron deficiency in peaty soils, thus, adequate fertilization is required before planting to avoid the problem. Less boron fertilization is required on mineral soils (Whitlock, 1979).

Figure 2.6 - Celery cracked stems. Source: http://www.ipt.us.com/produce-inspection-resources/inspectors-blog/defect-identification/celery-cracks).

Pithiness: Is one of the major problems negatively affecting quality and shelf-life of celery (Saltveit and Mangrich, 1996). It manifests with parenchyma tissue becoming less dense, whitish and fibrous, resulting in formation of aerenchyma (**Figure 2.7**). Current evidence indicates that pithiness can be induced by pre-harvest factors. For instance, infrequent drip irrigation during summer periods has been shown to significantly increase pithiness (Breschini and Hartz, 2002; Saltveit and Mangrich, 1996). Also, mechanical perturbation and Ethephon application increased pithiness, especially at the basal part of the petioles (Pressman *et al*., 1984).

A **B**

Figure 2.7 - Fresh celery cross-sections: (A): no pithiness; (B): evident pithiness (photos provided by G's Fresh Ltd).

2.5 The browning disorder in fresh-cut vegetables

Since fresh-cut produce began to be sold on the market, processors have always faced problems related to quality problems and reduced shelf-life. One of the most relevant challenges is to stop the products turning brown after cutting. Fresh appearance and colour are the most important parameters through which consumers evaluate visual quality of fresh-cut products on the market (Kays, 1999). In this regard, processors have always tried to develop methods to avoid cut-end discolouration, such methods include proper refrigeration and appropriate packaging. Some processors have experimented with chemical approaches like application of edible coatings and water baths even though quality improvements where low compared to additional costs (Laminkanra, 2002).

Enzymatic browning is a physiological disorder occurring in fresh-cut vegetables and fruits during processing and storage which dramatically reduces their quality and shelf-life. It is important to distinguish enzymatic browning from non-enzymatic browning. The latter is triggered by heating after processing of food with high carbohydrate content (biscuits, bread, etc.) and it does not involve enzymes. Types of non-enzymatic browning include Maillard reaction, sugar pyrolysis and caramelization. On the other hand, enzymatic browning develops at both ambient and low temperatures on fresh fruits and vegetables and, as the name indicates, it involves enzymes. Enzymatic browning on cut ends of fresh cut products, including celery, can negatively affect appearance and sensory parameters like flavours, odours, texture and nutritional value (Komthong *et al*., 2006) with serious consequences on consumer preferences that tend to view affected products as "non-fresh" and impacts on sales. This results in considerable economic losses for the growers and the fresh-cut produce industry. Economic losses can be more severe especially if browning occurs in the early stage of product shelf-life, when the costs of processing, packaging and storage have been incurred. For this reason, it is important to understand the underlying biochemical and physiological mechanisms involved in this disorder with the aim of applying further strategies to avoid, or at least, reduce its incidence and severity.

2.5.1 Browning-related enzymes

Postharvest browning is thought to be due to the enzymatic activity of polyphenol oxidase (PPO) (1,2-benzenediol: oxygen oxidoreductase) which utilizes phenolic compounds as substrate and molecular oxygen as co-substrate in order to produce *o*-quinones. PPO contains copper in its active site, which is fundamental for its catalytic activity. The overall reaction occurs in two steps: hydroxylation of monophenols to diphenols mediated by monophenol oxidase, and subsequent oxidation of diphenols to *o*-benzoquinones mediated by diphenol oxidase (**Figure 2.8**).

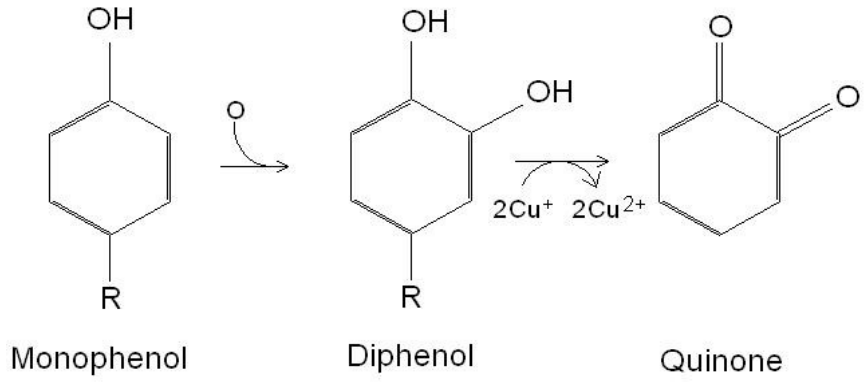


Figure 2.8 - Mechanism of the overall reaction converting monophenols to quinones catalyzed by PPO (Queiroz *et al*., 2008).

Afterwards, *o*-benzoquinones, through undergoing non-enzymatic polymerization, are transformed into melanins; insoluble molecules having antimicrobial properties, which form barriers to prevent pathogen infections. Melanins are the pigments responsible for the unappreciated brown colour on cut or damaged surfaces of fresh products. The reaction does not occur in normal conditions because PPOs and phenolic compounds are compartmentalized from each other in unwounded cells. PPOs are present in the plastids and in the soluble fraction of the cell while phenolic compounds are enclosed in the vacuole (Mayer and Harel, 1979). As soon as tissues are damaged and cells are broken, substrate and enzyme come in contact and the reaction occurs. The reaction is dependent on pH as it does not occur under acidic (pH<5) or alkaline (pH>8) conditions. PPOs are responsible for the enzymatic browning of some vegetables including artichoke (Leoni *et al*., 1990), head lettuce (Fujita *et al*., 1993), cocoa bean (Lee, 1991) and cabbage (Nagai and Suzuki, 2001). Yagar (2004) isolated PPO from celery roots and found that it was active towards catechol, pyrogallol and $_{L}$-Dopa yet was not active towards Tyrosine, resorcinol and *p*-cresole (**Table 2.7**). The enzyme showed the highest activity with pyrogallol.

Table 2.7 - Affinity of six different phenolic substrates to PPO extracted from celery roots (Yagar, 2004).

Substrate	**Activity (units/mL)**	**K_m (mM)**	**V_{max} (units/mL)**	**V_{max}/K_m**
Catechol	290	8.3	400.0	48.19

Pyrogallol	560	4.5	666.6	148.13
L-Dopa	185	6.2	222.2	35.83
Tyrosine	n.a	-	-	-
Resorcinol	n.a.	-	-	-
p-Cresole	n.a.	-	-	-

The peaks of PPO activity were found towards pyrogallol at 25 °C, catechol at 40 °C and L-Dopa at 45 °C (**Figure 2.9**). The ideal pH range for all the three substrates was from 7.0 to 7.5. Overall, the affinity dramatically decreased out of this range, except for L-Dopa which maintained a high activity from 7.5 to 9.0 (**Figure 2.10**).

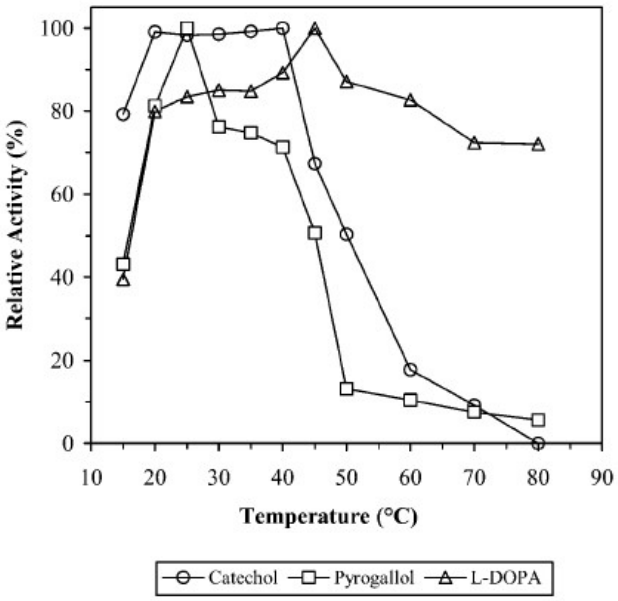


Figure 2.9 - Effect of temperature on celery root PPO activity towards three different phenolic substrates (Yagar, 2004).

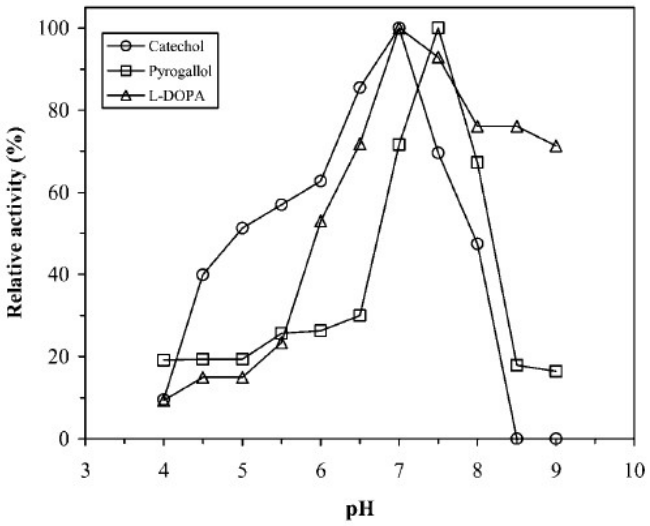


Figure 2.10 - Effect of pH on celery root PPO activity towards three different phenolic substrates (Yagar, 2004).

As well as PPO, two other enzymes are believed to play a key role in enzymatic browning of fresh-cut vegetables: peroxidase (POD) and phenylalanine ammonia-lyase (PAL). POD is an important enzyme which helps to protect plants from the harmful effects of Reactive Oxygen Species (ROS) as a consequence of biotic and abiotic stresses. ROS are extremely reactive compounds deriving from biochemical activities involving oxygen, this means that they are normally present in cells but they can cause severe damaging effects if their concentration is excessive (Vicuna, 2005). POD contains a heme cofactor and its role is to remove hydrogen peroxide (H_2O_2); a toxic by-product of primary metabolism; from cells. The reaction needs phenolic compounds, which are converted into *o*-quinones and then to brown pigments (Subramanian *et al*., 1999).

The other enzyme involved in postharvest browning is PAL, which catalyzes the deamination of the aromatic amino acid L-phenylalanine to produce *trans*-cinnamic acid and ammonia. This reaction is the first committed step in the phenylpropanoid pathway (**Figure 2.11**). The phenylpropanoid pathway provides plants with important secondary metabolites like hydroxycinnamic alcohols (coumaryl alcohol, coniferyl alcohol and sinapyl alcohol), which are the building blocks of lignin (Boerjan *et al*., 2003), and other compounds involved in plant defense against biotic and abiotic stresses. These compounds include coumarins, flavonoids, lignans and tannins (Vogt, 2010). Due to the

position of PAL in the pathway, its activity controls the rate at which phenolic molecules are produced. PAL contributes to the browning disorder because its activity is positively correlated to the synthesis of phenolic compounds, which are substrates of PPO for production of quinones (He and Luo, 2007). PAL activity is induced by excision of plant tissues and the resultant wounding (Saltveit, 2000) (**Figure 2.12**) and its levels can significantly fluctuate in short time intervals in response to a large variety of external stimuli (Camm and Towers, 1973). Ke and Saltveit (1989) reported that wounding induces PAL activity not only in damaged cells close to the wound but also in adjacent cells (2.5 cm away). This indicates that the damage signal is transferred from wounded to unwounded tissues. PAL activity was found to increase before browning manifested in minimally processed lettuce (Couture *et al*., 1993).

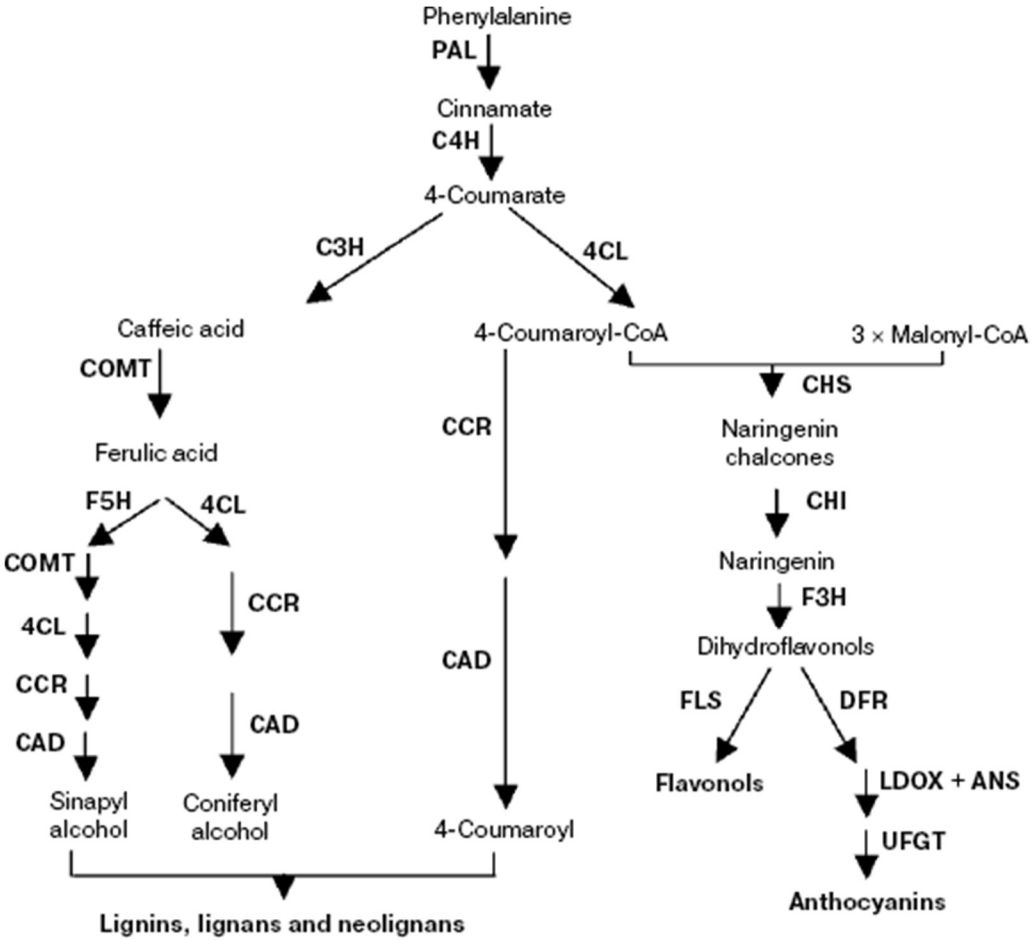


Figure 2.11 - The phenylpropanoid metabolism in plants (Du *et al*., 2009). Abbreviations: PAL, phenylalanine ammonia-lyaise; 4CL, 4-coumarate-CoA ligase; C4H, cinnamate-4-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase;

F3H, flavonone 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; LDOX, Leucoanthocyanidin dioxygenase; C3H, coumaroyl-quinate/shikimate 3-hydroxylase; COMT, caffeic acid:5 hydroxyferulic acid O-methyltransferase; CCR, cynnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; F5H, ferulate 5-hydroxylase; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase.

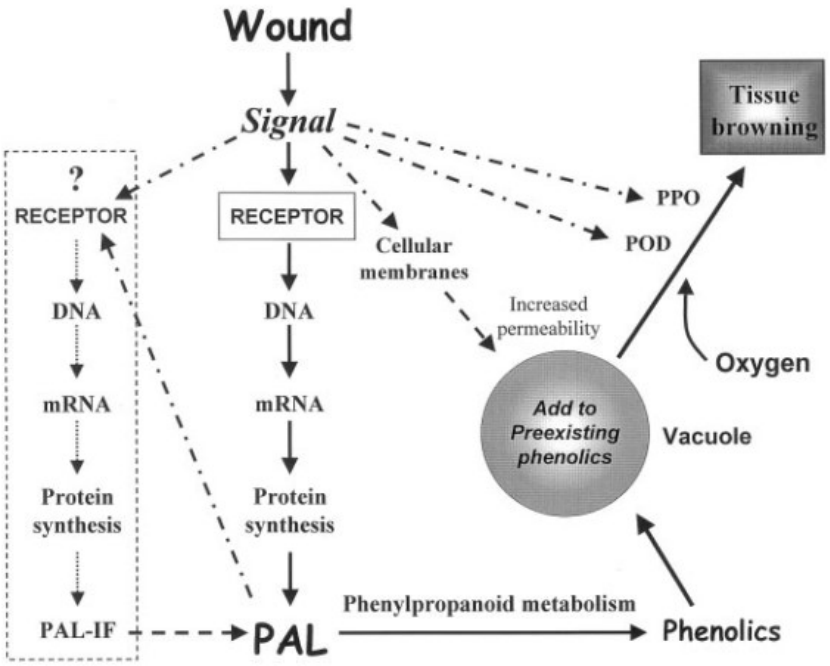


Figure 2.12 - The interrelationships between tissue wounding and all the involved biochemical factors leading to browning in cut leaves of lettuce (Saltveit, 2000).

2.5.2 Phenolic compounds

Phenolic compounds are a class of organic chemicals, ubiquitous in plants, composed by an aromatic ring bonded with one or more hydroxyl groups. They are divided into simple phenols or polyphenols depending on the number of phenolic units the molecule is made of (**Table 2.8**). Some examples include benzoic acids, flavonoids, coumarins and tannins (Khoddami *et al*., 2013). Since phenolic compounds are able to scavenge free radicals and transfer hydrogen atoms, they consequently function as natural antioxidants (Afanas'ev *et al*., 1989; Amarowicz e*t al*., 2004).

Table 2.8 - Classification of phenolic compounds according to their chemical structure (Balasundram *et al*., 2006).

Chemical class	Chemical structure
Simple phenolics, benzoquinones	C_6
Hydroxybenzoic acids (i.e. gallic, vanillic and syringic acids)	C_6 -C_1
Acethophenones, phenylacetic acids	C_6-C_2
Hydroxycinnamic acids, phenylpropanoids (caffeic, ferulic, sinapic and *p*-coumaric acids)	C_6-C_3
Naphthoquinones	C_6-C_4
Xanthonoids	C_6-C_1-C_6
Stilbenoids, anthraquinones	C_6-C_2-C_6
Flavonoids, isoflavonoids	C_6-C_3-C_6
Lignans, neolignans	$(C_6–C_3)_2$
Biflavonoids	$(C_6–C_3–C_6)_2$
Lignins	$(C_6–C_3)_n$
Condensed tannins (proanthocyanidins or flavolans)	$(C_6–C_3–C_6)_n$

The abundance and profile/diversity of phenolic compounds in plants depends on genotype (genus, species and cultivar) and environment (agronomic factors, handling and storage conditions) (Thomàs-Barberàn and Espìn, 2001). Some phenolic compounds are indicators of the presence of postharvest disorders. For instance, Saltveit (2000) reported that chlorogenic acid (5-caffeoylquinic acid), isochlorogenic acid (3,5-dicaffeoylquinic acid), caffeoyltartaric acid and dicaffeoyltartaric acid were found to be associated with tissue browning in fresh-cut lettuce. All these compounds are synthesized from cinnamic acid which is hydroxylated to form *p*-coumaric acid, and then, caffeic acid. Caffeic acid can bond with quinic acid or tartaric acid to from a large variety of phenylpropanoids (Dixon and Paiva, 1995). By contrast, Mai and Glomb

(2013) found that the total concentration of phenolic compounds was not correlated with enzymatic browning in Iceberg lettuce. This is an indication that other molecules can be involved in the "disorder". In celery, no research about correlations between phenolic compounds and browning appearance on cut surfaces has been conducted. Yao *et al*., (2010) studied the phenolic composition of 9 celery cultivars, and they identified three different flavonoids (apigenin, luteolin and kaempferol). The most present of these was found to be apigenin, followed by luteolin and kaempferol (**Table 2.9**).

Table 2.9 - The concentration of flavonoids in nine varieties of *Apium graveolens* L. (mg 100 g^{-1} DW) (Yao *et al*., 2010).

Cultivars	**Flavonoids**		
	Apigenin	**Luteolin**	**Kaempferol**
95-6	79.42 ± 0.77	62.43 ± 0.59	1.06 ± 0.03
24-1-6	108.15 ± 0.82	38.62 ± 0.16	3.32 ± 0.07
33-1-3	61.64 ± 0.39	65.91 ± 0.24	2.31 ± 0.05
Shandong celery	124.73 ± 0.41	42.86 ± 0.37	1.48 ± 0.06
27-1-12	130.59 ± 0.40	42.12 ± 0.14	2.01 ± 0.03
Jinquin Nr 1	74.33 ± 0.19	28.81 ± 0.18	2.87 ± 0.04
Xin taishiquin	73.88 ± 0.55	36.34 ± 0.63	2.47 ± 0.02
Xiangmao	95.75 ± 0.42	31.27 ± 0.33	0.64 ± 0.06
Shengjie celery	142.85 ± 0.33	24.83 ± 0.29	2.11 ± 0.03

Yao *et al*., (2010) also determined the three different phenolic acids, caffeic, ferulic and *p*-coumaric acid. The dominant one was found to be *p*-coumaric acid, followed by ferulic and caffeic acid.

Table 2.10 - The concentration of phenolic acids in 9 varieties of *Apium graveolens* L. (mg $100g^{-1}$ DW) (Yao *et al*., 2010).

Cultivars	Phenolic acids		
	Caffeic acid	**Ferulic acid**	***p*-Coumaric acid**
95-6	16.68 ± 0.36	28.12 ± 0.68	95.53 ± 5.81
24-1-6	10.45 ± 0.08	23.56 ± 1.34	80.80 ± 7.15
33-1-3	13.82 ± 0.55	10.94 ± 1.25	102.75 ± 4.62
Shandong celery	11.94 ± 0.23	21.36 ± 0.17	80.18 ± 4.51
27-1-12	7.15 ± 0.57	94.33 ± 0.92	86.37 ± 2.67
Jinquin Nr 1	10.72 ± 0.38	28.06 ± 0.14	98.29 ± 4.50
Xin taishiquin	16.12 ± 0.03	31.66 ± 0.16	99.12 ± 1.26
Xiangmao	10.25 ± 0.71	31.27 ± 0.34	111.04 ± 3.13
Shengjie celery	30.26 ± 0.11	93.05 ± 0.25	100.18 ± 2.35

In this study the authors reported very unusual high levels of phenolic compounds (

Table 2.10). Since the paper lacks some hard detail about methods and procedures it is not possible to detect further errors. It is likely that miscalculations could have been done or wrong units of measure could have been adopted.

Mai and Glomb, (2014) found that, besides the enzymatic polyphenolic browning, other reactions could be involved in the formation of brown pigments. The authors isolated different lettucenin sesquiterpenes, which were found to be key chromophores in the cut-browning of Iceberg lettuce.

2.6 Main factors influencing browning and related enzymes

2.6.1 Genetic factors

Considering the genetic differences between crop species, there seems to be similarities in the mechanisms around browning disorder in some minimally processed vegetables. Degl'Innocenti *et al*., (2007) studied the browning disorder and relative key enzymes (PPO, POD and PAL) in cut leaves of three different crop species: lettuce (*Lactuca sativa* var. *capitata* L.), escarole (*Cichorium indivia* var. *latifolium*) and rocket salad (*Eruca sativa* Mill.), upon 4 °C storage. The results showed that the first two species were found to be markedly sensitive to leaf browning while rocket salad was not affected. This resistance was also associated with higher ascorbic acid content and a reduction of this compound during storage, while in lettuce and escarole ascorbic acid content remained relatively constant.

Browning susceptibility can also vary not only among crop species, but also among cultivars of the same species. For instance, a study carried out by Atkinson *et al*., (2013) on 28 different accessions representing lettuce cultivars of different crop types and geographical origin of the wild relative *Lactuca serriola*, showed significant differences between accessions for intensity and extent of browning. Interestingly, accessions were found to be more susceptible to browning at early postharvest stages but more susceptible to pinking during late postharvest stages.

Genetic-dependent browning was confirmed in another study conducted by Cantos *et al*., (2001) on lettuce (*Lactuca sativa* L.). The authors tested the effect of wounding (excision into 2-cm x 2-cm pieces) in total six cultivars upon storage in polypropylene perforated bags for 7 days at 5 °C (Iceberg Mikonos, I. Green Queen, I. Asdrùbal, Little Gem Sandra, Romaine Cazorla and R. Modelo). They found that Iceberg Mikonos was the most susceptible cultivar to browning while Romaine Cazorla was the least susceptible. They also found that no correlations existed between browning and other biochemical attributes (PPO, POD and PAL activities, total and individual phenolic compounds accumulation).

Phenolic compound metabolism was found to vary among seven cultivars of carrot (*Daucus carota* L.). The effect of genetic factors seems to play a role in the enzymatic

browning of the sap and in the accumulation of isocoumarin (Leja and Stodolak, 1997). The fact that phenolic composition of plants depends on genotype is demonstrated by the fact that phenolic compounds are used in taxonomy. Different plant species, and also different cultivars of the same species, synthesize specific phenolic molecules responsible of their quality. For example, some lettuce cultivars are very deficient in flavonoids, while others are very rich in flavonols, anthocyanins and caffeic acid derivatives (Du Pont *et al*., 2000).

2.6.2 Agronomic factors

2.6.2.1 Harvest maturity

Current literature has not reported on any studies concerning the effect of plant developmental stage on browning at cut surfaces of green celery. However, Guerra *et al*., (2010) investigated postharvest discolouration on entire petioles of the self-whitening Golden Clause celery cv. according to different harvest maturity stages. Harvest dates were 93 (HD1) and 124 (HD2) days after transplantation and samples were analyzed 1 h after harvest. The authors found that browning potential (BP) at 320 nm and total quinone content (TQ) were higher in HD2 instead of HD1. On the other hand, no significant differences were found in hue angle. In addition, pithiness was higher at HD2. More detailed results are reported in **Table 2.11**.

Table 2.11 - Chemical characteristics of celery petioles according to two different harvest dates (AU: Artificial Unit) (Guerra *et al*., 2010).

Harvest date	BP (AU per g of fresh tissue)	TQ (AU per g of fresh tissue)
HD1	0.10 ± 0.043	0.02 ± 0.005
HD2	0.25 ± 0.095	0.04 ± 0.005

According to Yommi *et al.*, (2012), delayed harvest of celery cv. Golden Boy significantly increased plant size but it was associated with low quality petioles, basically due to an increase in fibrosity of tissues. These findings have been confirmed by another study carried out by Yommi *et al*., (2013) on the same celery cv. The authors found that celery harvested at late physiological stages caused a decrease in the quality

of petioles, mainly attributable to undesirable changes in texture (crunchiness, hardness, fibrousness and juiciness).

A study conducted on lettuce (*Lactuca sativa* L.) var. Grand Rapids by Chutichudet *et al*., (2011) evaluated the effect that different horticultural maturity stages have on PPO activity, leaf color and total quinones. The authors found that PPO activity sequentially increased when plants entered the older stages, reaching the maximum content at 73 days after planting (DAP). Regarding total quinone content the results showed that the peak level was reached at 28 DAP. Also, leaf colour in terms of L* (lightness) and b* (yellowness) significantly changed according to plant maturity. At early plant growth of 28 DAP, both L* and b* showed their highest level, then these values tended to decrease at 42 DAP through the 73 DAP. All these findings seemed to reveal that, as the lettuce aged, the plants were more susceptible to browning. According to Hilton *et al*., (2009), increasing maturity increased "pinking" in Iceberg lettuce cv. Saladin, although it did not seem to have an effect on the degree of cut-end browning.

2.6.2.2 Fertilization

Currently, there are no studies about the influence of nutrients on browning in celery, yet some research has been conducted on lettuce. It is reported that nitrogen fertilization can reduce processed leaf shelf life. This was confirmed by previous studies which found that poor nitrogen applications can significantly improve shelf-life (Poulsen *et al*., 1995). Yet, in other studies, the link between browning and nitrogen fertilization is conflicting. According to D'Antuono and Neri (2001), quality traits were not significantly affected with addition of nitrogen. In another study, postharvest quality was not influenced by different nitrogen management systems in Romaine and Iceberg lettuce (Hartz and Breschini, 2001). Such contradictory results may be caused by the variability of nitrogen application during crop trials. In addition, the soil nitrogen concentration can vary to great extent as it depends on a wide range of factors like soil type, cropping system and type of irrigation employed. Calcium application decreased discolouration in cv. Saladin lettuce at low K:N ratios (Hilton *et al*., 2009). Another study conducted by Khumjing *et al*. (2001) on lettuce cv. Grand Rapids showed that plants treated with 2% calcium chloride ($CaCl_2$) had the lowest PPO activity and phenolic compound concentration, which corresponded to the lowest browning level at

harvest. On the same cultivar, Chutichudet *et al*. (2009) evaluated the effect of calcium in terms of gypsum ($CaSO_4$. 2 H_2O) applied by soil dressing and found that plants treated with 100 mg/kg had the lowest PPO level at 3 weeks after transplantation, yet no effect on phenolic compounds were detected at harvest stage.

2.6.2.3 Irrigation

Currently, there is little research concerning the impact of irrigation management on postharvest browning of fresh-cut celery, yet, there is some evidence on fresh-cut lettuce (*Lactuca sativa* L.), another crop which also suffers from cut surface discolouration. In particular, two studies on this crop have been published. The most recent was carried out by Luna *et al*., (2013) investigating the influence that various low flow subterranean drip irrigation managements have on postharvest quality of fresh-cut Romaine lettuce (*Lactuca sativa* var. *longifolia*) according to the Standard Irrigation Regime (SIR) (266-320 mm) of this crop in the field. The results showed that when the midribs were stored in air at 7 °C, cut edge browning was significantly affected. Cut edge browning was significantly reduced when samples were irrigated with -35% SIR, and was markedly increased when irrigated with +75% SIR. In addition, the authors reported that phenolic content was significantly affected. In more detail, phenolic content increased under all irrigation regimes as storage time increased, except for the midribs treated with +75% SIR, in which the initial content was maintained during storage. This is probably due to the lower PPO activity of highly watered samples. Similar research conducted by Luna *et al*., (2012) on fresh-cut Iceberg lettuce (*Lactuca sativa* var. *capitata*) showed that PAL activity dramatically increased in midrib tissue of highly irrigated plants. PPO activity increased simultaneously with cut-end browning development, especially at high irrigation regimes (+25% and 50% SIR). Phenolic compounds, especially caffeic acid derivatives, increased in the less irrigated midribs due to the low PPO activity. POD was found to be the least affected enzyme.

2.6.3 Physical factors

2.6.3.1 Mechanical damage

Previous literature reports that mechanical damage of fresh-cut vegetables induces the synthesis of enzymes involved in the phenylpropanoid metabolism, the accumulation of phenolic compounds and tissue browning (Brecht, 1995; Saltveit, 1997). A study

conducted by Loaiza-Velarde (2003) showed that in celery excised petioles, wounding induces a 12-fold increase in PAL activity by 36 h in 5-mm excised petioles. After that, PAL activity decreased rapidly reaching levels similar to that found initially by 96 h. The authors reported that browning potential increased rapidly by 36 h as PAL activity increased (**Figure 2.13**). Browning potential increase was associated with evident browning at vascular surfaces.

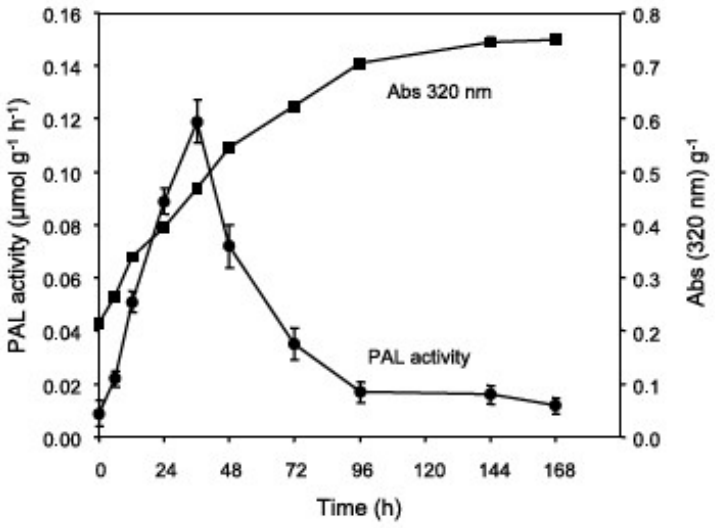


Figure 2.13 - Effect of wounding on the subsequent activity of PAL after 24 h and browning potential after 48 h at 10°C (Loaiza-Velarde *et al*., 2003).

In Romaine lettuce var. Col stored at 0 °C, it was observed that the most intensively damaged tissues presented two peaks of PAL activity, while in whole and less intensively damaged, only one peak was detected. Simultaneously, browning developed in the most damaged leaves (Pereyra *et al*., 2005).

According to Campos-Vargas and Saltveit (2002), cutting leaves of Romaine lettuce produces a signal that induces the synthesis of PAL, the accumulation of phenolic compounds and tissue browning in cells near the damaged site. Leaf age was found to affect the response of leaves to putative chemical wound signals (jasmonate, methyl-jasmonate, abscisic acid and ethylene). Exposure to these three chemicals had no effect on PAL activity, accumulation of phenolic compounds or discoloration in mature leaf tissue. By contrast, they induce high levels of PAL in young leaves.

2.6.3.2 Light

Current research shows that the quantity and quality of light can influence browning and the activity of related enzymes in fresh-cut vegetables. Zhan *et al*., (2013) demonstrated that continuous exposure to high intensity light (2000 lux) significantly suppressed PPO, POD activity and browning index in 5-cm long celery sticks (cv. Kaifeng Glass Crispy) cut with stainless sharp steel knife and stored for 8 days at 7 °C. By contrast, PAL activity was higher in light conditions than in darkness (**Figure 2.14**). This pattern was very similar in another study carried out by Zhan *et al*., (2012) on fresh-cut romaine lettuce. The inhibitory activity of light could be explained by its photo-oxidative effect, which results in the inactivation of the enzymes (Manzocco *et al*., 2009).

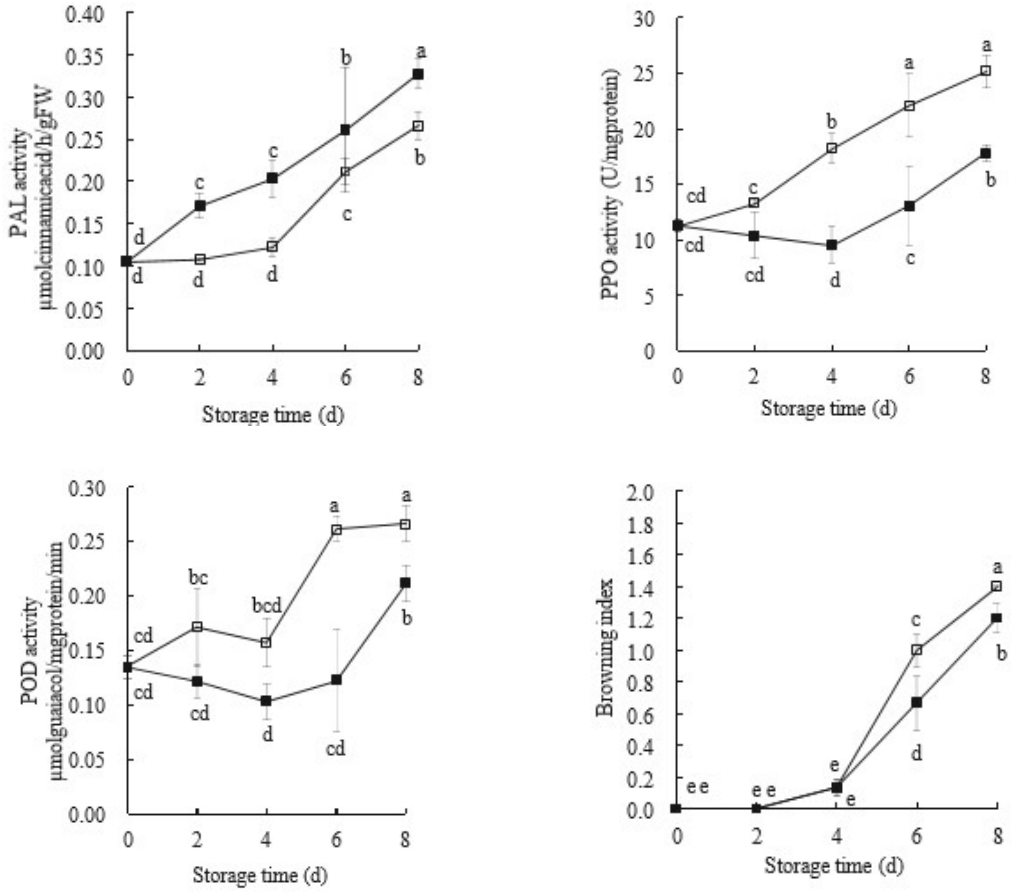


Figure 2.14 - Effects of light exposure on the activity of PAL, PPO, POD and browning index of fresh-cut celery stored for 8 days at 7 °C. White squares indicate high intensity light (2000 lux) while the black indicates darkness (0.2 lux) (Zhan *et al*., 2013).

According to Chutichudet and Chutichudet (2011) pre-harvest shading conditions created by using shade netting saran can significantly affect phenolic compounds, quinone content, PPO activity and browning appearance in lettuce cv. Grand Rapids. In more detail, they found that plants grown under 60% shading sunlight presented the lowest PPO activity, phenolic content, quinone content and browning.

Wavelengths out of the visible spectrum can also influence browning. For instance, ionizing irradiation (Gamma-rays) applied from 0.5 to 2 kGy increased phenolic content in Romaine lettuce, Iceberg lettuce and endive in midrib and non-midrib tissues, with the increase being more marked in midrib tissues. Yet, irradiation caused browning in midrib tissues (Fan, 2005). UV-C treatments showed to extend shelf-life of minimally processed Red Oak Leaf lettuce by reducing bacteria population. Yet, irradiating samples at 7.11 kJ m^{-2} induced tissue browning after 7 storage days at 5 °C (Allende *et al*., 2006).

2.6.3.3 Temperature

It is well known that cold temperature plays a pivotal role in slowing down deterioration, and then, increasing shelf life of fresh produce. The reason is because cold temperature lowers metabolic rates in fruits and vegetables during postharvest storage. Optimal storage temperatures and times depend on various factors like crop, type of edible tissue and time between harvest and storage. UC Davis (2012) affirmed that celery can maintain good quality if stored at 0 °C and 98-100% relative humidity for 5-7 weeks. A study conducted by Viña and Chaves (2006) showed that cv. Golden Boy celery stored for 28 days had the lowest browning potential at 0 °C instead of 4 °C or 10 °C. Also, higher temperatures can slow down decay processes in celery, if applied at particular intensities and timings. For instance, Loaiza-Velarde *et al*., (2003) demonstrated that heat shock treatments have a marked impact on browning potential and PAL activity in fresh-cut celery petioles. In particular, heat treatments at 20-40 °C for 60 secs, slightly decreased PAL activity 24 h after wounding with a razor blade, while temperatures of 50-70 °C had a more marked effect in PAL activity reduction. In parallel, browning potential responded in an almost similar way, decreasing at temperatures of 20-40 °C and then falling sharply at 40-70 °C (**Figure 2.15**).

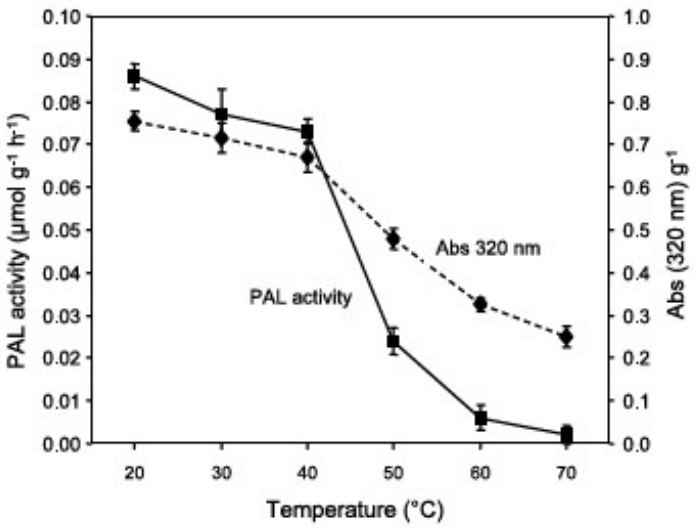


Figure 2.15 - Effect of 60 sec treatment at 20-70 °C on the activity of wound-induced PAL after 24 h, and browning potential after 48 h in 5 mm excised celery petioles stored at 10 °C (Loaiza-Velarde, 2003).

Beneficial results mediated by high temperature were confirmed by research carried out by Viña and Chaves (2008), who studied the effects of two types of heat treatment, water immersion at 50 °C for 90 sec and dry air at 48 °C for 1 h, on the postharvest quality of celery cut with a sharpened knife in 4-cm long sticks. The authors found that both treatments clearly reduced browning potential and chlorogenic acid concentration upon storage at 0°C for 28 days. Heat treatments were shown to be beneficial also in fresh-cut lettuce. According to Saltveit and Qin (2008), heating the ends of cut leaves after removal of the central axis at 55 °C for about 10 secs significantly reduced the accumulation of phenolic compounds and browning without any decay development. Heat shock treatment at 45 °C for 5 min delayed the increase of wound-induced PAL activity in Romaine lettuce without any decay development (Campos-Vargas *et al*., 2005). High temperatures have a deactivating effect on proteins, and then, on browning-related enzymes. This is probably the reason why heat shock treatments reduce cut edge discolouration.

2.6.4 Postharvest factors

2.6.4.1 Chemical effectors

Several chemical compounds can be used to avoid postharvest browning. The most important ones are sulphiting agents, even though the toxic effects on human health led the World Health Organization to recommend reducing their use as much as possible or, if applicable, completely avoiding it. Cysteine, a sulphur-containing amino-acid, is sometimes used to counteract browning as it reacts with *o*-quinones to form colourless molecules (Iyidogan and Bayiindirli, 2004), yet there is some evidence that it can cause some undesirable side-effects like production of off-flavours and off-odours (Garcia and Barrett, 2000). Ascorbic acid, an antioxidant contained in many fruits and vegetables, was found to be an effective additive. It is commonly applied on products as a salt (calcium ascorbate). Its anti-browning mechanism works by reducing *o*-quinones to convert them into colourless compounds and chelating the copper ion in PPO structure, which is important for its catalytic activity. The application of 4-hexylresorcinol (4-HR) was found to be effective at inhibiting PPO activity at low concentrations without negatively affecting taste and odour, and it is classified as Generally Regarded As Safe (GRAS) (He and Luo, 2007). Sodium chlorite ($NaClO_2$) has been demonstrated to be a highly effective anti-browning agent. The anti-browning properties can be explained by two mechanisms of action: direct PPO inactivation and oxidation of PPO phenolic substrates (He *et al*., 2008). Ozonated water has proved to inactivate PPO and respiration rate of fresh-cut celery without affecting sensory quality. It has been reported that a concentration of 0.18 ppm had the best preservative effect (Zhang *et al*., 2005). The same positive effect on browning control and quality retention was also noticed in fresh-cut lettuce (Beltràn *et al*., 2005). Monocarboxylic acids and their salts can inhibit phenolic metabolism induced by tissue wounding and subsequent discolouration in fresh-cut romaine lettuce. The effectiveness of these organic compounds increased as the number of carbons in the molecule chain increased (Saltveit *et al*., 2005). On the same crop, also application of n-alcohols significantly reduced tissue browning, with effectiveness increasing from ethanol to heptanol. The treatments were most effective during the first 2 h after wounding and ineffective after 6 h after wounding (Choi *et al*., 2005).

2.6.4.2 Controlled atmosphere (CA)

Controlled atmosphere (CA) is a food storage method that helps fresh produce to reduce decay processes over storage time through the utilization of altered composition of atmospheric gases in combination with regulated temperatures and humidity. CA systems have commonly been used to improve postharvest quality of fruits as well as fresh-cut vegetables. Gómez and Artés (2004) reported that CA helped to avoid browning of butt cut ends of cv. Trinova celery, maintaining the green appearance appreciated by consumers. In more detail, browning was prevented when stalks were stored at 4 °C with CA characterized by 5 kPa O_2 and at least 5 kPa CO_2, even though at CA with 5 kPa O_2 and 25 kPa CO_2 little discolouration of internal petioles was noticeable. The authors also found that CA reduced respiration rate by 30%, inhibited stalk elongation, controlled decay and slightly reduced pithiness.

The beneficial effects of CA on fresh cut vegetables are confirmed by other studies. According to Darezzo *et al*., (2003), when Iceberg lettuce cv Lorca was stored at 5 °C for 15 days, CA treatments 3% O_2 + 10% CO_2 and 3% O_2 + 12% CO_2 resulted in retarding browning on midrib tissues without affecting sensory characteristics and overall appearance. CA systems maintain green colour because they lower oxygen levels. Consequently, PPOs have less substrate for the browning reaction to occur.

2.6.4.3 Packaging

The most widespread type of packaging used on fresh products after harvest is the modified atmosphere packaging (MAP). It is used for slowing down quality loss and maintaining shelf life (Kader *et al*., 1989). The MAP preservation principle is simple: the air surrounding the food in the package changes in its composition to prolong the freshly state of products. The gases present in the MAP are N_2, O_2 and CO_2 and their concentration vary depending on product type, temperature and packaging material. Gòmez and Artés (2005) showed that when cv. Trinova celery stalks were packed in polypropylene (PP) and low-density polyethylene (LDPE) and stored at 4 °C for 15 days, significantly reduced browning on cut ends, the development of pithiness and microbial growth without decreasing sensory quality. The reason for the overall quality preservation is attributable to the high CO_2 levels reached within the package. By contrast, in another study of Rizzo and Muratore (2009), cv. D'Elne celery stalks

packed in microperforated polypropylene (MP) and in polyolefin with an antifogging additive (AF) found to lose colour intensity during storage at 4 °C for 35 days. However, colour measurements were performed directly on stick surfaces and not at cut ends.

2.6.5 Physiological factors

2.6.5.1 Ethylene

2.6.5.1.1 Definition and functions

Ethylene (C_2H_4) (**Figure 2.16**) is a gaseous plant hormone, having low molecular weight, which is associated with many aspects of plant growth and development as well as many responses to several stresses in higher plants. For instance, it has been demonstrated that environmental stresses like drought, mechanical damage, very high temperatures, heavy metals and pathogen attacks can increase ethylene biosynthesis (El Beltagy and Hall, 1974; Goeschl *et al*., 1966). Ethylene exerts its physiological effects at nanomolar concentrations (Bleecker and Kende, 2000) and it is responsible for fruit ripening, chlorophyll loss, lateral cell expansion, abscission of leaves, epinasty, somatic embryogenesis and stem shortening (Blankenship, 2000). Production of the plant hormone is stimulated by auxins and inhibited by red light (Burg, 1973).

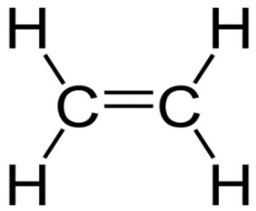


Figure 2.16 - Chemical structure of ethylene

2.6.5.1.2 Biosynthesis

The precursor of ethylene is the aminoacid L-methionine, which is continuously renewed in the Yang cycle (**Figure 2.17**). L-methionine is activated with the expense of an ATP molecule to form S-adenosylmethionine through the enzyme S-adenosylmethionine synthase. The next step is the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), which is a non-protein amino acid. This reaction is catalyzed by ACC synthase, which is a pyridoxal phosphate-dependent enzyme. ACC synthase is the

key enzyme in the regulation of ethylene biosynthesis and evidence suggests that it is encoded in a medium-size multigene family (Johnson and Ecker, 1998). ACC synthase also produces 5'methylthioadenosine (MTA) which is subsequently converted into methionine. Through this pathway, methionine is continuously recycled. Finally, ACC is oxidized to form ethylene, CO_2 and cyanide through the activity of the enzyme ACC oxidase. Cyanide is a toxic byproduct and is then removed by β-cyanoalanine synthase to prevent tissue toxicity.

The expression of ACC synthase genes is regulated by various signals. Previous literature suggests that in several plant species both positive and negative feedback regulation is present (Kende, 1993; Barry *et al*., 2000).

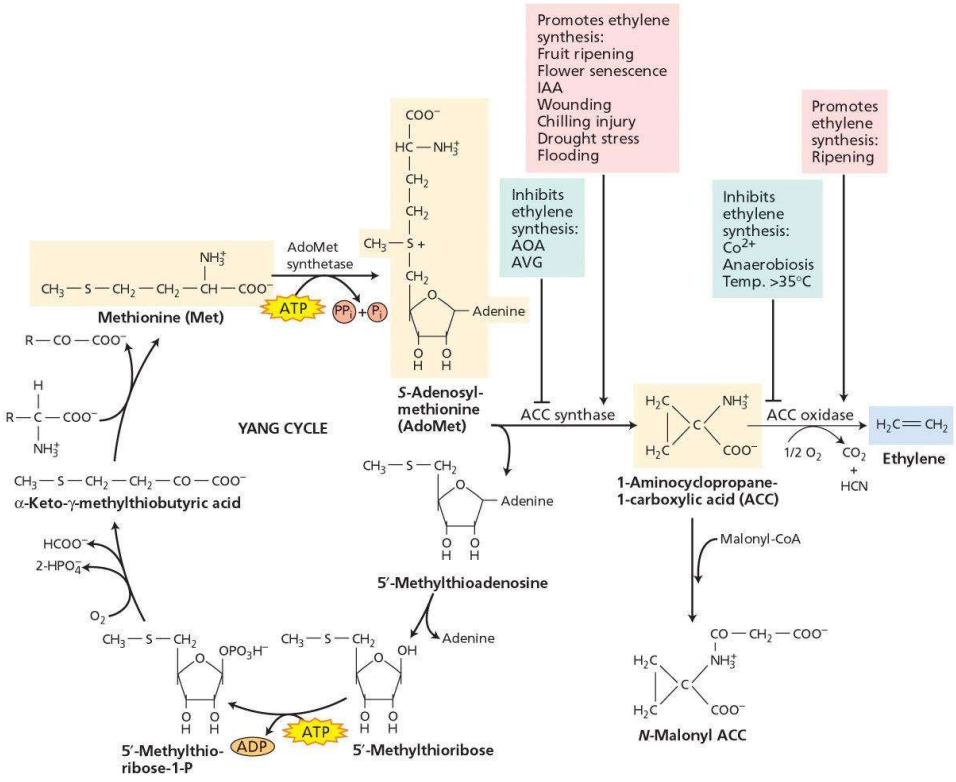


Figure 2.17 - Ethylene biosynthesis in plants (Wang *et al*., 2002)

2.6.5.1.3 Perception

In all plants, the ethylene-signaling pathway is activated by ethylene binding to specific receptors. These receptors consist of a multimember family constituted by five components, which in *Arabidopsis* were found to be ETR1, ETR2, ERS1, ERS2 and

EIN4 (**Figure 2.18**). These components regulate the pathway negatively, meaning that they deactivate the ethylene response when the hormone is absent (Hua and Meyerowitz, 1998). The ethylene receptors are mainly localized in the endoplasmic reticulum (ER) membrane. Considering that ethylene is capable of diffusing both in lipid and acqueous cell environments, it can interact with other compounds and other pathways effectively (Ju and Chang, 2012).

The basic structure of all the ethylene receptors consists of an N-terminal transmembrane domain in which the hormone is bound, a GAF domain involved in interactions between receptor types, and a C-terminal domain which serve to interact with the components of the pathway.

The ethylene receptor modular unit is a homodimer. More complex associations between homodimers and GAF domains can occur, allowing different receptor complexes to form in different plant tissues. This explains the different ethylene sensitivity existing among tissues of the same plant and among different plant species (Gao *et al*., 2008; Chen *et al*., 2010). Ethylene can bind with the receptor thanks to the copper ion, which is transported by RAN1 (Hirayama *et al*., 1999). RTE1 regulates ethylene response negatively by converting ETR1 from inactive to active signaling form (Resnick *et al*., 2008). RTE1 is localized at the ER, yet it has also been found on the Golgi apparatus membrane (Dong *et al*., 2008). When ethylene is absent, receptors activate CTR1, which is another negative regulator. It is a homodimer that is located at the ER membrane (Zhong *et al*., 2008). EIN2 plays a key role in the signaling cascade. It is a protein constituted of an N-terminal hydrophobic region and C-terminal hydrophilic one (Alonso *et al*., 1999). It is located on the ER membrane and its ethylene-dependent accumulation stabilizes EIN3, which is short-lived protein working as positive downstream component of the pathway (An *et al*., 2010).

When ethylene is not detected, the ethylene receptors activate the CTR1 protein kinase, which suppresses EIN2 function. Then, the transcription factors EIN3/EIL1 are degraded in the cell nucleus. On the other hand, when ethylene is detected, the ethylene receptors do not activate CTR1 protein kinase anymore, resulting in the proteolytic release of EIN2-C-END, which inhibits protein translation of the F-box proteins EBF1 and EBF2. Subsequently, EIN3 and EIL1 are stabilized and, therefore, can regulate an

extensive transcriptional cascade involving the ERF1 transcription factor (Chang, 2016).

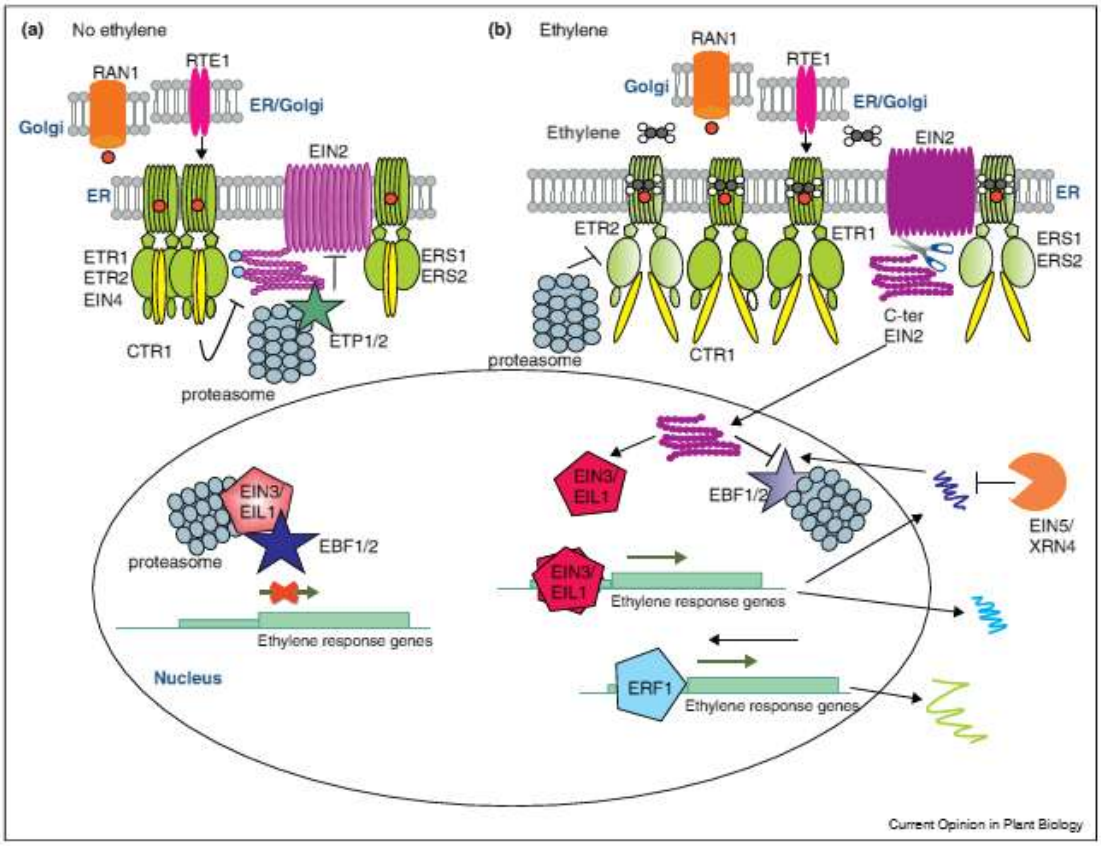


Figure 2.18 - Ethylene perception and signaling pathway (Merchante *et al*., 2013)

2.6.5.1.4 Effects of ethylene on fresh produce

Fruits and vegetables can be classified depending on their response to ethylene. Climacteric species produce ethylene as they ripen, and the harvested produce is capable of ripening during the postharvest period. These commodities tend to become sweeter and softer after harvest. Non-climacteric plants, such as leafy vegetables, do not continue to ripen after harvest; they will soften and rot, but this is due to moisture loss, decay, and tissue deterioration (Silva, 2008). Some examples of climacteric products include apple, banana, pear, apricot, mango, kiwi and tomato. On the other hand, examples of non-climacteric products include orange, cherry, watermelon, grape, pineapple and lemon (Sudheer and Indira, 2007). In some cases, the distinction of commodities in these two classes is not clearly defined. Paul *et al*., (2012) reported that

some fruits like guava, melon, Japanese plum, Asian pear and pepper had both climacteric and non-climacteric behavior depending on the cultivar or genotype.

Ethylene was found to be correlated with the stimulation of phenolic compounds related to lignification and browning in lettuce. For instance, Ke and Saltveit (1988) demonstrated that the phytohormone induced PAL activity, lignin production and the synthesis of soluble phenolic compounds related to browning disorder in iceberg lettuce. Also, Couture and Saltveit (1997) found that treating the same crop in continuous ethylene at 2 or 5 μL L^{-1} at 2.5 or 5 °C for 1 to 4 days significantly increased browning intensity, PAL and PPO activity after 6 to 10 days of storage. Regarding celery, there are currently no studies investigating the correlation between ethylene and postharvest browning. Furthermore, the effect that the plant hormone has on browning-related enzymes and molecules still remain unknown. It has only been reported that Ethephon application to celery cv. Florida 683 plants in protected environment induced parenchyma breakdown leading to pithiness, especially at the basal part of the stalks (Pressman *et al*., 1984).

Celery is classified as a non-climacteric crop and it produces very low quantities of ethylene. In more detail, less than 0.1 μL kg^{-1} h^{-1} at 20 °C (Kader, 2002). In a study conducted by Gòmez and Artés (2004) celery cv. Trinova produced less than 0.05 μL kg^{-1} h^{-1} when stored in air for the first 3 days of storage in a cold room at 4 °C.

2.6.5.2 1-Methylcyclopropene

1-Methylcyclopropene (1-MCP) (**Figure 2.19**) is a gaseous, odourless ethylene inhibitor having chemical formula C_4H_6. It is believed to express its inhibitory action by binding ethylene receptors irreversibly so that ethylene cannot consequently elicit its physiological action. Blankenship and Dole (2003) reported that 1-MCP affinity for the ethylene receptor is 10 times greater than that of ethylene. 1-MCP exerts its action at much lower concentrations compared to ethylene.

Figure 2.19 - Chemical structure of 1-methylcyclopropene

The effect of 1-MCP on plants depends on several factors like temperature, concentrations, developmental stage, treatment duration and temporal distance between harvest and treatment. In most studies 1-MCP is applied at temperature ranging from 20 to 25 °C, but can be used at lower temperatures in some commodities.

1-MCP has been used to improve postharvest quality of fresh vegetables. For instance, it was found to delay yellowing in pak choy (*Brassica rapa* var, *chinensis*) (Able e*t al*., 2002) and inhibit isocoumarin synthesis in carrot (*Daucus carota* L.) (Fan and Mattheis, 2000). A study carried out by Saltveit (2004) showed that exposing heads or leaves of iceberg lettuce to 1-MCP significantly decreased phenolic compounds and subsequent tissue browning stimulated by previous exogenous ethylene treatment. Regarding celery, current literature does not report studies about the effect that 1-MCP has on the postharvest quality of this crop. More research is therefore needed to investigate this aspect.

3. GENERAL MATERIALS AND METHODS

3.1 Sample preparation

When celery samples were consigned by G's Fresh Ltd (**Figure 3.1**), they were washed under running tap water to remove soil and subsequently dried with a soft paper towel. Samples were then labelled, placed on a plastic tray and photographed in different positions (**Figure 3.2**) to be ready for the assessments.

Figure 3.1 - Celery samples from G's Fresh Ltd

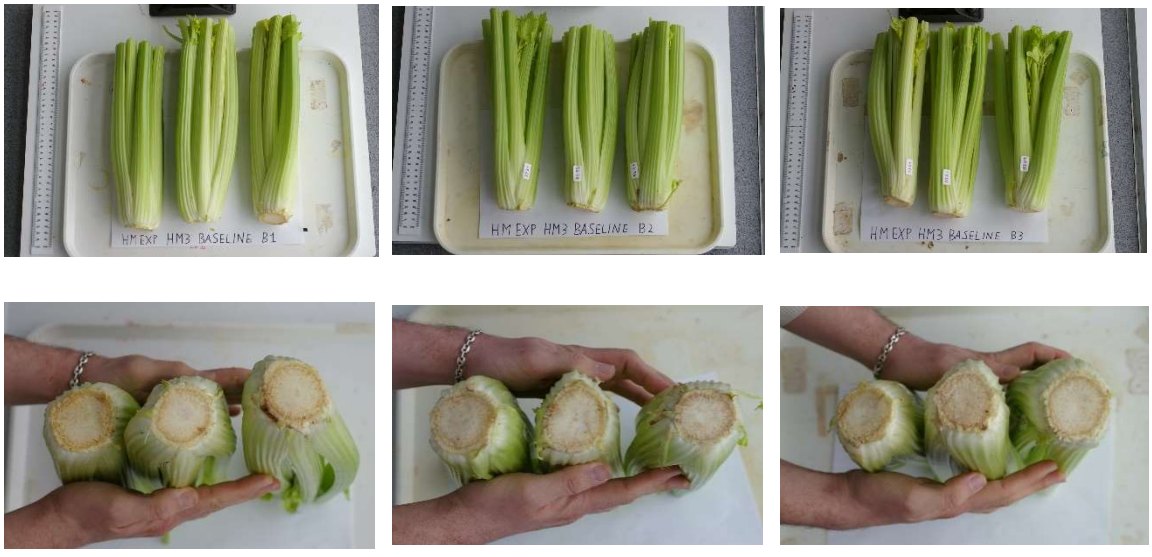


Figure 3.2 - Photos of celery samples taken in different positions before the visual assessments.

3.2 Visual assessments

3.2.1 Subjective colour

Samples were visually assessed for browning level at both cut petiole and butt ends of celery. A 1 to 4 visual scale was used to assign the proper score (**Figure 3.3** and **Figure 3.4**). Photos of celery samples were taken at both ends to be used as references. Visual scales used to assign browning were done by the author.

Level 1 Level 2

Level 3

Level 4

Figure 3.3 - Comparative visual scale for assessing browning at cut petiole ends of celery (1= absent, 2= slight, 3= evident, 4= severe) (photos taken by the author).

Level 1

Level 2

Level 3

Level 4

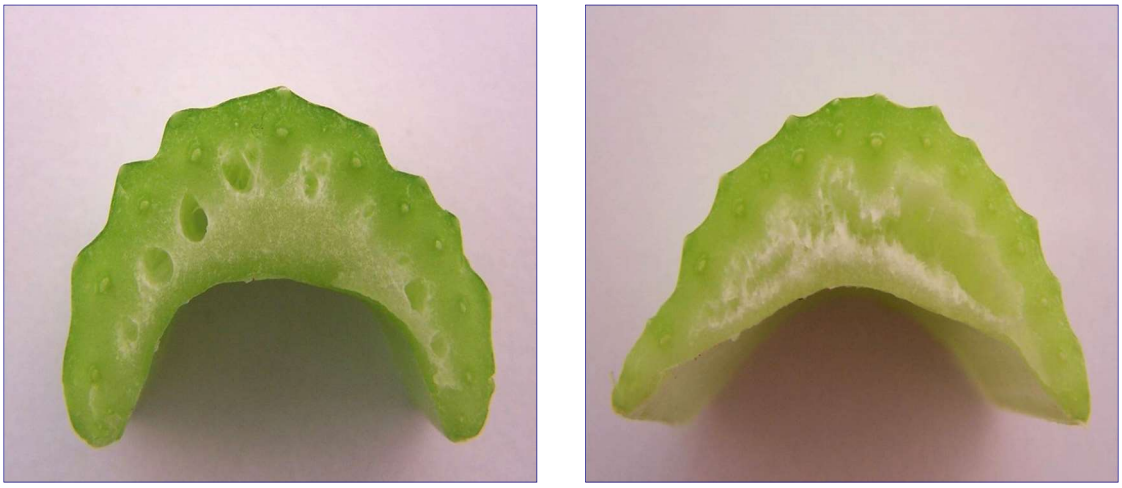
Figure 3.4 - Comparative visual scale for assessing browning at butt ends of celery (1= absent, 2= slight, 3= evident, 4= severe) (photos taken by the author).

3.2.2 Pithiness

Samples were visually assessed for pithiness at both cut petiole and butt ends of celery. A 1 to 4 visual scale was used to assign the proper score (**Figure 3.5**). Photos of celery samples were taken at both cut ends to be used as references. Visual scales used to assign pithiness score were provided by G'S Fresh Ltd. Prior to do the visual evaluations, samples were cut 5 mm from the apical cut petiole ends and 5 cm from the butt ends (called "spoon ends").

Level 1

Level 2

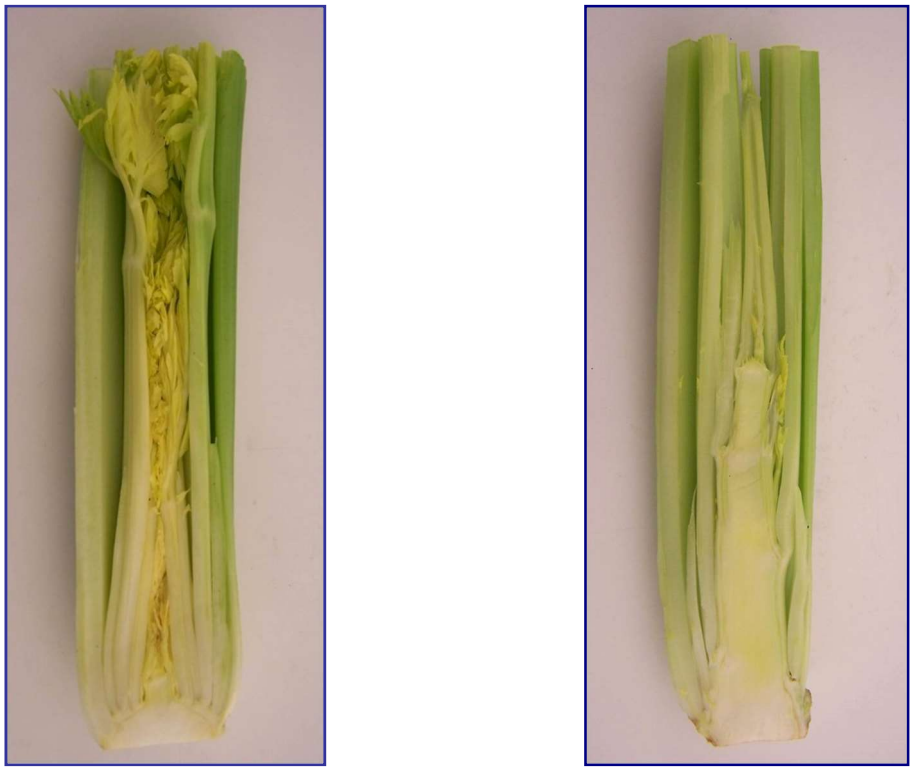


Level 3 Level 4

Figure 3.5 - Comparative visual scale for assessing pithiness at cut petiole and butt ends of celery (1= absent, 2= slight, 3= evident, 4= severe) (photos provided by G's Fresh Ltd).

3.2.3 Bolting

Bolting assessment was carried out by cutting samples longitudinally and looking at the basal part to observe the core length. A 1 to 2 visual scale, shown in **Figure 3.6** was used to assign the proper bolting score. Visual scales used to assign bolting score were provided by G'S Fresh Ltd.



Level 1 Level 2

Figure 3.6 - Comparative visual scale for assessing bolting level to celery samples (1= no bolting, 2= bolted plant) (photos provided by G's Fresh Ltd).

3.3 Physiological attributes

3.3.1 Respiration rate

Respiration rate of celery heads was measured as reported by Collings *et al*., (2013) with slight modifications. A Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA) (**Figure 3.7**) was used. Samples were incubated in air-tight plastic boxes (**Figure 3.8**) in continuous air flushed by an 80 HP pump (Hiblow, Techno Takatsuki Ltd., Philippines) to avoid modified atmosphere to 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 real time for 10 minutes over 3 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 at least a couple of minutes to be stabilized in the incubating boxes, measurements were taken at the second 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 temperature of 20 °C.

Figure 3.7 - Sample Respirometry System (photo taken by the author).

Figure 3.8 - Air tight plastic boxes connected with the Sable Respirometry System each containing one celery sample (photo taken by the author).

3.3.2 Objective colour

Objective colour was measured with a CR-400 Minolta colorimeter (**Figure 3.9**) and DP-400 data processor (Minolta Co. Ltd, Japan) 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 a surface the more unique and continuous as possible (**Figure 3.10**) to be easily processed by the colorimeter. Data were expressed in terms of chroma (C*), hue angle (H°) and lightness (L*) which were calculated automatically. Calibration of the instrument was done using a Minolta standard white tile CR-400.

Figure 3.9 - CR-400 Minolta colorimeter used for objective colour measurements (photo taken by the author).

Figure 3.10 - Cut petiole ends clustering for objective colour measurements (photo taken by the author).

3.4 Biochemical analysis

3.4.1 Sample preparation for biochemical analysis

After performing visual assessments 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 3.11**). Thereafter, cut portions were separately sealed in plastic bags and immediately snap-frozen in liquid nitrogen to stop biochemical reactions, weighted to obtain fresh weight (FW) and 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 3.12**) equipped with a vacuum pump (Vacuumbrand® RZ 2, Wertheim, Germany) for 7 days at -50 °C. After lyophilisation, samples were re-weighted to obtain dry weight (DW). The dry matter content was subsequently calculated (mg g^{-1} DW). Lyophilized samples were powdered with a mechanical pestle and placed into -40 °C freezers until usage for biochemical extractions.

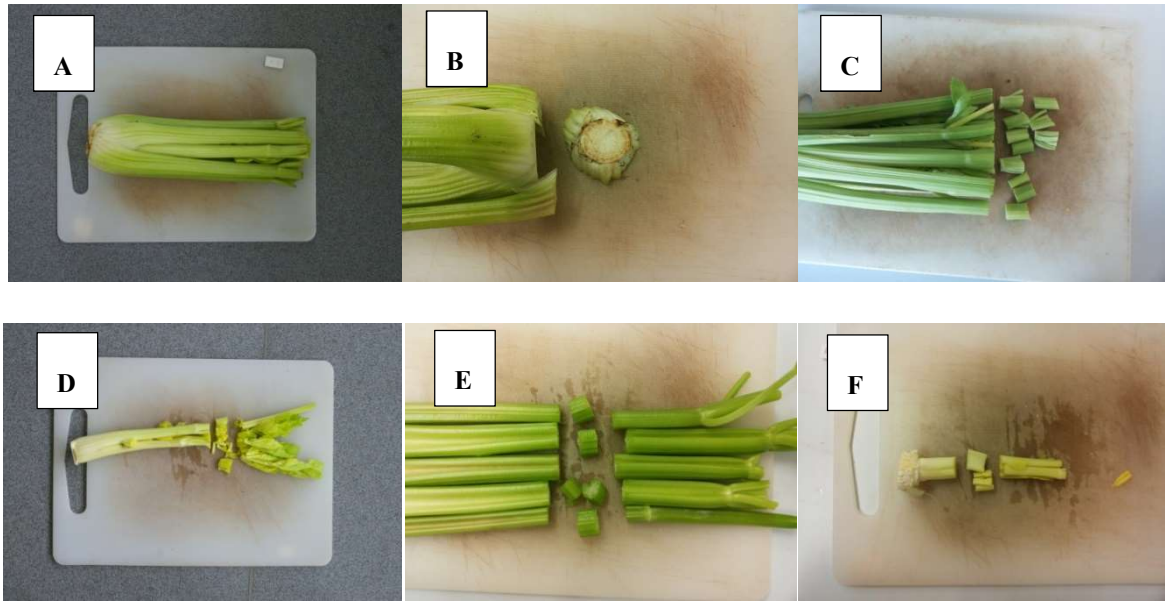


Figure 3.11 - Celery excised in different portions to snap-freeze in liquid nitrogen: celery head (A); butt end (B); apical outer petiole end (C); middle outer petiole end (D); apical inner petiole (E); middle inner petiole (F) (photos taken by the author).

Figure 3.12 - Freeze-drier used to lyofilize fresh celery samples (Coolsafe 55-9 Scanvac) (photo taken by the author).

3.4.2 Extraction and quantification of phenolic compounds

3.4.2.1 Extraction of phenolic compounds in celery

Samples were initially prepared as described in section **3.4.1**. The phenolic compounds were extracted from 50 mg of the lyophilized powder with 1 mL of a 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 secs every 15 min. 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.

3.4.2.2 Detection and quantification of phenolic compounds in celery using HPLC

Literature about detecting and identifying individual phenolic compounds in celery is scarce. In the majority of studies, generic methods are used (i.e. Folin-Ciocalteu reagent). Only Yao *et al.*, (2010) identified six different phenolic compounds (three simple phenolic acids and three flavonoids) in nine celery varieties using an HPLC system. HPLC methodology is key as it is efficient in separating and quantifying specific phenolic compounds, given the importance to examine their specific bioactivity independently.

An HPLC method from Giné Bordonaba and Terry (2008) was adopted to detect and quantify phenolic compounds in celery, with slight modifications. Identification of phenolic compounds was performed using an Agilent 1200 series HPLC (High Performance Liquid Chromatography) system (Agilent Technologies, West Berkshire, UK) (**Figure 3.13**) 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 stationery phase was an Agilent Zorbax Eclipse XDB-C18 column (4.6 mm x 150 mm, 5 µm particles size) (part no. 993967-902) fitted with a C18 Opti-guard column (part no. 820950-938). The mobile phase was formed by 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 analyse and present data. **Figure 3.14** and **Figure 3.15** show the phenolic compounds standards described by Yao *et al*., (2010) and detected using the above described method. Chlorogenic acid has been additionally included in the standards mixture as it is thought to be related to postharvest browning in several vegetables (Dong *et al*., 2016).

Figure 3.13 - HPLC-DAD system used to identify and quantify phenolic compounds (photo taken by the author).

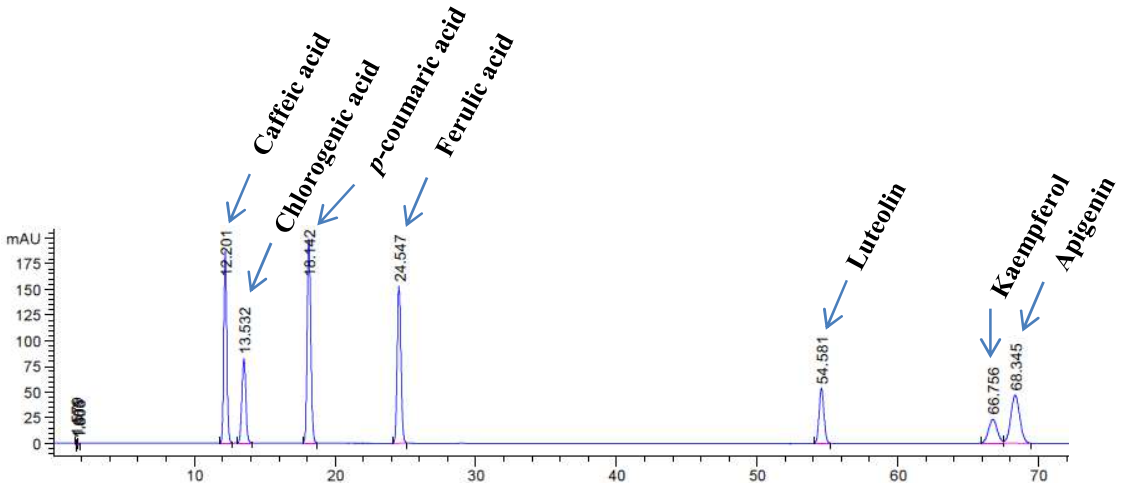


Figure 3.14 - Chromatogram showing the retention times (minutes) of the all the phenolic compounds standards in celery tissues at 320 nm wavelength DAD signal according to Yao *et al*., (2010) + chlorogenic acid.

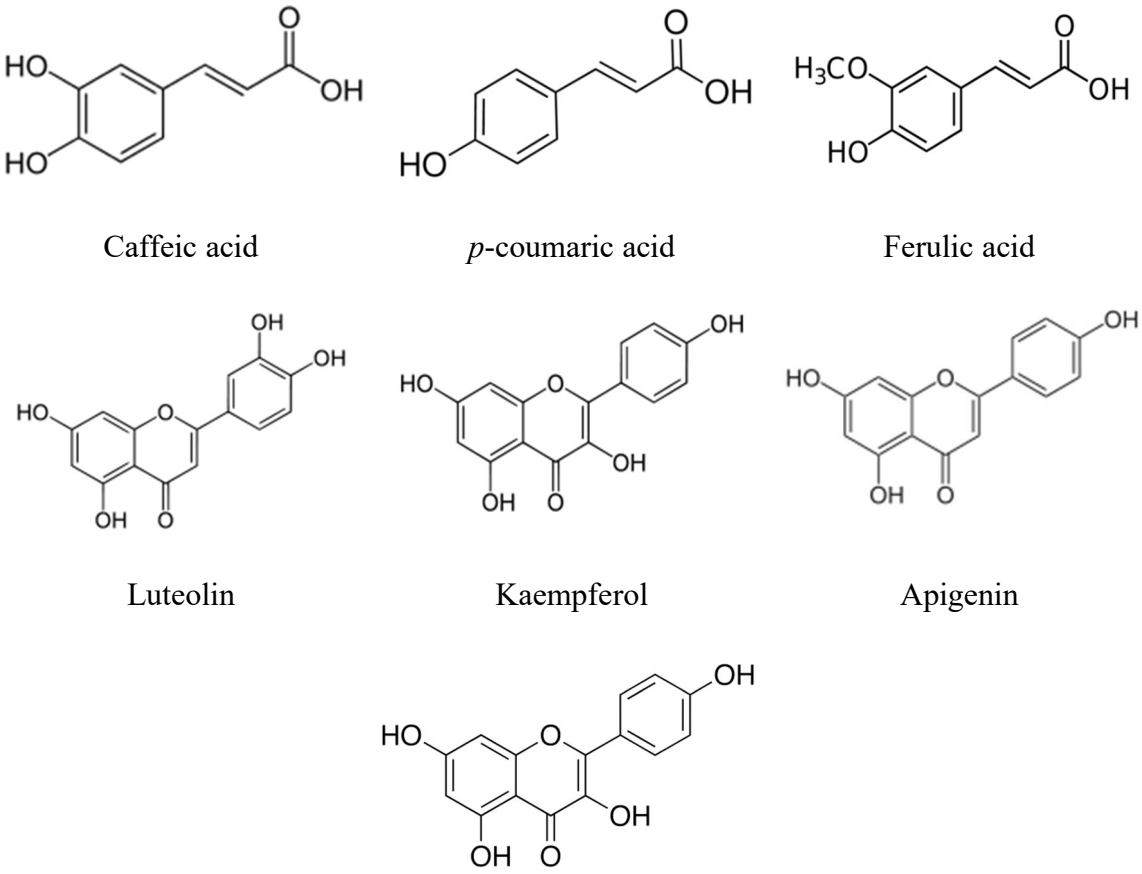


Figure 3.15 - Chemical structures of the phenolic compounds isolated by Yao *et al*., (2010) + chlorogenic acid.

3.4.3 Extraction, detection and quantification of non-structural carbohydrates

Non-structural carbohydrates 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 secs. 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 3.16**). The stationery phase was constituted by a Prevail carbohydrate Es (250 mm x 4.6 mm, 5 µm diametre particles). The mobile phase was formed by 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 compounds were identified and subsequently quantified by comparison with the respective calibration standards. Standards used were fructose, mannitol, glucose and sucrose.

Figure 3.16 - HPLC-ELSD system used to identify and quantify non-structural carbohydrates (photo taken by the author).

3.4.4 Extraction and quantification of plant growth regulators (PGRs)

Extraction of plant growth regulators (PGRs) was done as described by Ordaz-Ortiz *et al*., (2015) with slight modifications. Lyophilized celery tissue was weighed (100 mg) in 15 mL falcon tubes and mixed with 6 mL 75:20:5 methanol:water:formic acid solvent (v/v/v). Then, 30 µL of 400 ng mL^{-1} internal standard mixture solution (deuterated compounds) was added to the extraction mixture. Samples were vortexed for 2 mins and

shaken at 4 °C for 1 h, then centrifuged for 15 mins at 4500 rpm. Supernatant was removed and evaporated in a ScanVac freeze-dried overnight at -110 °C. Samples were then reconstituted with 400 µL of 10:99.9:0.1 acetonitrile:water:formic acid solvent, vortexed for 30 secs, centrifuged for 10 mins at 4500 rpm and filtered using 0.2 µm PTFE filters (Jaytee Biosciences Ltd, Kent, UK). The filtrate was put into deactivated silanized amber vials. All operations were done in dark environment to prevent compound inactivation by light. Detection and quantification of PGRs was performed using Ultra Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QToF) (Agilent Technologies) (**Figure 3.17**). Stationery phase was constituted by a thermostated Zorbax Rapid Resolution High Definition Eclypse Plus C18 column (2.1 x 50 mm, 1.8 µm particle size). The mobile phase was formed by two solutions: water + 0.1% formic acid (solution A) and acetonitrile + 0.1% formic acid (solution B). The program involved a linear increase/decrease of the following solutions: 0 min, 4% B; 1.5 min, 12.6% B; 7.0 min, 26% B; 10 min 40% B and hold for 0.5 min; 10.6 min 100% B and hold for 1 min. Lastly, the column was re-equilibrated for 1.35 min (4% B) for a total run time of 13 mins. The injection volume was 5 µL. The flow rate was 0.6 mL min^{-1}. Samples concentration was compared with calibration standard curves prepared using seven calibrations solution levels (10, 25, 50, 75, 100, 150, 300 ng mL^{-1}) of each deuterated standard compound. Deuterated standards compounds used were: abscisic acid, phaseic acid, dihydrophaseic acid and *trans*-zeatin riboside.

Figure 3.17 - UPLC-QToF system used to identify and quantify phytohormones (photo taken by the author).

3.5 Dry matter content

Dry matter content was measured with the following formula:

(Dry weight / Fresh weight) *100

Dry and fresh weight of the formula above was obtained summing all cut celery portions weights prepared for biochemical analysis (section **3.4.1**).

3.6 Postharvest treatments

3.6.1 Ethylene treatment

Samples were placed in water-sealed, 264 L polypropylene boxes covered with polypropylene lids and flushed with continuous exogenous ethylene at 10 μL L^{-1} concentration. The gas flow was controlled from a 50 L cylinder of 5000 μL L^{-1} certified standard concentration ethylene in nitrogen (BOC, Surrey, UK) and was reduced to 10 μL L^{-1} by mixing with pure flowing air and by using a gas regulating manifold (HNL Engineering Ltd, Stockton-on-Tees, UK). Pure air was flowing at 2mL*h^{-1} from a Controlled Atmosphere (CA) System. Flexible nylon pipes (6mm O.D., 4mm I.D.) were used to connect the boxes with the ethylene-flowing cylinder. In order to check if the ethylene concentration was stable in the boxes, periodical gas samples were withdrawn from each box as previously described by Terry *et al*., (2007). A 60 mL plastic syringe was used to withdraw the gas from the boxes. Thereafter, 20 mL of ethylene were injected into a Gas Chromatograph (GC) (Model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) (**Figure 3.18**) equipped with Flame Ionisation Detector (FID) and Porapak column (2m length, 6mm O.D., 4mm I.D) to measure the gas concentration. Calibration of the instrument was performed with a certified standard 10.3 μL L^{-1} ethylene cylinder). The excess gas was removed through the storage room ventilation system and directed outside the building.

Figure 3.18 - Gas Chromatograph (GC) equipped with Flame Ionisation Detector (FID) (photo taken by the author).

3.6.2 1-MCP treatment

1-MCP treatment was applied as reported by Downes *et al*., (2010) with slight modifications. A sample of 1.47 g 1-MCP powder (SmartFresh®, Rohm and Haas, product code: AFxRD-0014) were put in a 50 mL conical flask and subsequently sealed with Nescofilm (Bando Chemical Industries Ltd, Kobe, Japan). Then, 16.3 mL of warm water (50 °C) was mixed with the powder and shaken to release 1 µL L^{-1} concentration in the boxes. Water-sealed 264 L polypropylene chambers were utilized for the treatment. With the aim of circulating the gas, 8x8 cm electric fans (Nidec Beta SL, Nidec, Japan) were installed in the chambers. In order to check 1-MCP concentration, periodical gas samples were withdrawn from each treatment box by using a 60 mL syringe. 20 mL of gas were injected in the GC-FID with a stainless Chromosorb coloumn (6mm O.D., 4mm I.D.). Calibration of the instrument was performed with a certified standard 0.9 µL L^{-1} isobutylene cylinder (BOC, Surrey, UK).

3.7 General statistical analysis

Data was first subjected to normality test and plotted for residuals to verify assumptions for the Analysis of Variance (ANOVA). The STATISTICA software for Windows, Version 13.2 (StatSoft, Inc. UK), was used to generate the ANOVA tables and to identify statistically significant trends. The means between treatments were separated

with the Least Significant Difference (LSD), which they were stated to be significant at 95% confidence level (significance level was P= 0.05). ANOVA was used to analyse subjective colour, objective colour, pithiness bolting, respiration rate, dry matter content, phenolic compounds, non-structural carbohydrates and plant growth regulators. The ANOVA tables show the main effects of the treatments, plant tissues, storage time and the interactions between these factors. All the subjective assessments (subjective colour, pithiness and bolting) were collected as discrete, non-continuous data. This reflects in some limitations in the ANOVA analysis (i.e. little indication of sources of variation; nuances of the data tend to diminish in the size; more data points are required to make an equivalent inference of continuous data). To mitigate these problems, ANOVA analysis was done on the means of all ordinal subjective assessment scores, providing a higher number of data points onto make an equivalent inference of continuous data.

4. EFFECT OF CROP MATURITY STAGE ON THE VISUAL QUALITY, PHYSIOLOGY AND BIOCHEMISTRY OF FRESH-CUT CELERY

4.1 Introduction

Current literature reports on few studies investigating browning in fresh-cut celery. Almost the totality of these studies converged on the effects that postharvest factors have on postharvest discolouration and its relative biochemistry. For instance, Loaiza-Velarde *et al*., (2003) showed that the application of heat shock treatments significantly reduced browning potential and the wound-induced PAL activity in excised petioles. Gómes and Artés (2004) reported that storage in controlled atmosphere (CA) helped 25-cm celery stalks stored for 35 days at 4 °C to avoid cut butt end from postharvest discolouration without producing any undesirable off odours and off flavours. Another study conducted by Zhan *et al*., (2013) demonstrated that continuous light exposure (2000 lux) during storage significantly reduced the activity of PPO, POD and browning index in celery.

The studies above clearly indicate that the incidence of cut-end browning in celery can be reduced postharvest. On the other hand, no research has been carried out on the role of pre-harvest factors, which might determine susceptibility to postharvest browning. There is only one study conducted by Guerra *et al*., (2010) on the self-whitening cv. Golden Clause which showed that browning potential and total quinone content were significantly higher at late harvest maturity. Yet, more studies have been carried out on lettuce, which is a more important crop compared to celery and one which also suffers from cut-end browning. Chutichudet *et al*., 2011 reported that leaves of lettuce cv. Grand Rapids taken few minutes after cutting showed to have less bright green colour and higher PPO activity when harvested at late maturity stages. Similar results have been reported by Kang *et al*., (2008) who showed that browning rate of head lettuce was significantly higher in the over-mature heads compared to immature or mature.

4.2 Aim

The aim of this study was to investigate the effects of crop developmental stage on the visual quality, physiology and biochemistry of fresh-cut celery after harvest.

4.3 Specific objectives

- To determine the effects that the plant developmental stage has on cut-browning, pithiness, bolting and respiration rate.
- To investigate spatial and temporal variations of phenolic compounds in celery affected by different degrees of browning.

4.4 Hypothesis

The experiment was carried out with the following hypotheses:

4.4.1 Null hypothesis

Horticultural maturity has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

4.4.2 Alternate hypothesis

Horticultural maturity has no significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

4.5 Plant material and growing conditions

The experiment was conducted in collaboration with G's Fresh Ltd who provided all plant material. Three trials were conducted in total, two in Spain (Cartagena, Murcia) and one in UK (Stretham, Cambridgeshire). The first trial was conducted in the early Spanish spring season, the second in the late Spanish spring season and the third in the late UK season. The early Spanish season trial was conducted from the 10th March 2014 to the 7th April 2014 and celery plants were harvested every week from Puntal-Campo de Golf farm (coordinates: 37°36'42.55''N; 0°47'38.34''W) (**Figure 4.1**). Planting was

done in a clay-loam soil (clay: 33.8%, silt: 22.3% and sand: 43.9%), (EcoSur Laboratories, Murcia, Spain) with fine texture, pH 8.34 at 20 °C (

Table 4.1). The trial in the late Spanish season was conducted from the 5th May to the 2nd of June 2014 and plants were harvested every week from Barranquillo farm (coordinates: 37°44'13.86''N; 0°58'39.08''W) (**Figure 4.2**). Planting was done in a loam soil (clay: 29.4%, silt: 25.2% and sand: 45.4%), (EcoSur Laboratories, Murcia, Spain) with medium texture and pH 8.5 at 20 °C (**Table 4.2**). Finally, the trial in the late UK season was carried out from the 11th of August 2014 to the 8th of September 2014, and the crop was grown on Fenland peaty soil of Dimmocks Cote farm fields (coordinates: 52°19'57.7"N, 0°15'58.3"E) (**Figure 4.3**). In the Spanish trials, plants were covered with fleece from planting date until 24th April 2014 and were fertigated. Harvest was done by hand using a lightweight field knife. Optimum harvest date was decided 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. Plants were then packed into bags and corrugated boxes directly in the field. Thereafter, they were vacuum-cooled at 3 °C as per standard commercial practice and dispatched to the Plant Science Laboratory (PSL), Cranfield University, Bedfordshire. Further details are reported in **Table 4.3**.

Figure 4.1 - GPS image of Puntal-Campo de Golf Farm (Cartagena, Spain) where early season celery plants were grown and harvested (photo provided by G's Fresh Ltd).

Table 4.1 - Physico-chemical characteristics of Puntal-Campo de golf farm soil (EcoSur Laboratories, Murcia, Spain).

Physical characteristics	
Texture (USDA)	Fine
Clay (< 0.002 mm)	33.8 %
Sand (2-0.05 mm)	43.9 %
Loam (0.05-0.002 mm)	22.3 %
Chemical characteristics	
Active limestone	6.4 g/100g
Total carbonates ($CaCO_3$)	11.4 g/100g
Oxidable organic carbon	0.6 g/100g
Chlorides (Cl)	0.53 meq/100g
Oxidable organic matter	1.0 g/100g
Total organic nitrogen (Kjeldahl)	0.07 g/100g
pH (at 20 °C)	8.34
Sulphates (SO_4)	0.5 meq/100g
Calcium (Ca)	11.01 meq/100g
Magnesium (Mg)	1.36 meq/100g
Potassium (K)	1.35 meq/100g
Sodium (Na)	0.82 meq/100g
Phosphorus (P)	24.7 mg/kg
Boron (B)	1.8 mg/kg
Copper (Cu)	1.2 mg/kg
Iron (Fe)	4.5 mg/kg
Manganese (Mn)	8.3 mg/kg
Molybdenum (Mo)	0.04 mg/kg

Zinc (Zn)	3.2 mg/kg

Figure 4.2 - GPS image of Barranquillo Farm (Cartagena, Spain) where late season celery plants were grown and harvested (photo provided by G's Fresh Ltd).

Table 4.2 - Physico-chemical characteristics of Barranquillo farm soil (EcoSur Laboratories, Murcia, Spain).

Physical characteristics	
Texture (USDA)	Medium
Clay (< 0.002 mm)	29.4 %
Sand (2-0.05 mm)	45.4 %
Loam (0.05-0.002 mm)	25.2 %
Chemical characteristics	
Active limestone	13.0 g/100g
Total carbonates ($CaCO_3$)	36.8 g/100g
Oxidable organic carbon	0.8 g/100g
Chlorides (Cl)	0.25 meq/100g
Oxidable organic matter	1.3 g/100g

Total organic nitrogen (Kjeldahl)	0.05 g/100g
pH (at 20 °C)	8.53
Sulphates (SO_4)	0.4 meq/100g
Calcium (Ca)	9.54 meq/100g
Magnesium (Mg)	2.92 meq/100g
Potassium (K)	1.06 meq/100g
Sodium (Na)	0.70meq/100g
Phosphorus (P)	53.9 mg/kg
Boron (B)	2.0 mg/kg
Copper (Cu)	0.8 mg/kg
Iron (Fe)	2.5 mg/kg
Manganese (Mn)	5.7 mg/kg
Molybdenum (Mo)	0.07 mg/kg
Zinc (Zn)	2.1 mg/kg

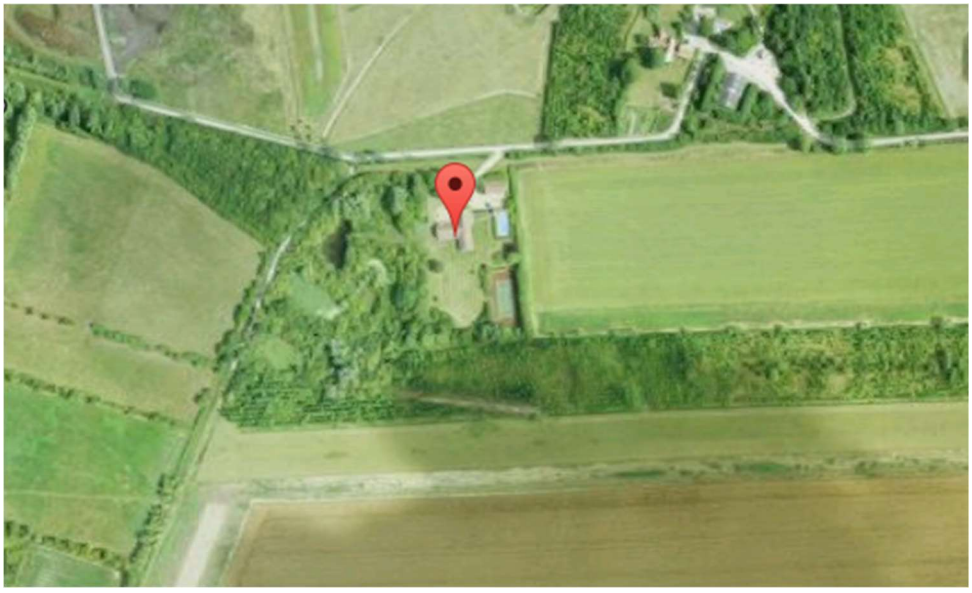


Figure 4.3 - GPS image of Dimmocks Cote Farm (Cambridgeshire, UK) where late UK season celery plants were grown and harvested.

Source: https://www.google.co.uk/maps/place/52°19'57.7"N+0°15'58.3"E.

Information on the physico-chemical characteristics of Dimmocks Cote Farm (Cambridgeshire, UK) soil are not reported in this Thesis as it was not possible for G's Fresh Ltd to provide relative data.

Table 4.3 - Pre-harvest information of celery grown in three different seasons.

Trial	Planting date	Optimum harvest date	cv. used	Transit time from field to PSL
Early Spanish season	6th November 2013	21st March 2014	Monterey	With fridge lorry for 3 days at 5 °C
Late Spanish season	18th February 2014	16th May 2014	Monterey	With fridge lorry for 3 days at 5 °C
Late UK season	19th May 2014	22nd August 2014	Victoria	With van at ambient temperature for 1 hour

Table 4.4 - Meteorological data of the celery trials carried out in three different growing seasons.

Trial	Location	Average temperature (°C)	Average relative humidity (%)	Total rainfall (mm)
Early Spanish season	Cartagena, Spain	12.5 ± 2.4	60.3 ± 13.2	41.8
Late Spanish	Cartagena,	16.3 ± 3.0	56.2 ± 11.0	6.0

season	Spain			
Late UK season	Stretham, UK	17.3 ± 2.8	69.8 ± 10.8	245.7

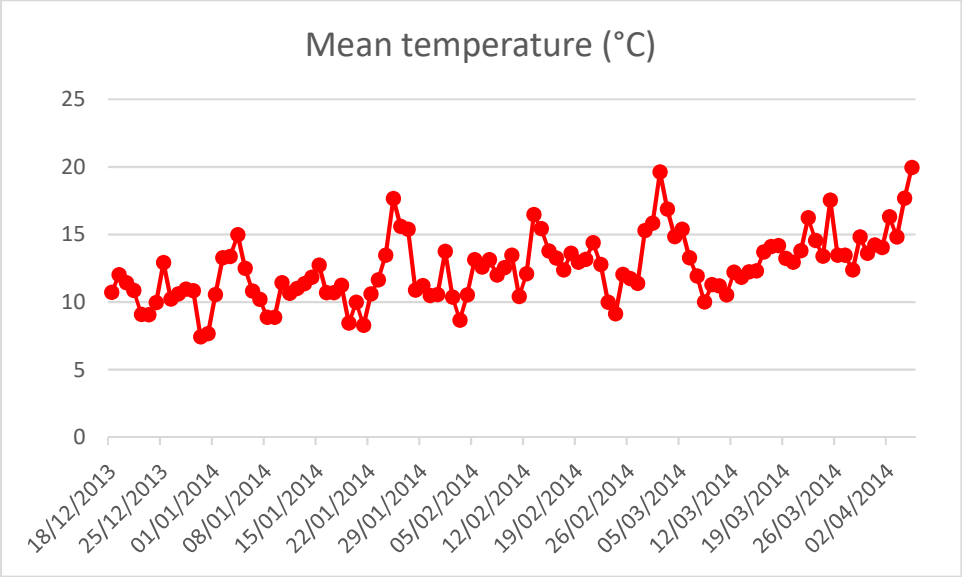


Figure 4.4 - Mean daily temperature of the field trial conducted in the Early Spanish season (data provided by G's Fresh Ltd).

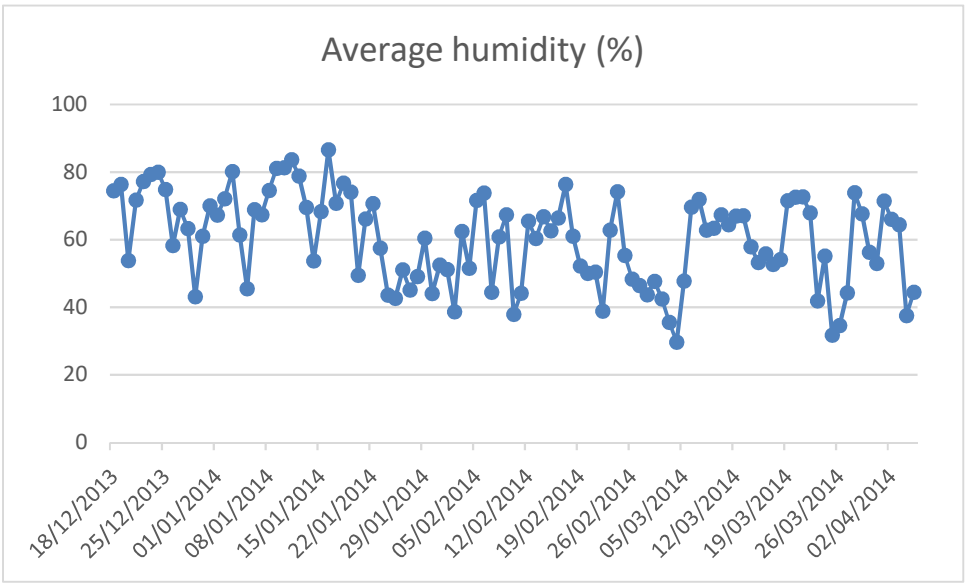


Figure 4.5 - Average daily relative humidity of the field trial conducted in the Early Spanish season (data provided by G's Fresh Ltd).

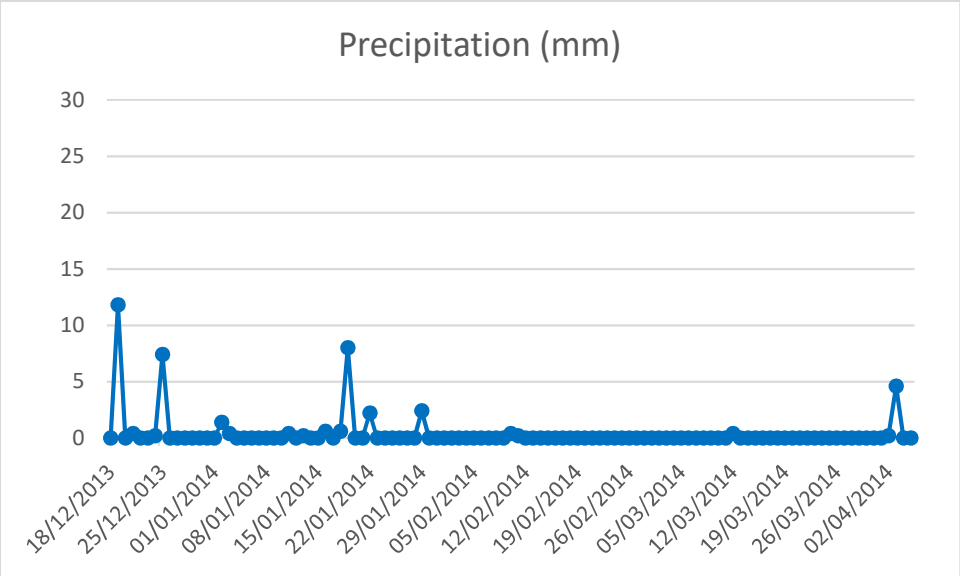


Figure 4.6 - Daily precipitation of the field trial conducted in the Early Spanish season (data provided by G's Fresh Ltd).

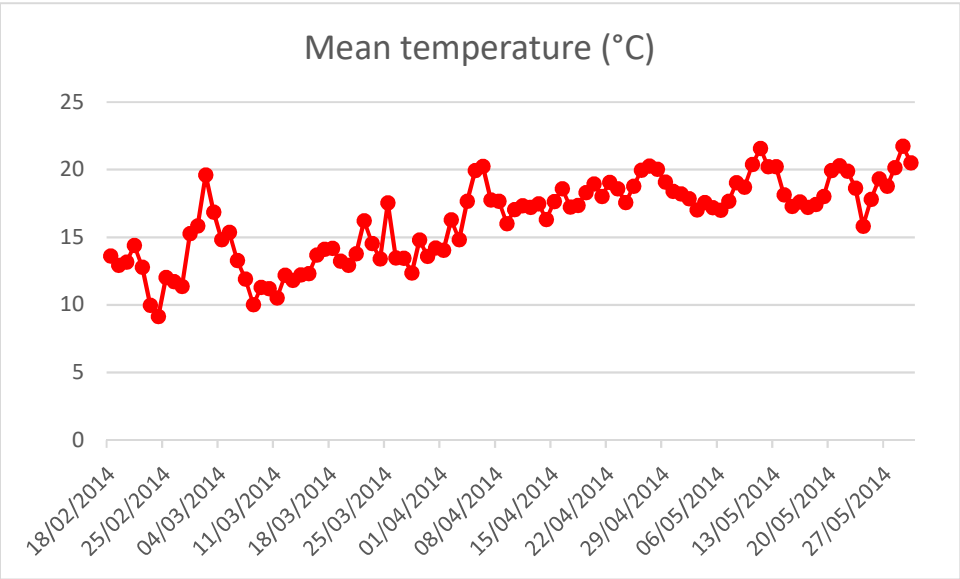


Figure 4.7 - Mean daily temperature of the field trial conducted in the Late Spanish season (data provided by G's Fresh Ltd).

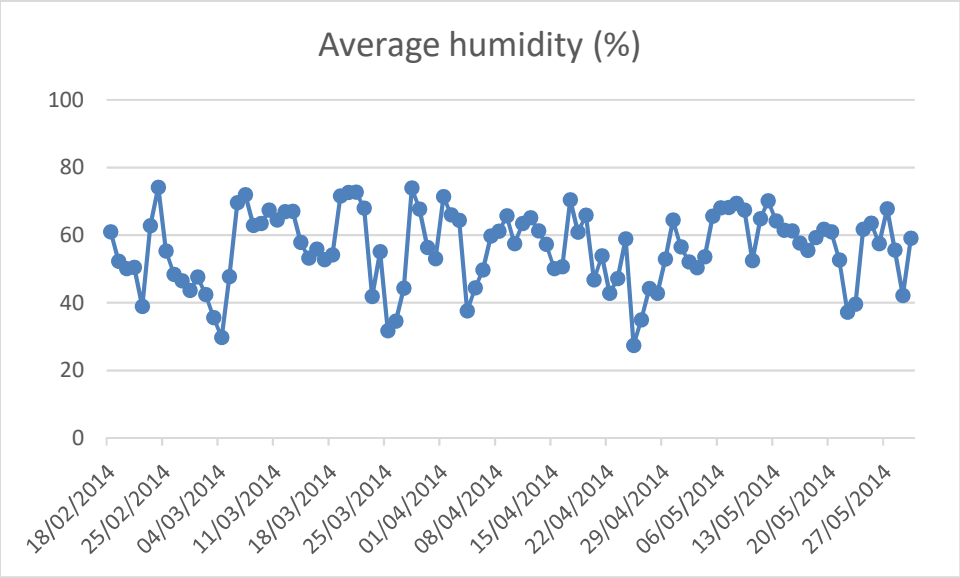


Figure 4.8 - Average daily relative humidity of the field trial conducted in the Late Spanish season (data provided by G's Fresh Ltd).

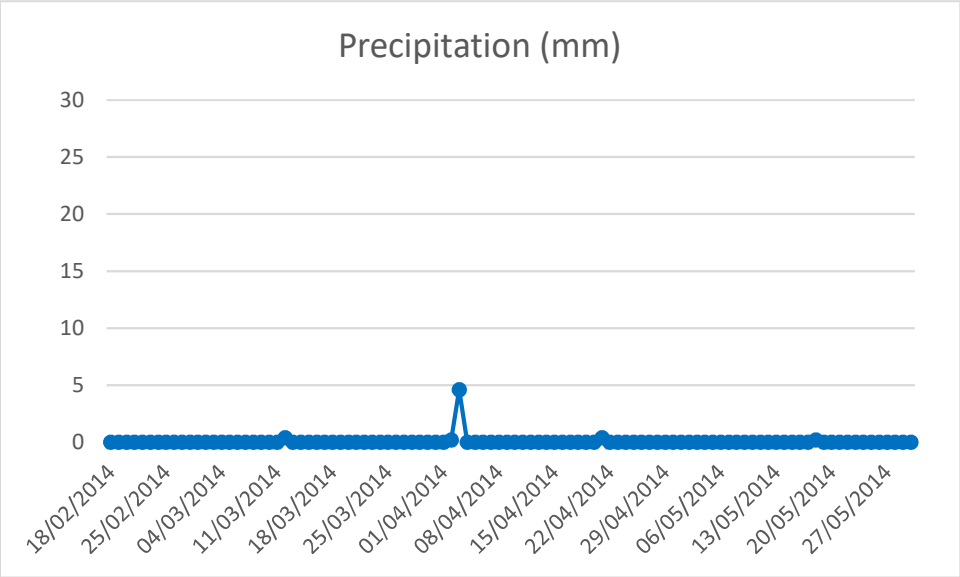


Figure 4.9 - Daily precipitation of the field trial conducted in the Late Spanish season (data provided by G's Fresh Ltd).

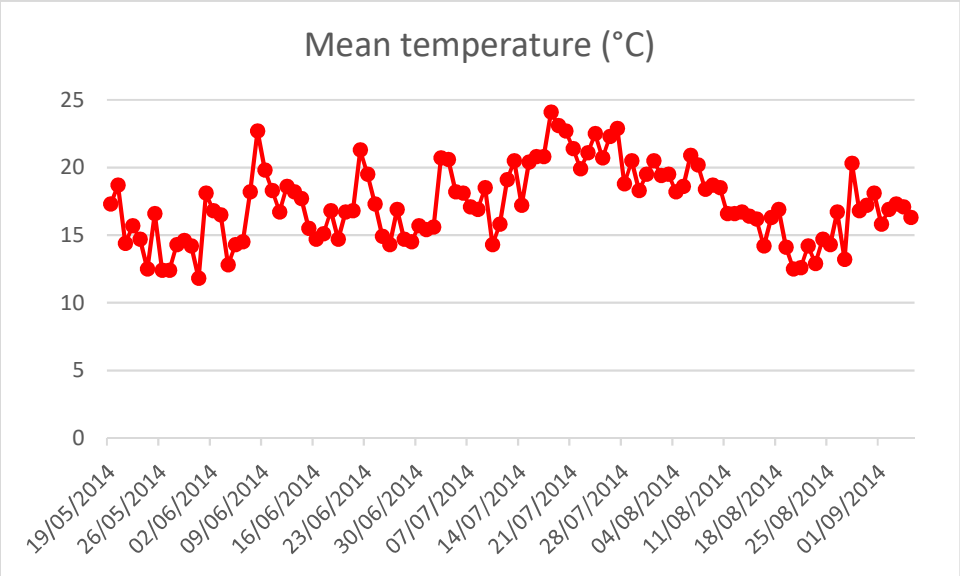


Figure 4.10 - Mean daily temperature of the field trial conducted in the Late UK season (data provided by G's Fresh Ltd).

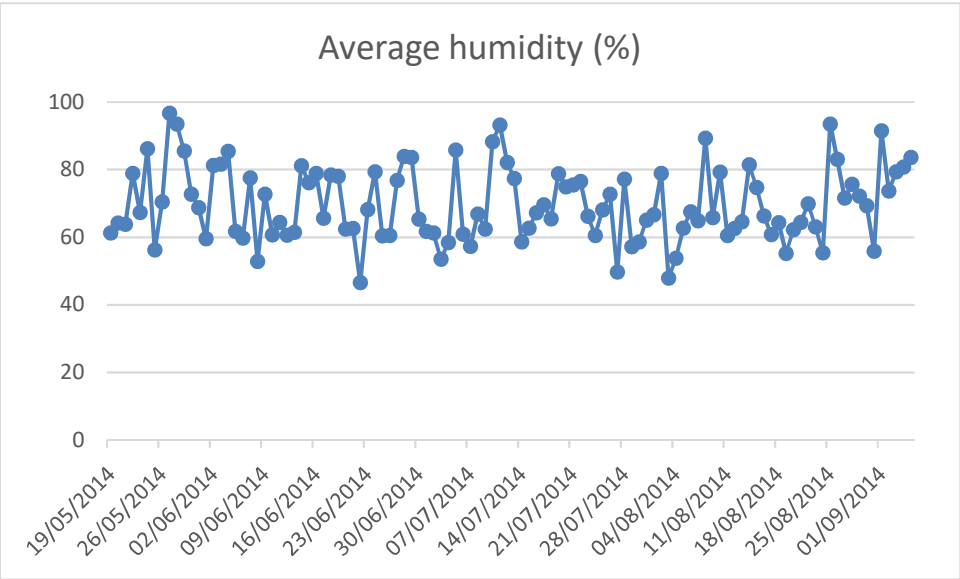


Figure 4.11 - Average daily relative humidity of the field trial conducted in the Late UK season (data provided by G's Fresh Ltd).

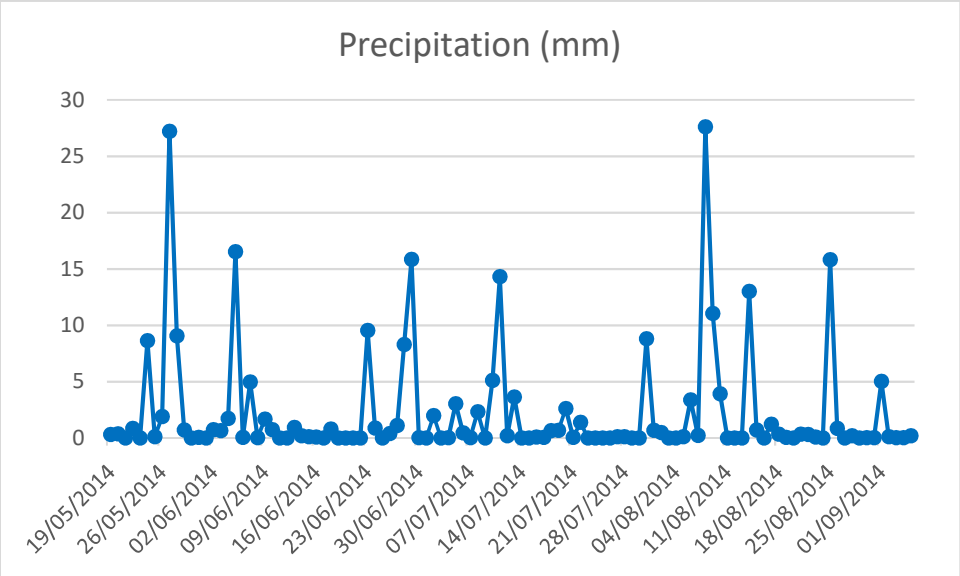


Figure 4.12 - Daily precipitation of the field trial conducted in the Late UK season (data provided by G's Fresh Ltd).

4.6 Experimental design and sampling

In all trials, the experimental field was divided into three portions: top, middle and bottom (**Figure 4.13**) and samples were randomly harvested from each portion, representing a block. Plants were harvested two weeks before optimum harvest date (HM1), one week before optimum harvest date (HM2), at optimum harvest date (HM3), one week after optimum harvest date (HM4) and two weeks after optimum harvest date (HM5).

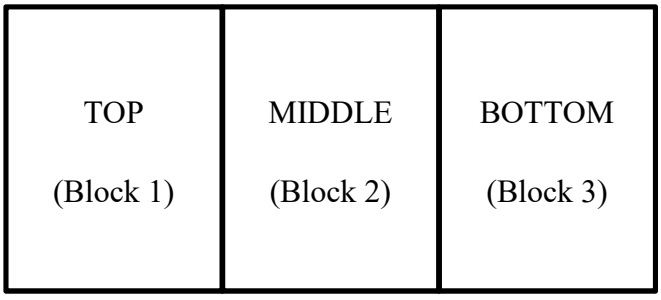


Figure 4.13 - Apportionment of the experimental field to create three blocks

After transit, samples were sent to the PSL, placed into three different crates in a storage room at constant temperature of 20°C and 56% relative humidity (RH). Storage

temperature was set up at 20 °C to encourage browning, hence, to create worst case scenario. Thereafter, samples were subjected to visual, physiological and biochemical analysis at regular time intervals. In more detail, samples were analysed on receipt (baseline), after 6 storage days (sampling day 1) and after 12 storage days (sampling day 2). Three replicates were taken from each crate for a total of 9 replicates for each sampling day. The detailed experimental layout is shown in the **Figure 4.14**. Due to an occurred consignment problem, HM1 early Spanish growing season celery samples were not included in the results.

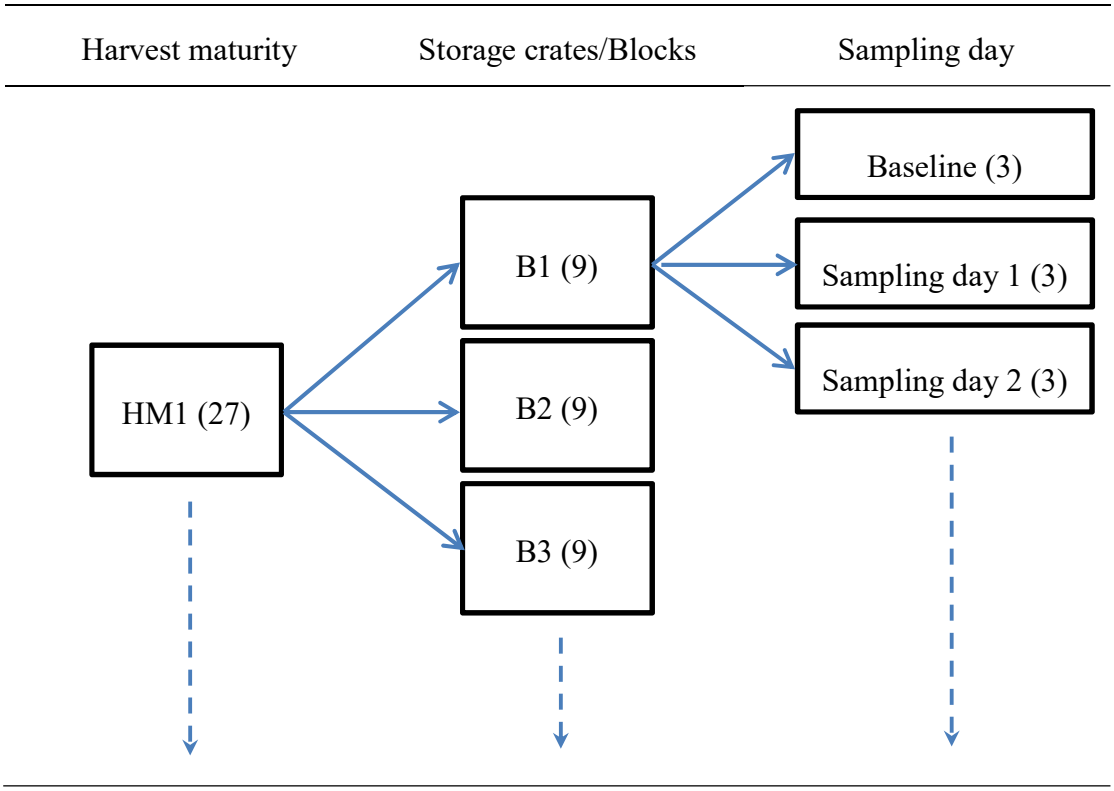


Figure 4.14 - Experimental layout (number of samples is indicated in brackets)

4.7 List of visual assessments, physiological attributes and biochemical analysis taken

In this experiment, the following analysis were taken: subjective colour (section **3.2.1**), pithiness (section **3.2.2**), bolting (section **3.2.3**), respiration rate (section **3.3.1**), objective colour (section **3.3.2**), extraction and quantification of phenolic compounds (section **3.4.2**).

4.8 Statistical analysis

Data analysis was done with the software STATISTICA, file version 13.2. (StatSoft, Inc. UK). Means were firstly checked for their residuals and secondly submitted to Analysis of variance (ANOVA) 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 crop maturity stage and storage time as factors. Respiration rate, subjective colour, objective colour, pithiness, bolting and phenolic compounds 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.

4.9 Results

4.9.1 Respiration rate

Results showed that respiratory activity of celery generally increased over storage, especially in the samples grown in the late Spanish season, regardless of crop maturity stage. Values range from 3 to 14 mL CO_2 kg^{-1} h^{-1} (**Figure 4.15**). Contrarily to all other samples, respiration rate of the most immature plants grown in the late UK season dropped over time. This unexpected value could be attributable to a fault in the equipment.

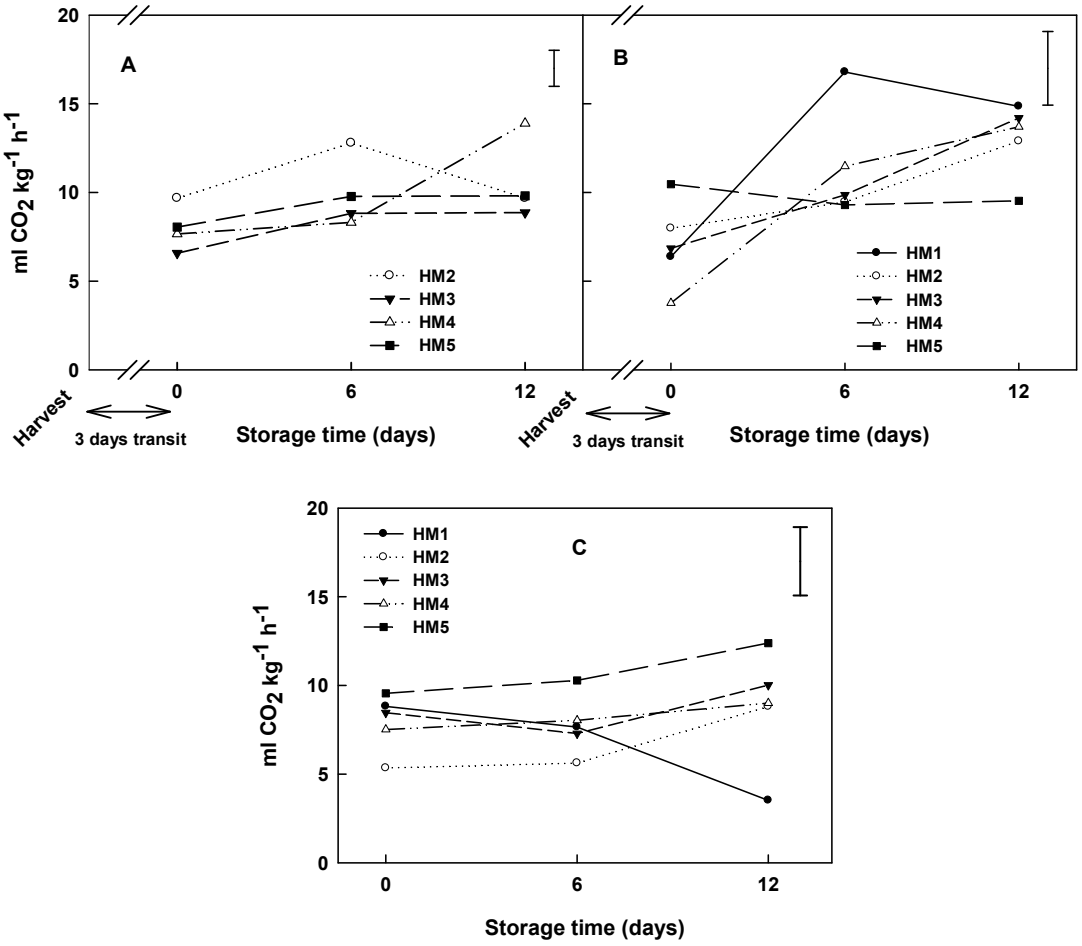


Figure 4.15 - Respiration rate of celery grown in three different seasons (A: early Spanish season; B: late Spanish season; C: late UK season) and belonging to five different harvest maturities (HM1: two weeks before optimum harvest date; HM2: one week before optimum harvest date; HM3: optimum harvest date; HM4: one week after optimum harvest date; HM5: two weeks after optimum harvest date). LSD bars are shown.

4.9.2 Bolting

In the celery samples belonging to the early Spanish season, bolting increased with harvest maturity, with the most evident increase occurring between the most immature plants (HM1) and the ones harvested 1 week before optimum date (HM2) (**Table 4.5**). Thereafter, values slightly increased until reaching the maximum score in the most

mature plants (HM5). On the other hand, no bolting was observed in the late Spanish and UK growing season plants.

Table 4.5 - Bolting score of celery at different harvest maturity stages and belonging to three different growing seasons.

Harvest maturity	Bolting score		
	Early Spanish growing season	**Late Spanish growing season**	**Late UK growing season**
Two weeks before optimum after date (HM1)	n.a.	1.00	1.00
One week before optimum after date (HM2)	1.05	1.00	1.00
Optimum harvest date (HM3)	1.74[a]	1.00	1.00
One week after optimum harvest date (HM4)	1.93[ab]	1.00	1.00
Two weeks after optimum harvest date (HM5)	2.00[b]	1.00	1.00

4.9.3 Pithiness

Results herein showed two important points. Firstly, there was a significant increase in pithiness over storage, especially within the initial six days in the Spanish-derived crops (**Figure 4.16**). On the other hand, pithiness developed less sharply in the UK celery. Secondly, the "disorder" significantly intensified with increasing stages of crop maturity (see Tables B4, B5, B12, B13, B19 and B20 in Appendix B). The most over-mature

samples were assigned the highest score overall. In the UK grown crop, there was a significant increase in the pithiness level after six days. This pattern was particularly evident at cut petiole ends rather than butt ends. On the other hand, the most over-mature plants (HM5) did not follow this pattern, increasing steadily over storage.

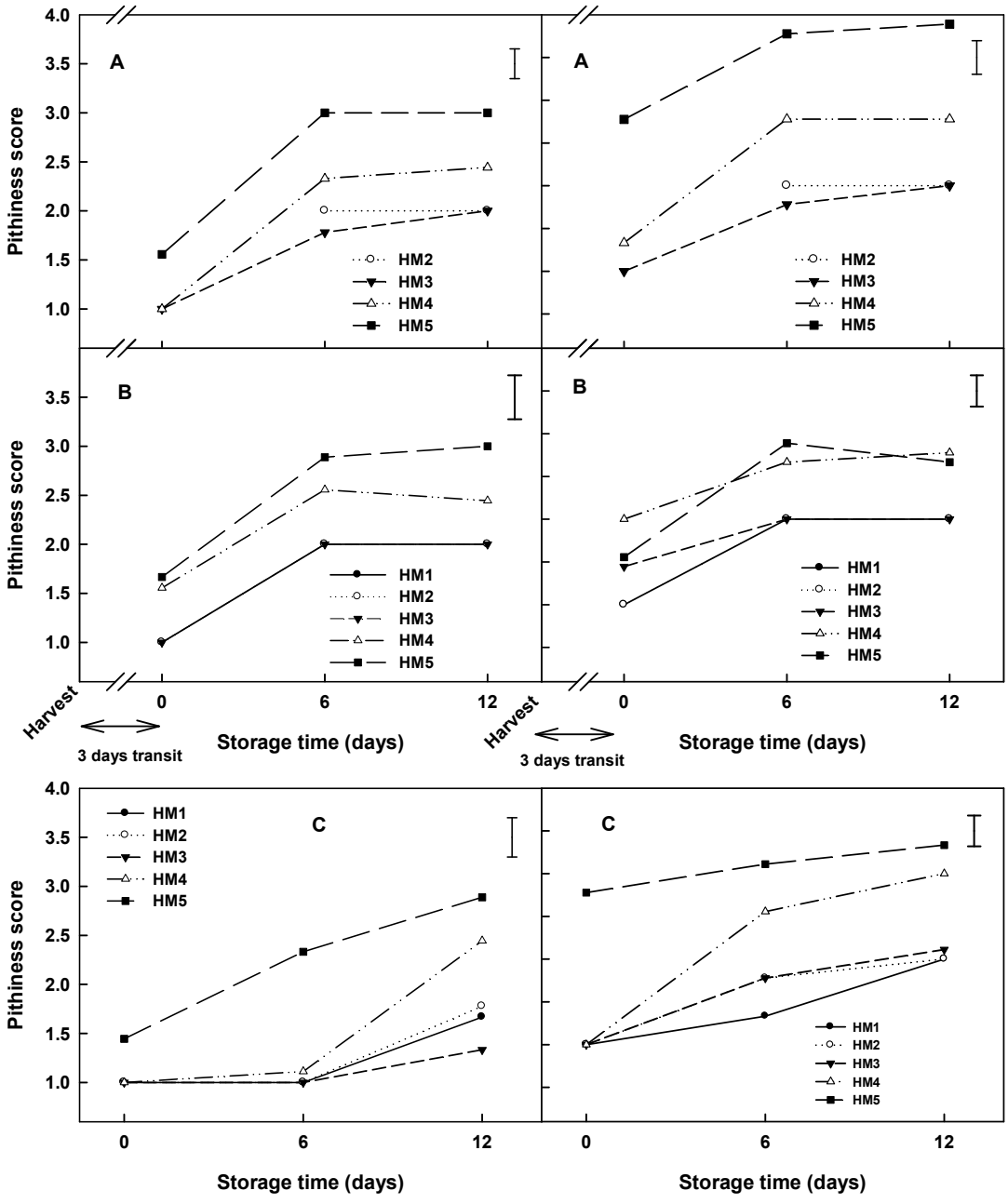


Figure 4.16 - Pithiness score of celery at cut petiole ends (on the left) and butt ends (on the right), grown in three seasons (A: early Spanish season; B: late Spanish season; C:

late UK season) and belonging to five different harvest maturities (HM1: two weeks before optimum harvest date; HM2: one week before optimum harvest date; HM3: optimum harvest date; HM4: one week after optimum harvest date; HM5: two weeks after optimum harvest date). LSD bars are shown.

4.9.4 Cut-end browning

Results showed two important patterns. Firstly, browning intensified over storage at both butt and cut petiole ends of celery; developing more sharply during the first 6 days and less sharply until the end of storage (**Figure 4.17**). In general, this pattern was more evident at cut petiole ends rather than butt ends. The highest average browning score was observed at cut petiole ends of celery grown in the early Spanish season, while the lowest score was observed at butt ends of the late UK growing season celery. Secondly, results indicated that browning incidence significantly increased as the stage of crop maturity increased (see Tables B2, B3, B10, B11, BB17 and B18 in Appendix B). Objective colour results confirmed subjective colour findings. In fact, there was a significant decrease in hue angle with storage time and crop maturity (see Tables B7, B8, B14, B15, BB21 and B22 in Appendix B) (**Figure 4.18**), meaning that cut surfaces turned towards darker colour.

High negative significant correlations were found between cut petiole ends browning and cut petiole ends hue angle of celery grown in all the three seasons (Early Spanish season R^2 = -0.89; Late Spanish season R^2 = -0.90; Late UK season R^2 = -0.87) (results in Appendix E).

There were also high negative significant correlations between butt ends browning and butt ends hue angle of celery grown in all the three seasons (Early Spanish season R^2 = -0.89; Late Spanish season R^2 = -0.78; Late UK season R^2 = -0.83) (results in Appendix E).

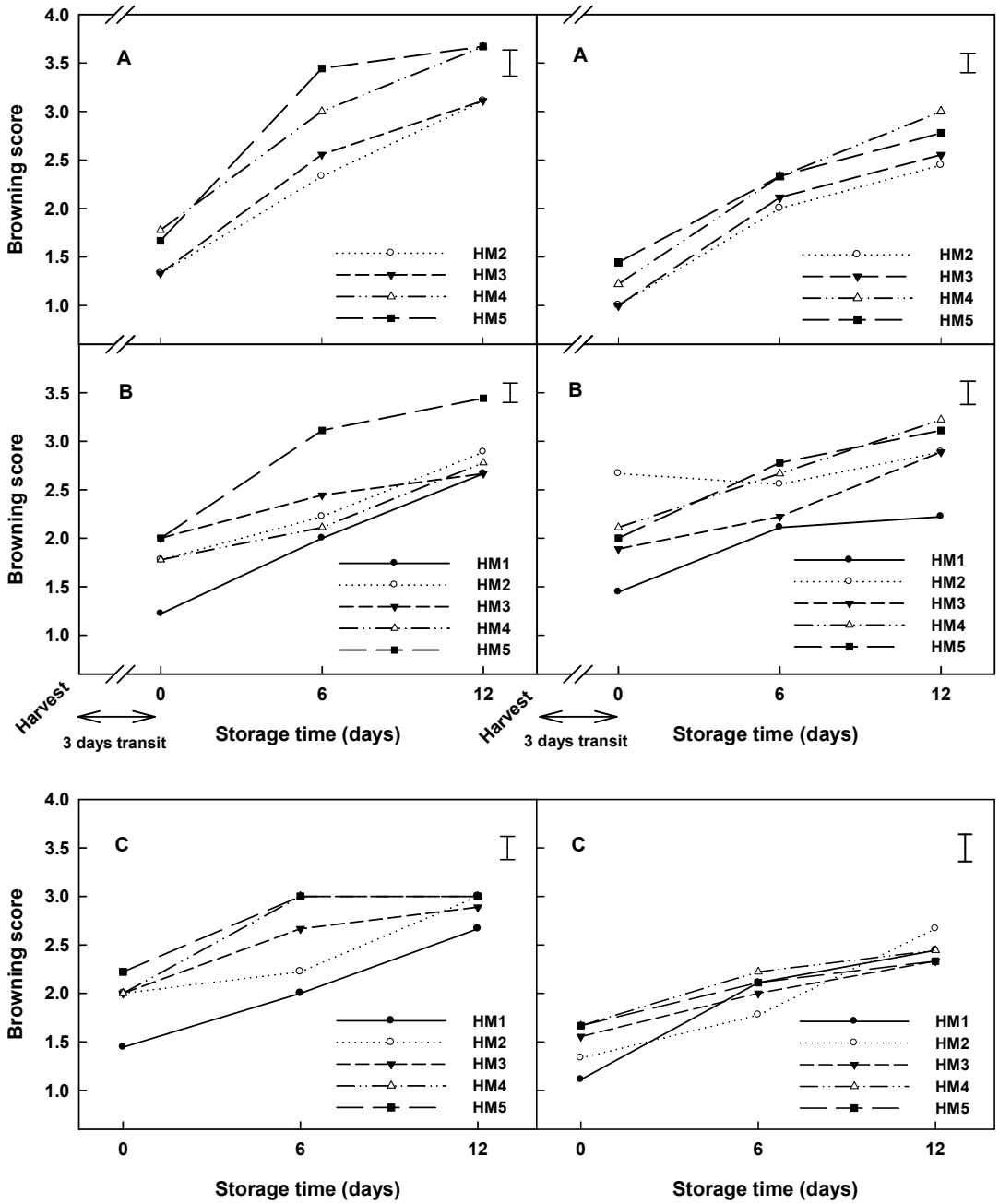


Figure 4.17 - Subjective colour score of celery at cut petiole ends (on the left) and butt ends (on the right), grown in three seasons (A: early Spanish season; B: late Spanish season; C: late UK season) and belonging to five different harvest maturities (HM1: two weeks before optimum harvest date; HM2: one week before optimum harvest date; HM3: optimum harvest date; HM4: one week after optimum harvest date; HM5: two weeks after optimum harvest date). LSD bars are shown.

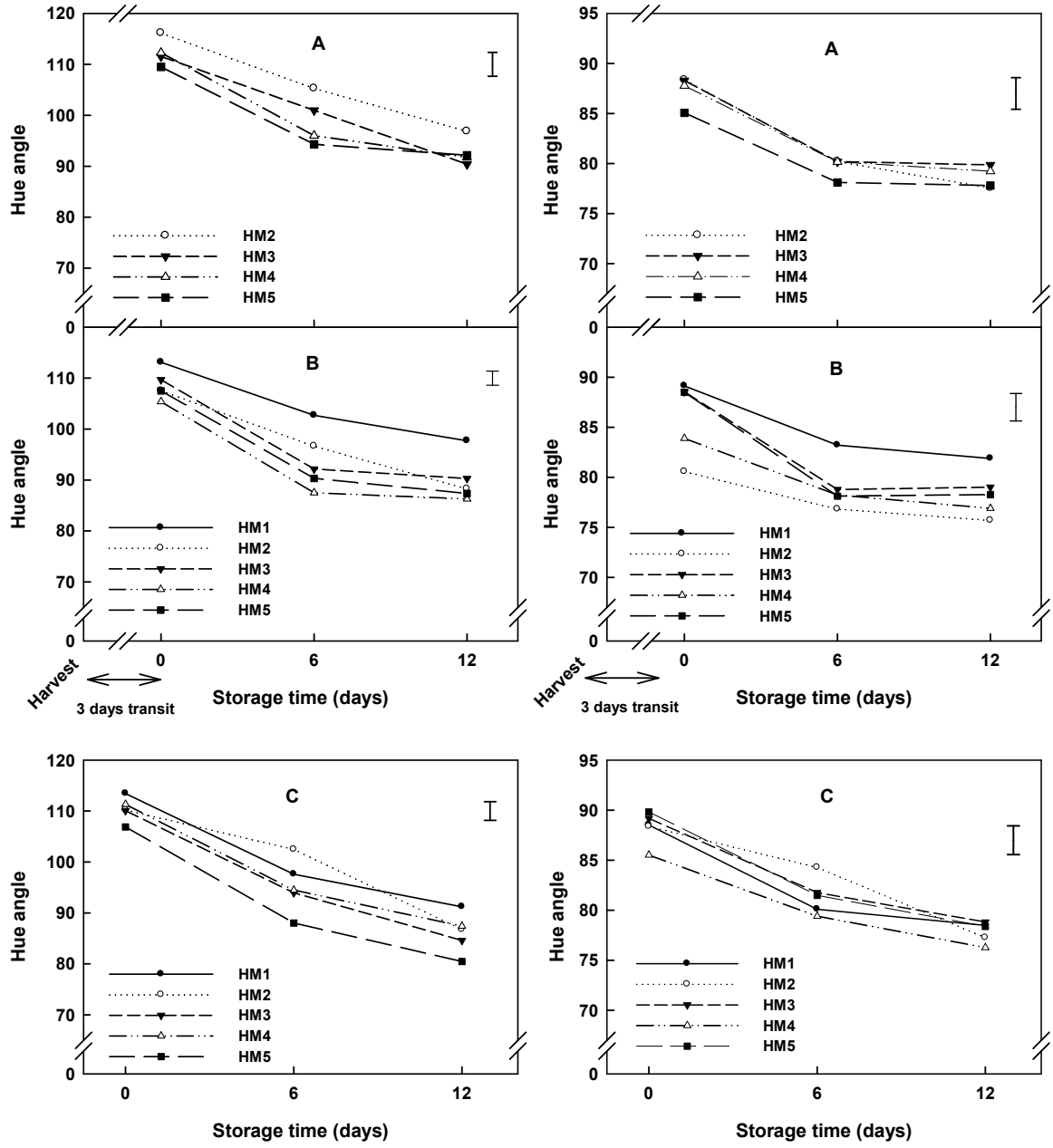


Figure 4.18 - Objective colour of celery at cut petiole ends (on the left) and butt ends (on the right), grown in three seasons (A: early Spanish season; B: late Spanish season; C: late UK season) and belonging to five different harvest maturities (HM1: two weeks before optimum harvest date; HM2: one week before optimum harvest date; HM3: optimum harvest date; HM4: one week after optimum harvest date; HM5: two weeks after optimum harvest date). LSD bars are shown.

4.9.5 Isolation and quantification of phenolic compounds

Significant levels of phenolic compounds were found in butt ends and cut petiole ends tissues of celery. On the contrary, middle outer, apical inner and middle inner petioles tissues showed very low levels of those molecules (**Figure 4.19**, **Figure 4.20** and **Figure 4.21**).

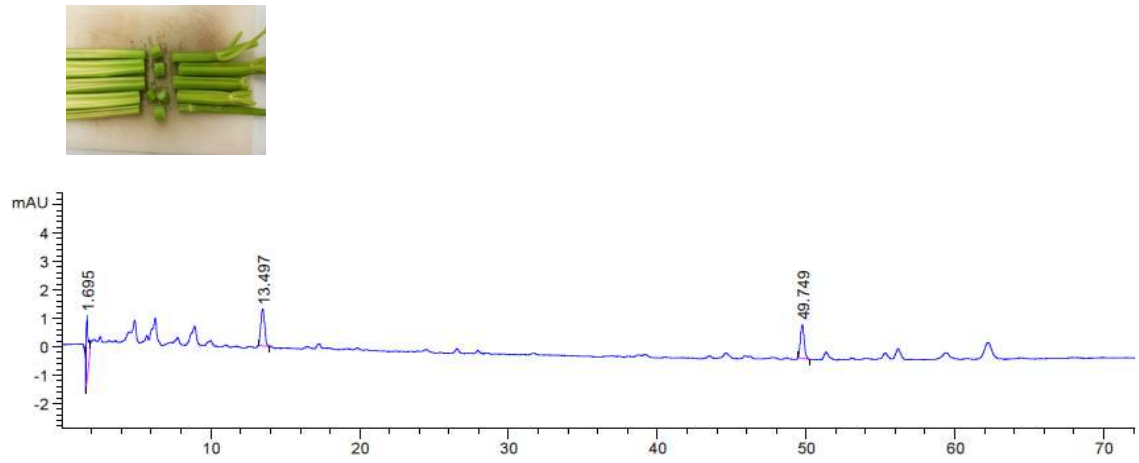


Figure 4.19 - Chromatogram showing the retention times (minutes) of the phenolic compounds in Monterey cv. celery at middle outer petiole ends and at 320 nm wavelength DAD signal.

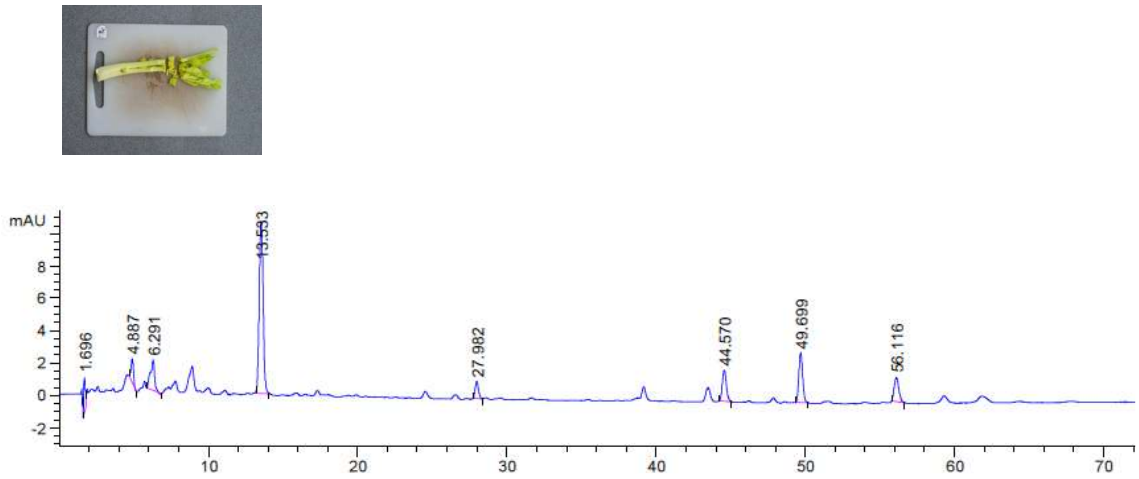


Figure 4.20 - Chromatogram showing the retention times (minutes) of the phenolic compounds in Monterey cv. celery at apical outer petiole ends and at 320 nm wavelength DAD signal.

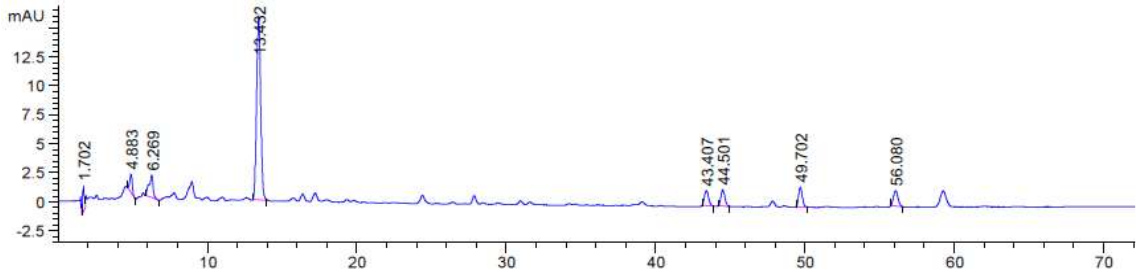
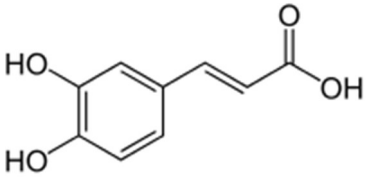
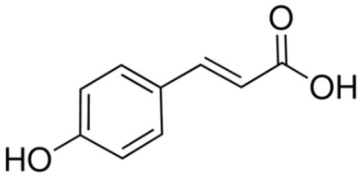


Figure 4.21 - Chromatogram showing the retention times (minutes) of the phenolic compounds in Monterey cv. celery at apical inner petiole ends and at 320 nm wavelength DAD signal.

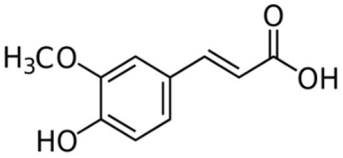
Yao *et al*., (2010) identified six phenolic compounds in different celery varieties. More precisely, the authors reported the presence of luteolin, apigenin and kaempferol; which belong to the class of flavonoids, and caffeic, ferulic and *p*-coumaric acid; which belong to the class of phenolic acids (**Figure 4.22**).



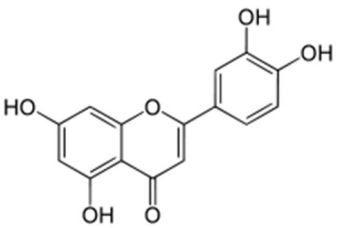
Caffeic acid



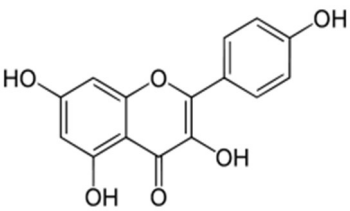
p-coumaric acid



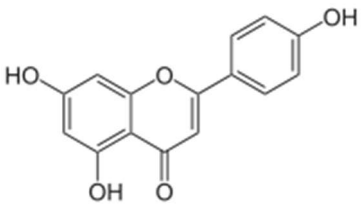
Ferulic acid



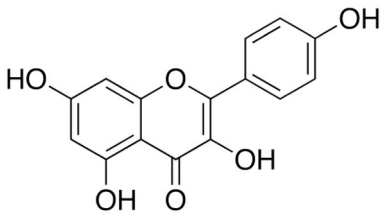
Luteolin



Kaempferol



Apigenin



Chlorogenic acid

Figure 4.22 - Chemical structures of the phenolic compounds isolated by Yao *et al*., (2010).

According to these findings, compounds standards were purchased and subsequently analysed with HPLC-DAD to detect the respective retention times. Results showed that all molecules identified by Yao *et al*., (2010) were completely absent in Monterey celery cv. analysed tissues. Secondly, several unknown phenolic compounds were isolated. The most important are displayed in **Figure 4.23**, **Figure 4.24**, **Figure 4.25** and **Figure 4.26** below.

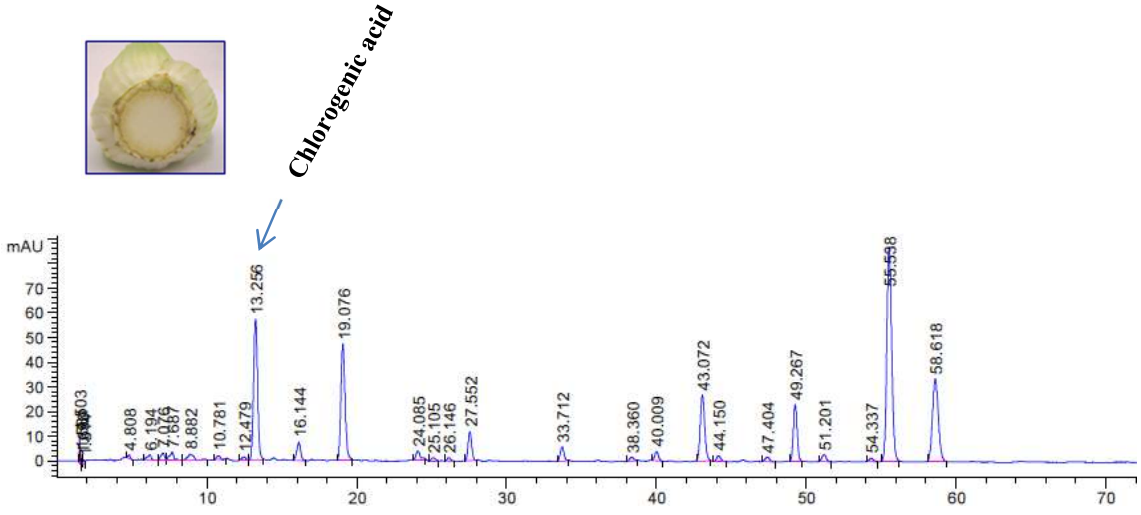


Figure 4.23 - Chromatogram showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery butt ends with no browning at 320 nm wavelength DAD signal.

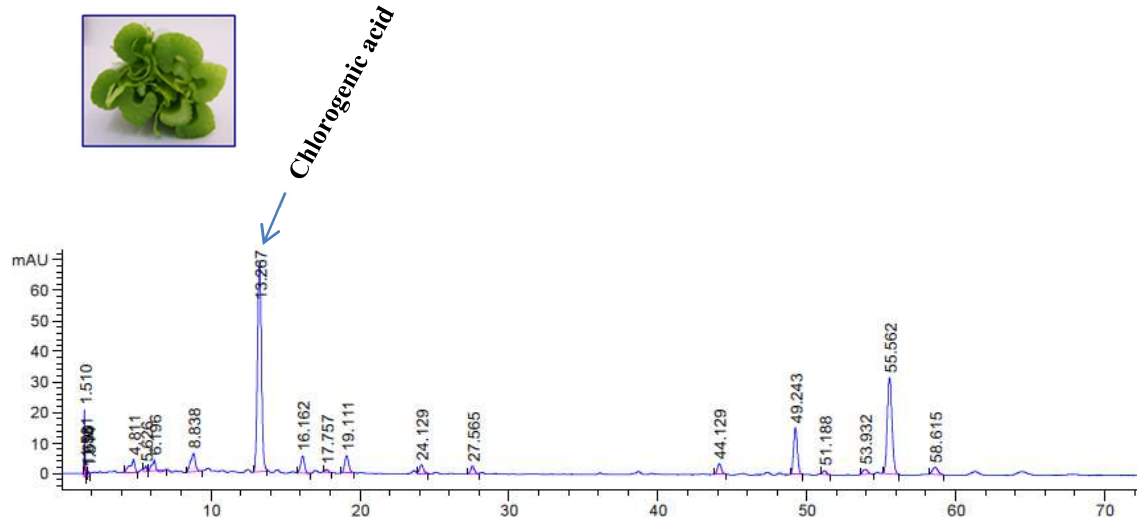


Figure 4.24 - Chromatogram showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery apical cut petiole ends with no browning at 320 nm wavelength DAD signal.

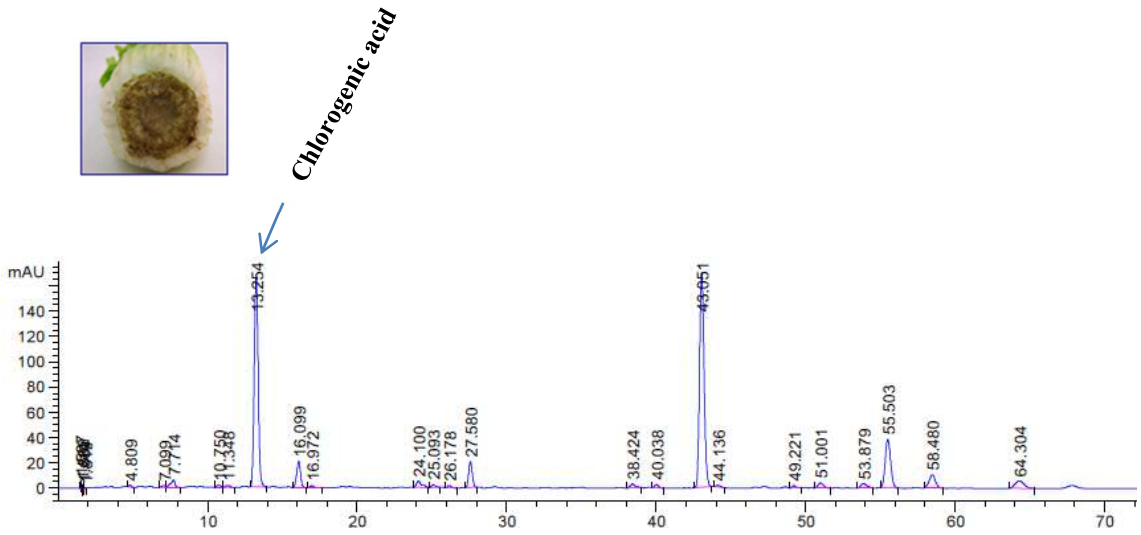


Figure 4.25 - Chromatogram showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery butt ends with severe browning at 320 nm wavelength DAD signal.

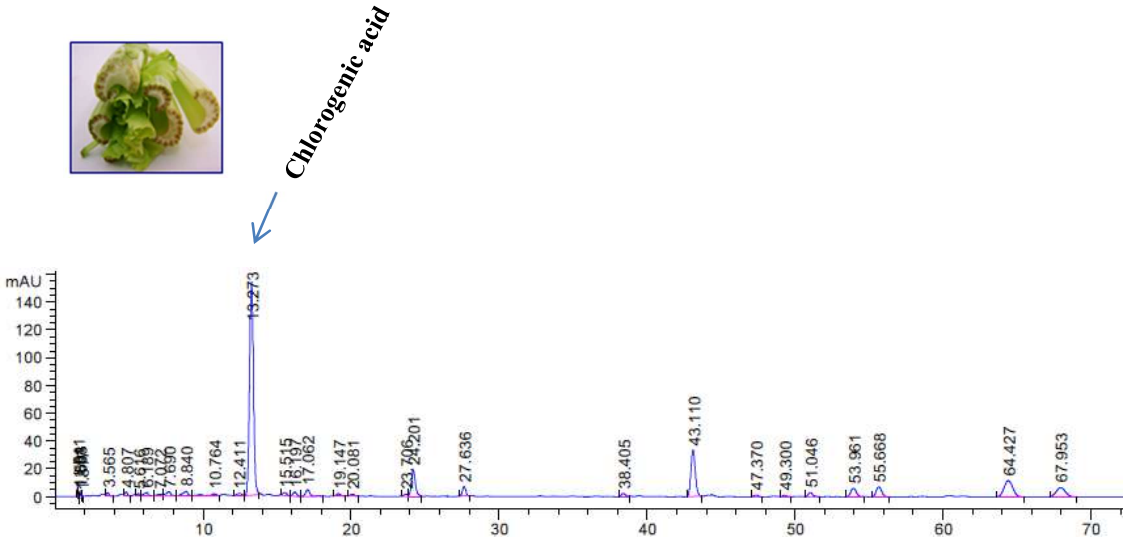


Figure 4.26 - Chromatogram showing the retention times (minutes) of the all the phenolic compounds in Monterey cv. celery apical cut petiole ends with severe browning at 320 nm wavelength DAD signal.

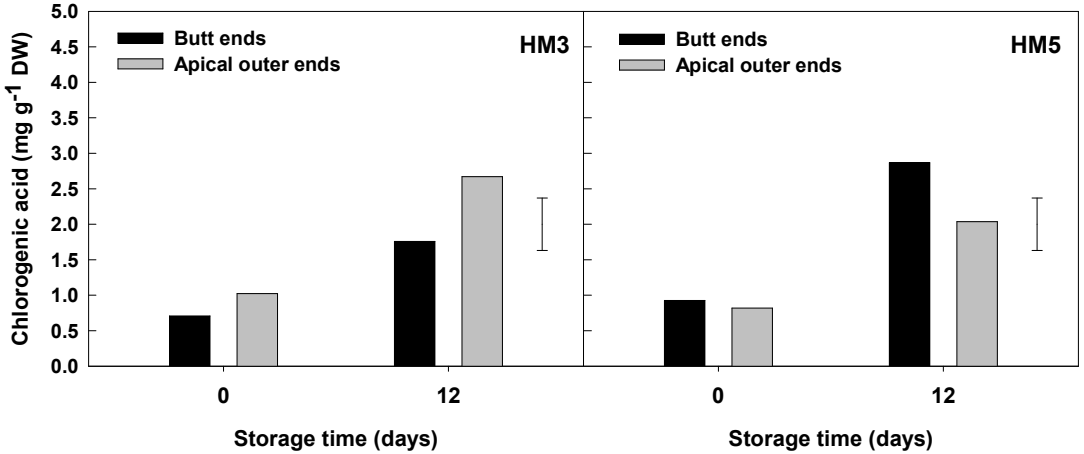


Figure 4.27 - Effect of crop maturity stage on the chlorogenic acid content at butt ends and apical outer ends of celery (cv. Monterey) grown in the Early Spanish season and stored in air at 20 °C. HM3: optimum harvest date; HM5: two weeks after optimum harvest date. LSD bars are shown.

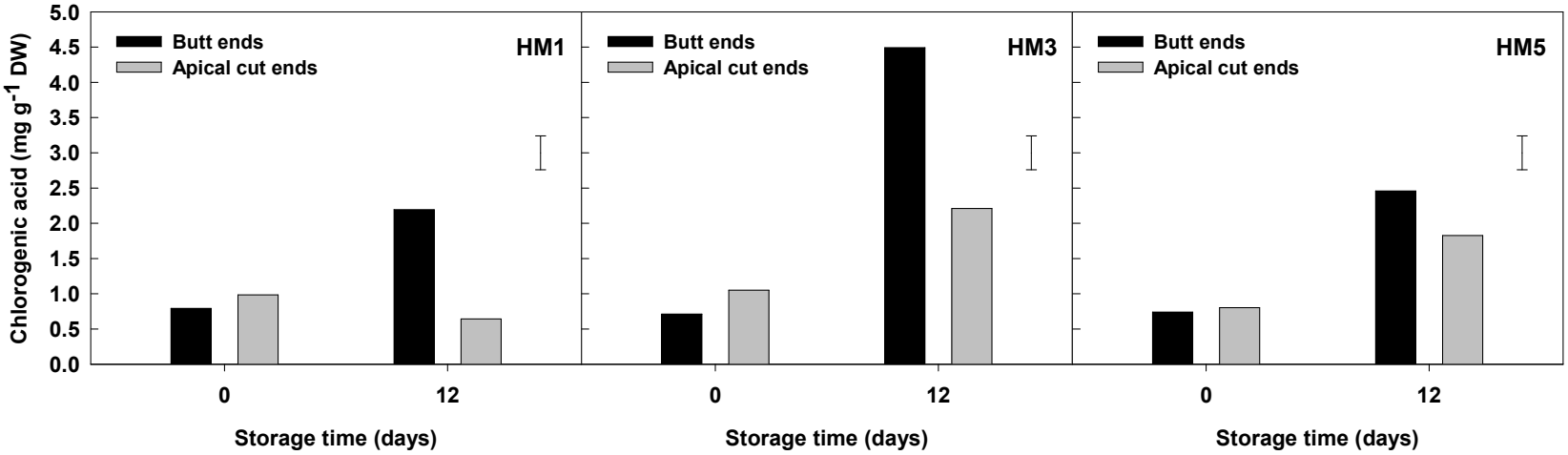


Figure 4.28 - Effect of crop maturity stage on the chlorogenic acid content at butt ends and apical outer ends of celery (cv. Monterey) grown in the Late Spanish season and stored in air at 20 °C. HM1: two weeks before optimum harvest date; HM3: optimum harvest date; HM5: two weeks after optimum harvest date. LSD bars are shown.

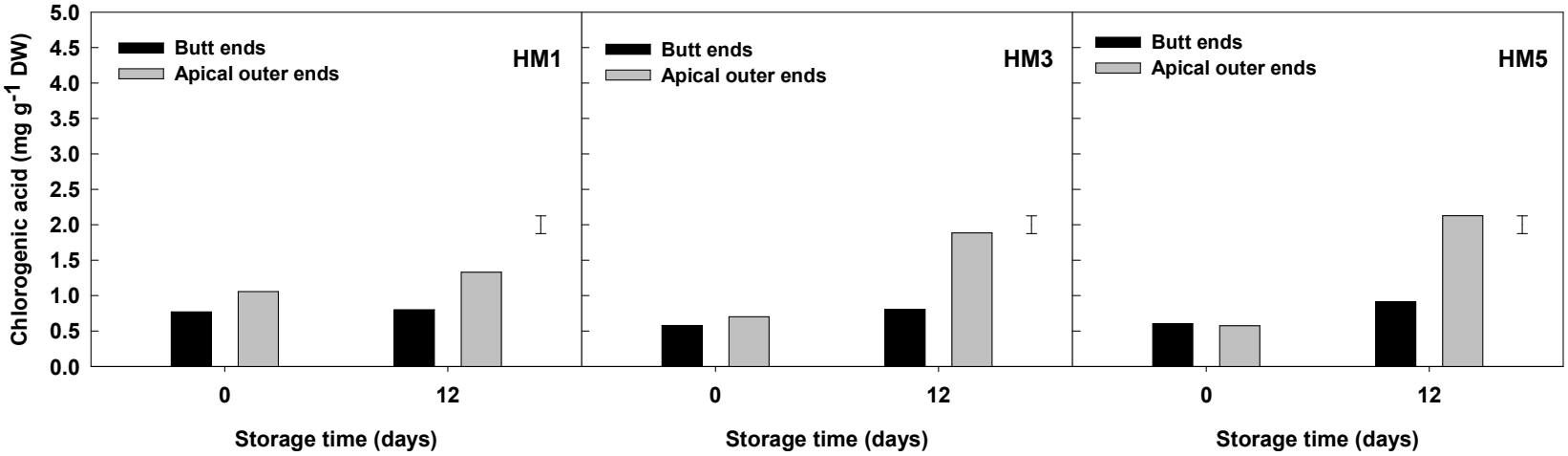


Figure 4.29 - Effect of crop maturity stage on the chlorogenic acid content at butt ends and apical outer ends of celery (cv. Victoria) grown in the Late UK season and stored in air at 20 °C. HM1: two weeks before optimum harvest date; HM3: optimum harvest date; HM5: two weeks after optimum harvest date. LSD bars are shown.

Results showed that concentration of chlorogenic acid ranged between 0.6 to 4.5 mg g^{-1} dry weight (**Figure 4.27**, **Figure 4.28** and **Figure 4.29**). On average, it was the lowest in the most immature celery plants (HM1) grown in the late UK season (**Figure 4.29**) and it was the highest in the optimum harvested celery plants (HM3) grown in the late Spanish season (**Figure 4.28**). In general, chlorogenic acid clearly increased over storage time at both butt and apical cut petiole ends. In more detail, it enhanced from 0 to 12 days at apical cut ends in the late UK grown plants while it remained stable at butt ends. On the other hand, it increased at both cut ends in the Spanish plants. Comprehensively, no significant differences were found in the concentration of chlorogenic acid between butt and apical cut ends at day 0, whereas they were detected at the end of storage. Generally, late UK season celery plants contained the lowest levels of the phenolic compound. Furthermore, it contained the lowest levels in the plants harvested two weeks before optimum date (HM1) overall.

High positive significant correlations were found between chlorogenic acid content and cut-end browning of celery grown in all the three seasons (Early Spanish season R^2 = 0.78; Late Spanish season R^2 = 0.60; Late UK season R^2 = 0.56) (results in Appendix E).

4.10 Discussion

This study was premised on the hypothesis that horticultural maturity has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery. Results arising from this experiment corroborated this hypothesis. As a matter of fact, results shown in this study have demonstrated two key points. Firstly, cut-end browning was positively correlated with stage of crop maturity in celery. Similar findings were obtained by Guerra *et al*., (2010) whose experimental work on the self-whitening Golden Clause celery cv. found that browning potential of leaf stalks was significantly higher in celery harvested 124 days after transplantation compared to those harvested 94 days after transplantation. Cut-end browning has been poorly investigated in celery, yet it has been extensively studied in an economically important crop like lettuce. A study conducted by Chutichudet *et al*., (2011) on the Grand Rapids lettuce cultivar showed that browning appearance in leaves

measured in terms of L* and b* significantly increased with plant developmental stage. Also, PPO activity increased as with crop maturity and it reached the maximum levels at the latest harvesting date (73 days after planting). These results are in accordance with a previous research carried out by Kang *et al*., 2008 who also revealed that browning and decay rate of head lettuce (*Lactuca sativa* L. var. *capitata*) stored at 5 °C for 5 weeks was higher in over-mature products rather than immature and mature ones. Cut-end browning is thought to be due to the enzymatic activity of PPOs, which oxidase phenolic compounds to produce brown pigments (Queiroz *et al*., 2008). PPOs and phenolic compounds are normally compartmentalized in plant cells, hence, the browning reaction can only occur in case tissues are broken or damaged (for instance, after harvest and during handling). One of the consequences of plant senescence is loss of membrane integrity. It is likely that in these conditions, even though tissues have already been cut, substrate and enzyme have more possibility to come in contact, which leads to increased discolouration.

The second point arising from this experimental work is that plant developmental stage affected pithiness in a similar way to cut-end browning. Pithiness development was generally comparable to that of browning but only with regard to early and late Spanish growing season trials. On the contrary, in the late UK trial, the development of pithiness differed, remaining relatively stable in the first 6 days of storage and then clearly raising until 12 days of storage. This pattern could be attributable to the different timings and temperatures of sample transit. In the late UK trial, as previously specified in the methodology section, samples were transported from the farm to the laboratory in about 1.5 hours at ambient temperature, while in the Spanish trials, samples took about 3 days to be dispatched, even though at lower temperatures. This could have led to differences in the pith breakdown after harvest. This assertion is confirmed by a study conducted by Pressman *et al*., (1984) who reported that mechanical perturbation can cause the onset of pithiness in celery, especially at the basal part of the stalks. Thus, it could be reasonable to assume that the mechanical disturbance occurred during transit of Spanish samples could have contributed to the enhancement and progress of this postharvest "disorder". Pithiness increased with plant developmental stage, with the most over-mature celery plants reaching the maximum score. In previous studies on celery, it can be noticed that pithiness was stimulated by several factors which result in the induction

of senescence (Pressman *et al*., 1984; Breschini and Hartz, 2002; Guerra *et al*., 2010). Therefore, it is plausible that the harvest maturity delay may have triggered the induction of senescence in a similar way.

Results arising from this experimental work also showed that celery plants only bolted in the trial conducted in the early Spanish growing season. This is unsurprising as the effect of cold temperatures occurred during such growing season (data provided by G's Fresh Ltd) could probably have triggered the transition of plants from the vegetative to the reproductive phase. In addition, considering that celery is a short-long day plant (Pressman and Negbi, 1980), the passage from a short to long photoperiod could have additionally contributed to promote this plant survival mechanism.

Chlorogenic acid was the unique phenolic compound identified in celery tissues. The other compounds isolated by Yao *et al*., (2010) (caffeic acid, *p*-coumaric acid, ferulic acid, luteolin, apigenin and kaempferol) have not been detected in the celery varieties used to carry out this experimental work. This discrepancy could be due to the different plant varieties used to conduct the two experiments. In effect, Yao *et al*., (2010) used Chinese celery whilst in the present work European celery was used.

Quantitative results from this experiment showed that chlorogenic acid increased over storage in all plants belonging to Spanish and UK trials. Interestingly, such increase is accompanied by an intensification of cut-end browning, suggesting a probable involvement of the compound in the development of the postharvest "disorder". These findings contrast with those of Viña and Chaves (2006) who reported that the chlorogenic acid sharply decreased at different storage temperatures (0, 4 and 10 °C) for 28 days. This contradiction could be attributable to differences in materials and methods of the two experiments. As a matter of fact, the previous authors used the self-whitening cv. Golden Boy whilst in this work, green celery varieties were utilized. Moreover, in this study, storage temperature was 20 °C, which could have stimulated degradative/decaying processes, leading to increased production of phenolic compounds. Howsoever, there are few studies investigating the evolution of chlorogenic acid during storage in celery. Levels of this phenolic compound seem to depend on several factors. For instance, Gil-Izquerdo, Gil, Conesa and Ferreres (2001) found chlorogenic acid concentration to increase in artichoke at 2 to 7 °C storage temperature

range. The authors also found the increase being more evident in internal bracts rather than external bracts.

4.11 Conclusions

According to the finding arising from the present experimental work, it was found that the hypothesis of postharvest browning in fresh-cut celery being affected by horticultural maturity was true. Plant senescence processes may play an important role in the stimulation of the biochemical mechanisms that lead to the reduction of postharvest quality in celery. In summary, it seems that harvesting immature celery significantly improves its postharvest visual and organoleptic quality, rendering the product more appreciated by consumers. On the other hand, it should also be considered that such quality benefits can potentially be mitigated, or even cancelled, by the negative side-effects deriving from changing the standard agronomic practices adopted by horticultural companies. For instance, it is expected that harvesting celery at an earlier date could result in reduced yield. Also, the typical taste and aroma (especially that of “Fenland celery” protected with the PGI status from the European Union) could change. These two possible scenarios can result in unpredictable implications on the profit of growers and horticultural companies in general.

5. EFFECT OF ETHYLENE AND 1-MCP ON THE VISUAL QUALITY, PHYSIOLOGY AND BIOCHEMISTRY OF FRESH-CUT CELERY

5.1 Introduction

Ethylene is an important plant hormone involved in many processes in plants and it is correlated with many physiological responses to biotic and abiotic stresses (El Beltagy and Hall, 1974; Goeschl *et al*., 1966). It has been demonstrated that the gas promotes the synthesis of various molecules involved in phenylpropanoid metabolism and subsequent production of phenolic compounds related to browning in celery (Heredia and Cisneros-Zevallos, 2009), although the correlation between ethylene and browning incidence on cut surfaces of this crop is still unknown. Celery is classified as having medium sensitivity to ethylene after harvest. This was confirmed by Pressman *et al*., (1986) who found that exposure of celery plants to Ethephon for 2 days in the field caused pithiness, especially in the basal part of the petioles. Yet, no studies have investigated the role of ethylene on the browning mechanism in celery.

1-Methylcyclopropene (1-MCP) is usually applied postharvest in many crops to contrast the effects of ethylene as it prevents the hormone from exerting its physiological effects by binding to receptors on cell membranes (Merchante *et al*., 2013). Saltveit (2004) reported that exposing heads or leaves of iceberg lettuce stored at 2.5 °C to 1-MCP significantly decreased phenolic compounds and tissue discoloration stimulated by previous ethylene treatment. Currently, the visual and physiological effects of 1-MCP on celery have not yet been investigated. It is expected that the utilization of 1-MCP in celery storage may help to understand the role that endogenously-produced ethylene has on postharvest discolouration in this crop.

5.2 Aim of the study

The aim of this study is to investigate the effects of both ethylene and 1-MCP on the visual quality, physiology and biochemistry of fresh-cut celery.

5.3 Specific objectives

- To determine the effects that 10 μL L^{-1} exogenously applied ethylene has on cut-end browning, pithiness and respiration rate.
- To investigate variations in the metabolism of sugars as affected by ethylene and 1-MCP treatments.

5.4 Hypothesis

The experiment was carried out with the following hypotheses:

5.4.1 Null hypothesis

Continuous ethylene supplementation and 1-MCP treatment has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

5.4.2 Alternate hypothesis

Continuous ethylene supplementation and 1-MCP treatment has no significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

5.5 Plant material and growing conditions

The plant material, growing conditions and sample transit conditions are the same described in the previous chapter, section **4.5**.

5.6 Experimental design and sampling

In all trials, the experimental field was divided into three portions: top, middle and bottom (see paragraph **4.5** of previous chapter) and samples were randomly harvested from each portion, representing a block. Plants were harvested two weeks before optimum harvest date (HM1), at optimum harvest date (HM3) and two weeks after optimum harvest date (HM5).

After transit, samples were sent to the PSL and placed into crates in a storage room for 12 days at constant temperature of 5 °C and 95% relative humidity. Each batch was

divided into three sub-batches: one for storage in continuous flowing air, one for storage in continuous flowing ethylene at 10 μL L^{-1} concentration and the last for storage in continuous flowing air after 1-MCP treatment at 1 μL L^{-1} concentration for 24 h at 20 °C. For each storage condition, samples were placed inside three different water-sealed 264 L polypropylene boxes (**Figure 5.1**). Samples were periodically processed for visual and physiological analysis. In more detail, they were processed after 3 (sampling day 1), 6 (sampling day 2) and 10 storage days (sampling day 3). For each harvest maturity, baselines were taken from the crop maturity experiment described in the previous chapter. The detailed experimental layout is shown in **Figure 5.2**. Due to a consignment problem, HM1 early Spanish growing season celery samples were not included in the results.

Figure 5.1 - 264 L capacity water-sealed polypropylene boxes used to store celery samples in different postharvest conditions (photo taken by the author).

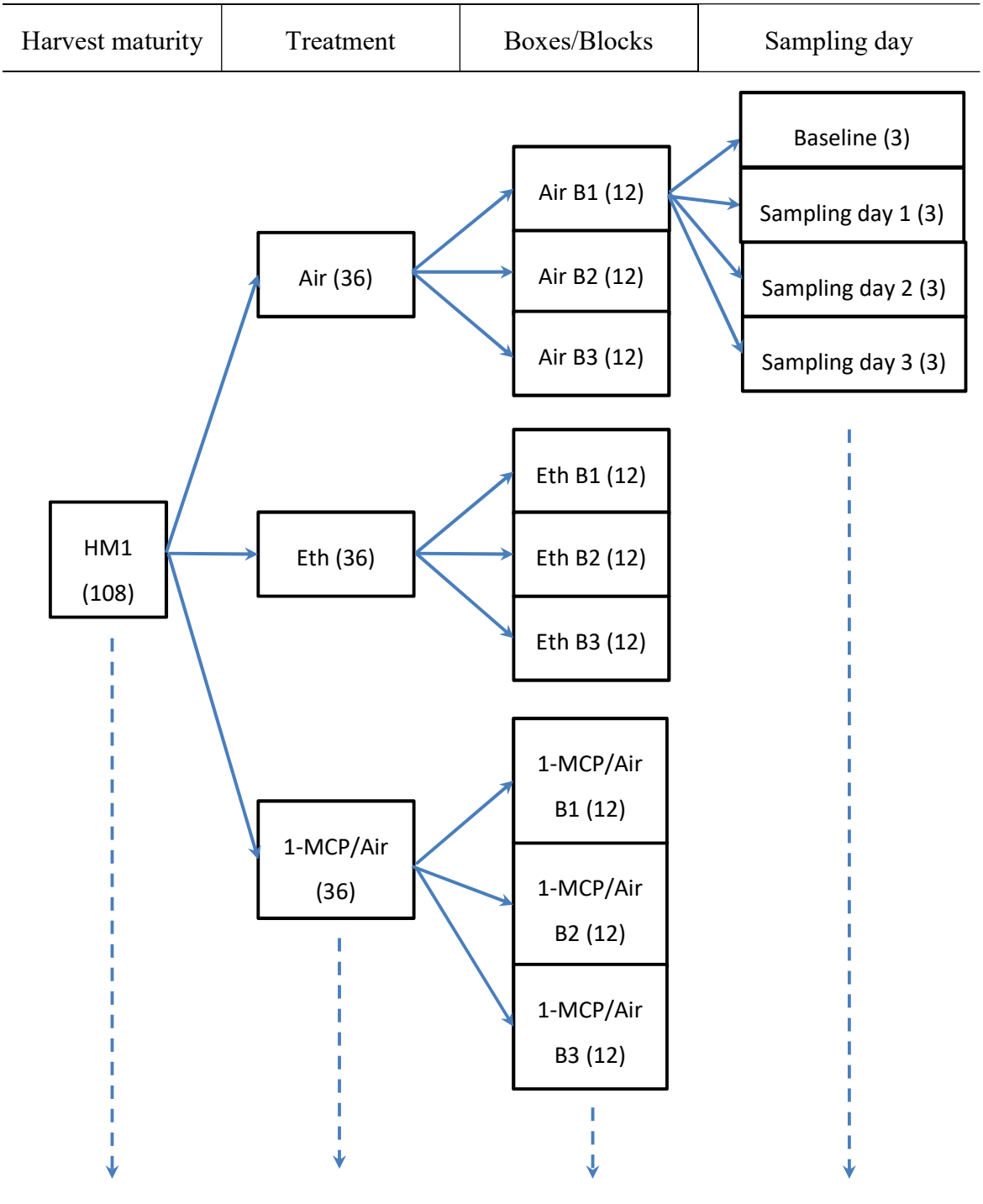


Figure 5.2 - Experimental layout (number of samples is indicated in brackets)

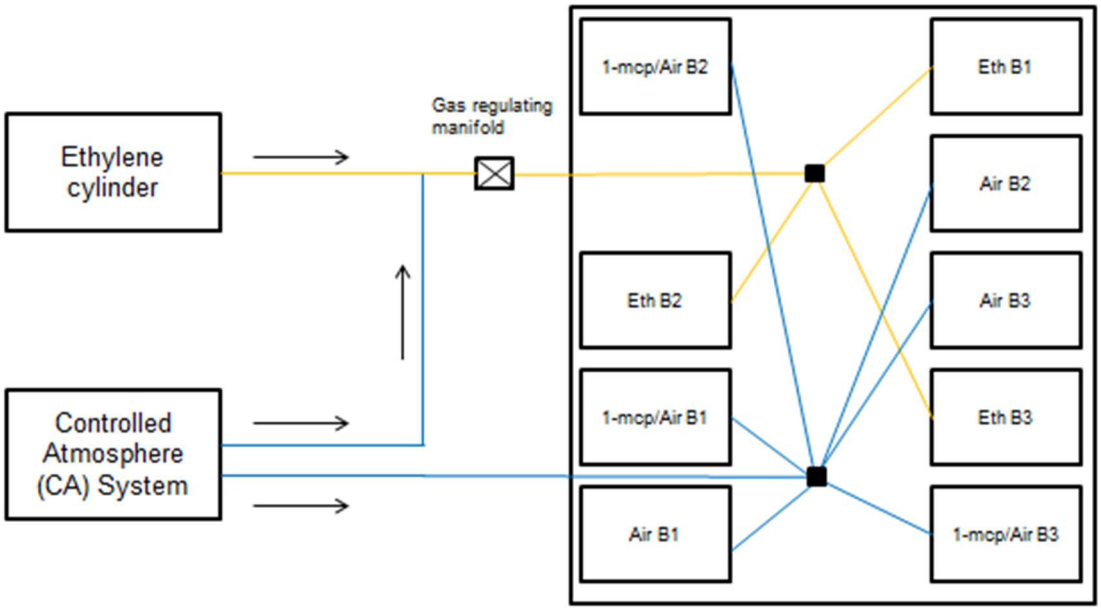


Figure 5.3 - Experiment setup

5.7 List of visual assessments, physiological attributes and biochemical analysis taken

In this experiment, the following analysis were taken: subjective colour (section **3.2.1**), pithiness (section **3.2.2**), bolting (section **3.2.3**), respiration rate (section **3.3.1**), objective colour (section **3.3.2**), extraction and quantification of non-structural carbohydrates (section **3.4.3**).

5.8 Statistical analysis

Data analysis was done with the software STATISTICA, file version 13.2 (StatSoft, Inc. UK). Means were firstly checked for their residuals and secondly submitted to Analysis of variance (ANOVA) 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 crop maturity stage, postharvest gaseous treatments and storage time as factors. Respiration rate, subjective colour, objective colour, pithiness and non-structural carbohydrates 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.

5.9 Results

5.9.1 Respiration rate

In general, respiration rate had a similar range in the samples grown to all the three seasons, with results for Early Spanish growing season being the most stable and having the narrowest range (**Figure 5.4**). On the other hand, respiratory values for celery belonging to the late Spanish season oscillated the most (**Figure 5.5**). Overall, a clear pattern emerged, where within each crop maturity stage, there was a significant increase in the respiration rate of ethylene-treated samples, whilst there was a significant decrease in the respiratory activity of 1-MCP-treated in the plants grown all three seasons (see Tables C1, C8 and C15 in Appendix C). This effect was particularly evident in the samples harvested in the late UK growing season (**Figure 5.6**). In addition, in ethylene-treated samples, values increased over storage, while in the 1-MCP-treated they initially decreased but they slightly heightened after 6 days of storage. The samples showing the most evident burst in the respiratory activity were the ones grown in the late Spanish season, harvested at optimum date (HM3) and flushed with continuous ethylene. Respiration rate was not significantly affected by the stage of crop maturity overall, regardless of postharvest treatments.

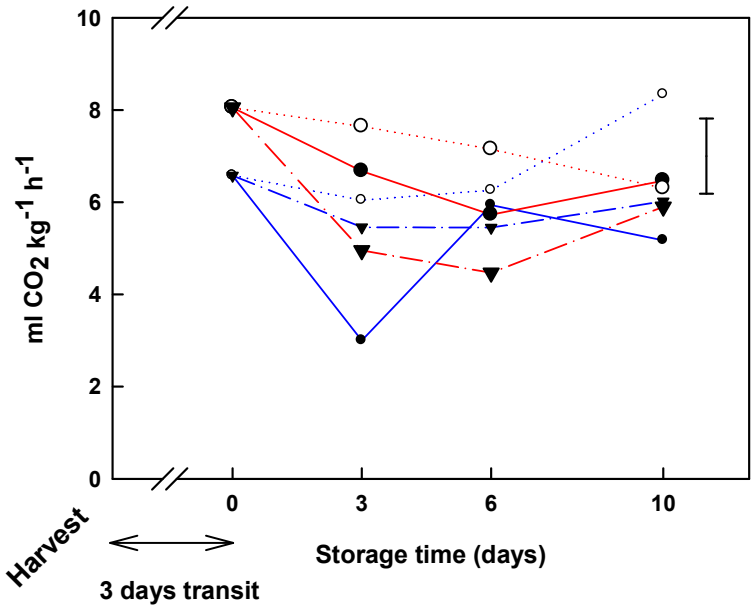


Figure 5.4 - Respiration rate of celery grown in the Early Spanish season and belonging to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red lines: two weeks after optimum harvest date) and stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○ symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24 h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

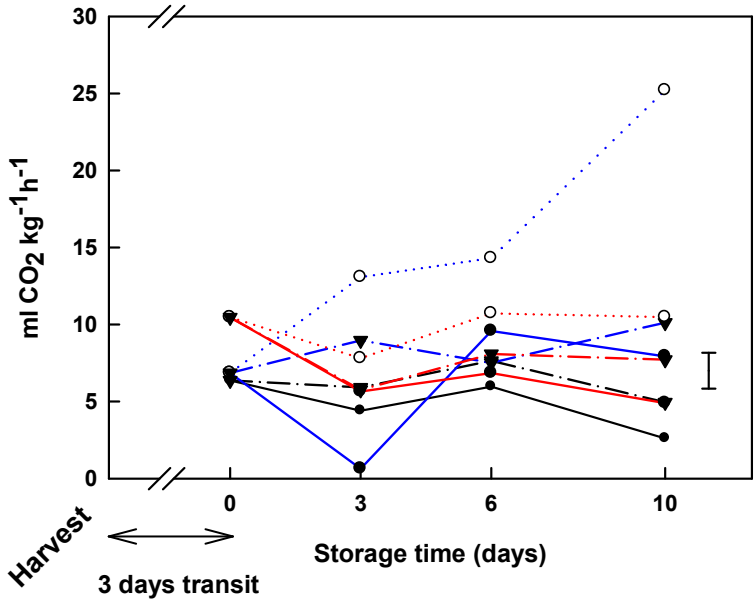


Figure 5.5 - Respiration rate of celery grown in the Late Spanish season and belonging to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red lines: two weeks after optimum harvest date) and stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○ symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

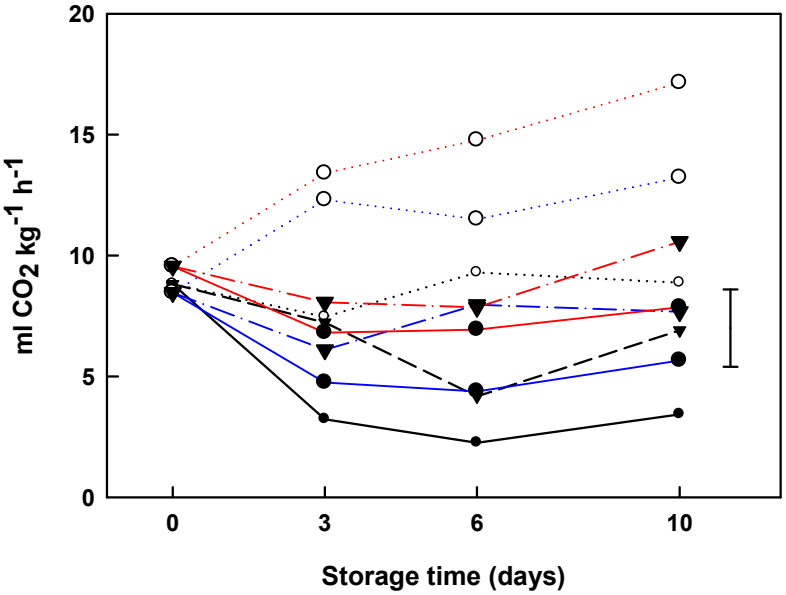


Figure 5.6 - Respiration rate of celery grown in the Late UK season and belonging to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red lines: two weeks after optimum harvest date) and stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○ symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24 h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

5.9.2 Pithiness

Results showed that pithiness was significantly affected by stage of crop maturity, yet, it was not significantly affected by postharvest gaseous treatments (see Tables C4, C5, C11, C12, C18 and C19 in Appendix C) (**Figure 5.7**). In addition, there was a significant increase in pithiness level over storage overall. Scores were particularly high in all the most over-mature samples (red lines). On the other hand, plants harvested at optimum harvest date and 2 weeks before optimum harvest date generally showed low levels of pithiness.

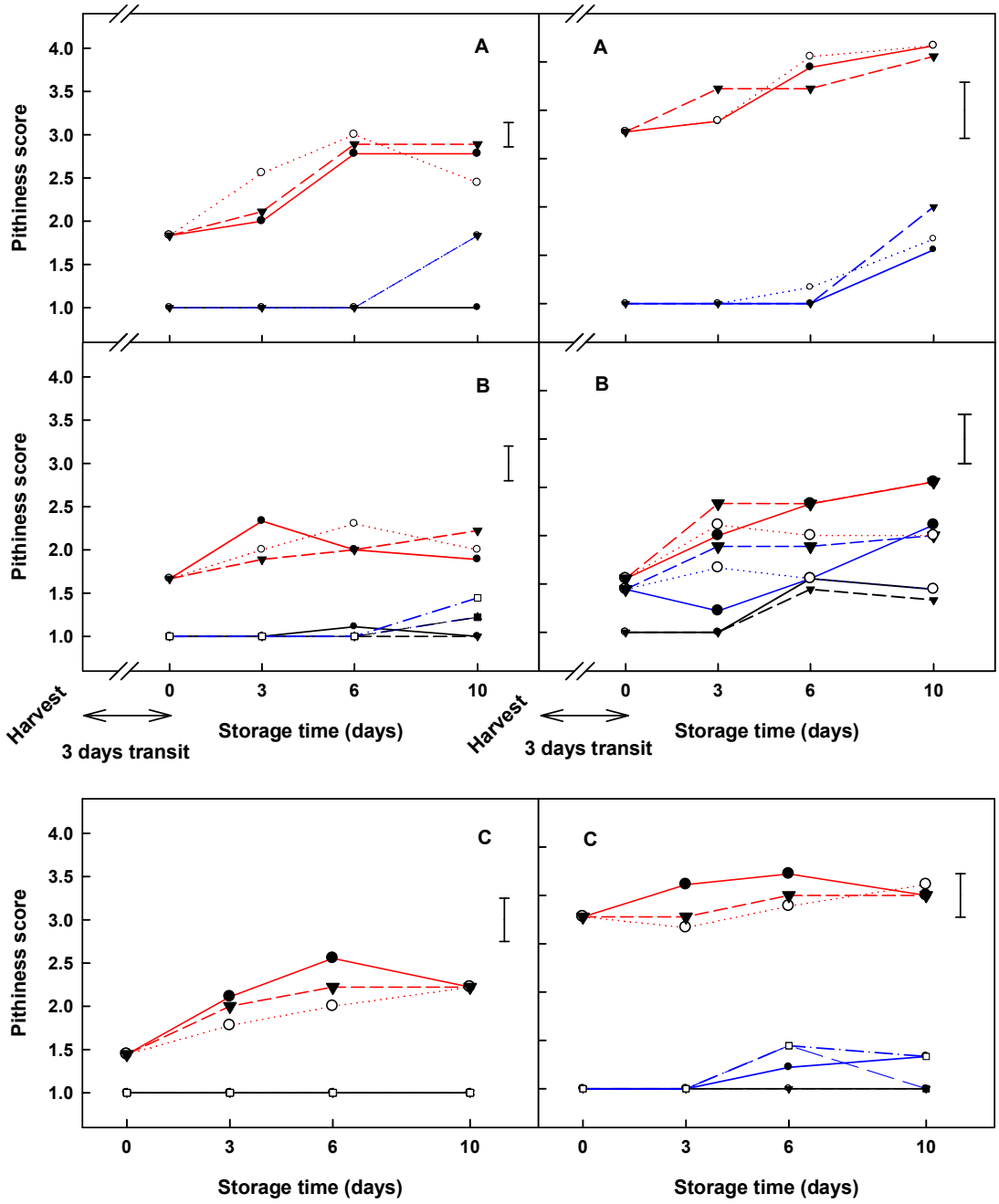


Figure 5.7 - Pithiness score of celery at cut petiole ends (on the left) and butt ends (on the right) and grown in three different seasons (A: Early Spanish season; B: Late Spanish season; C: Late UK season. Samples belong to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red lines: two weeks after optimum harvest date) and were stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○

symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24 h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

5.9.3 Cut-end browning

Similarly to pithiness, results herein showed two main patterns. Firstly, in confirmation with results from chapter 4, cut-end browning was significantly affected by plant developmental stage. The most marked difference occurred between the most immature (HM1) and the most over-mature (HM5) celery plants. Secondly, plants belonging to the same harvest maturity did not significantly develop browning regardless to ethylene and 1-MCP treatments in all the three seasons (see Tables C2, C3, C9, C6, C7, C10, C13, C14, C16, C17, C21 and C21 in Appendix C) (**Figure 5.8**).

Negative significant correlations were found between cut petiole ends browning and cut petiole ends hue angle of celery grown in all the three seasons (Early Spanish season R^2 = -0.54; Late Spanish season R^2 = -0.65; Late UK season R^2 = -0.84) (results in Appendix E).

There were also negative significant correlations between butt ends browning and butt ends hue angle of celery grown in all the three seasons (Early Spanish season R^2 = -0.67; Late Spanish season R^2 = -0.60; Late UK season R^2 = -0.71) (results in Appendix E).

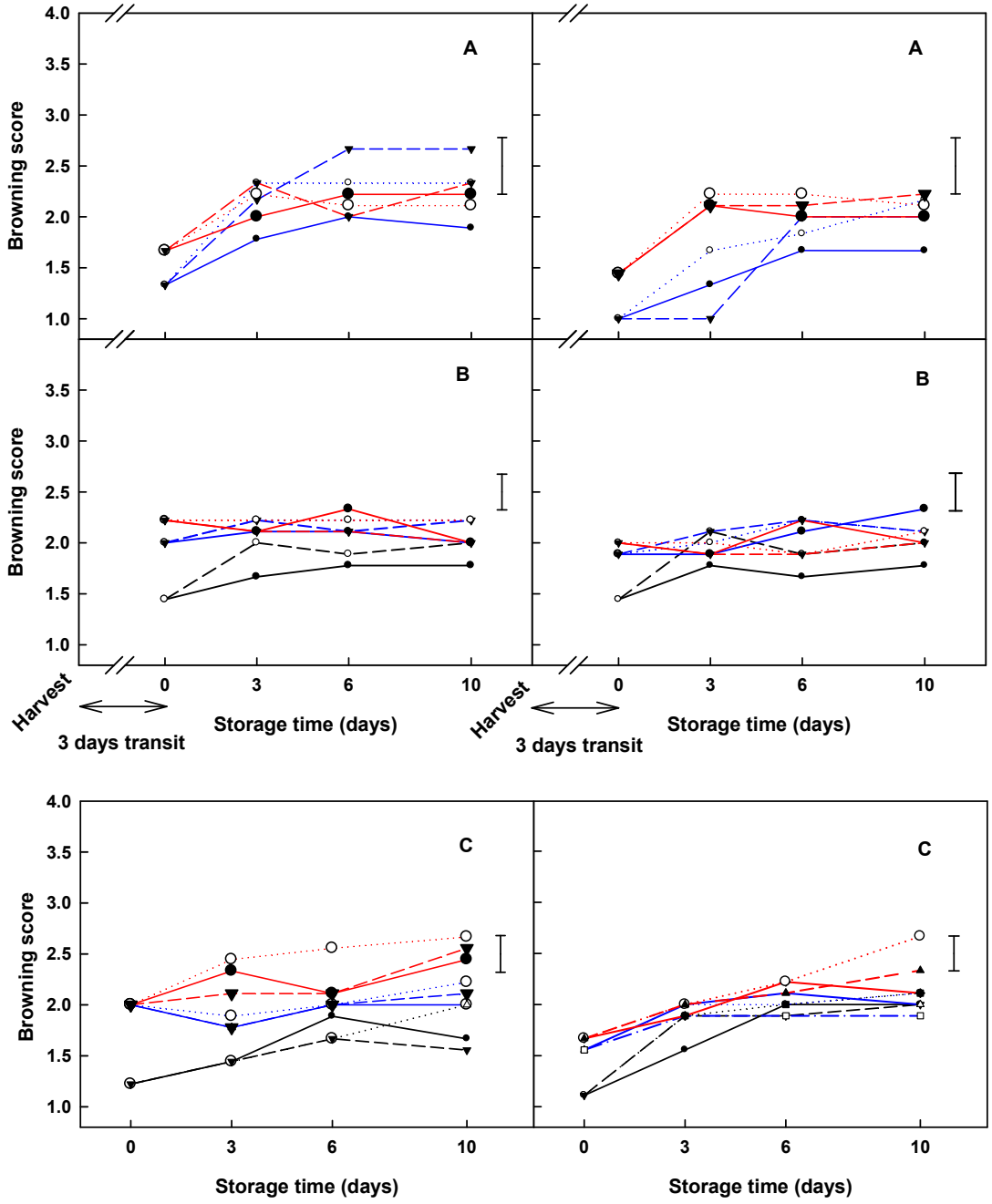


Figure 5.8 - Browning score of celery at cut petiole ends (on the left) and butt ends (on the right) and grown in three different seasons (A: Early Spanish season; B: Late Spanish season; C: Late UK season. Samples belong to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red lines: two weeks after optimum harvest date) and were stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○

symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24 h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

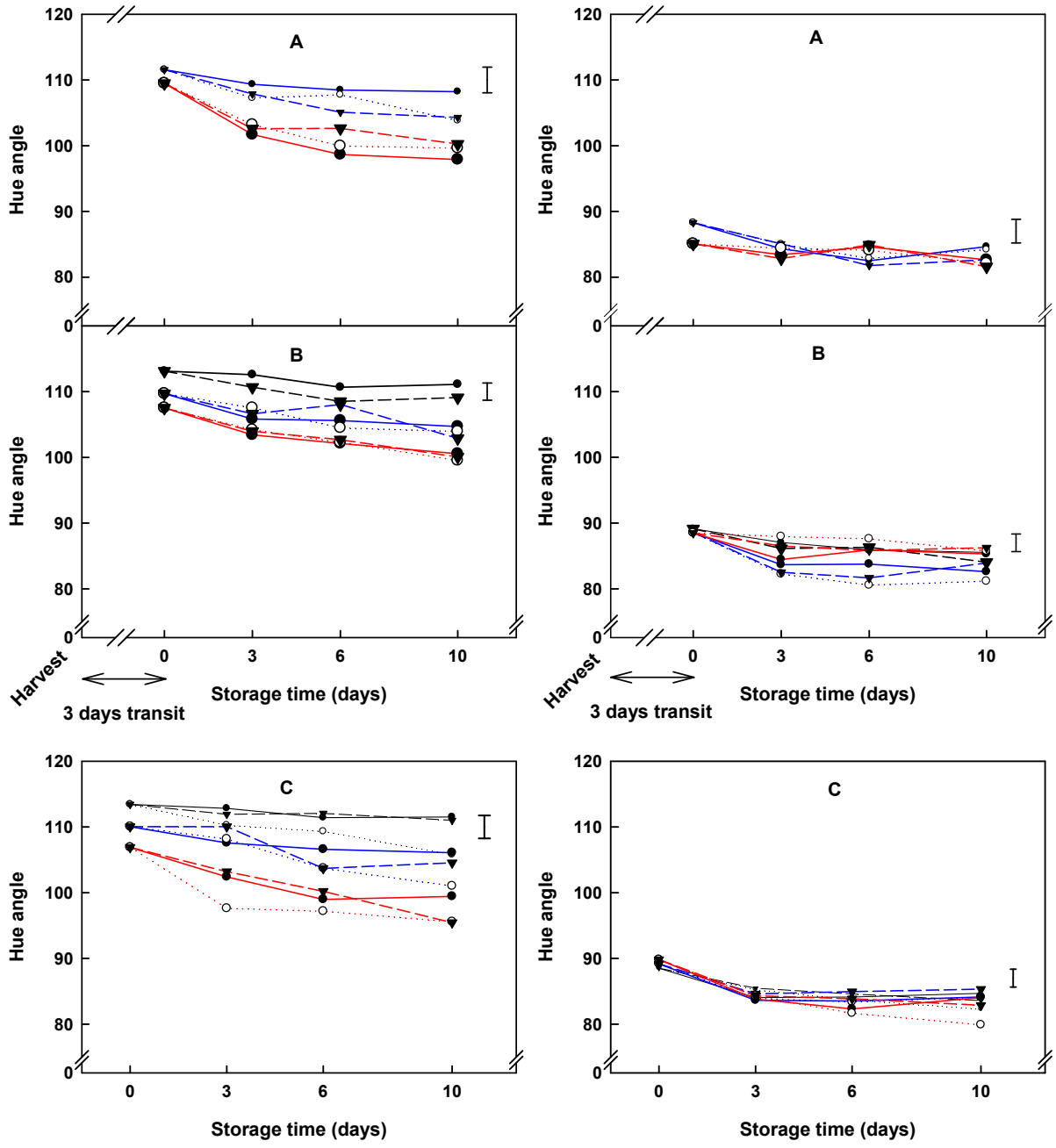


Figure 5.9 - Hue angle of celery at cut petiole ends (on the left) and butt ends (on the right) and grown in three different seasons (A: Early Spanish season; B: Late Spanish season; C: Late UK season. Samples belong to three different harvest maturities (black lines: two weeks before optimum harvest date; blue lines: optimum harvest date; red

lines: two weeks after optimum harvest date) and were stored in continuous air (point-dotted lines with ▼ symbol), 10 µL L^{-1} continuous ethylene (dotted lines with ○ symbol) and continuous air after 1 µL L^{-1} 1-MCP for 24 h at 20 °C (continuous lines with ● symbol). LSD bars are shown.

5.9.4 Sugars

HPLC-ELSD results showed that three sugars, were detected in celery: Fructose, glucose, sucrose. Mannitol, which was initially believed to be present, was not found in the celery tissues. An unknown compound has been detected but has not been identified (**Figure 5.10**).

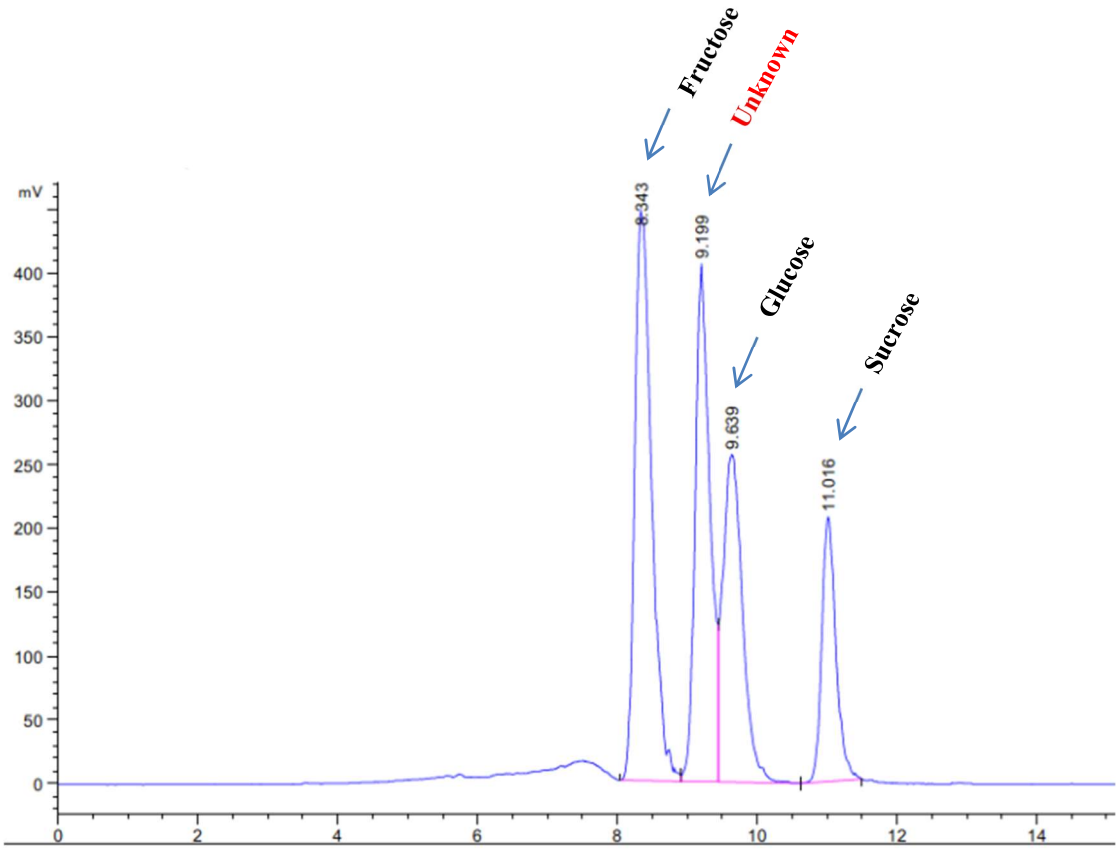


Figure 5.10 - Chromatogram showing the retention times (minutes) of all the non-structural carbohydrates isolated in butt ends and apical cut ends tissues of celery with the HPLC-ELSD.

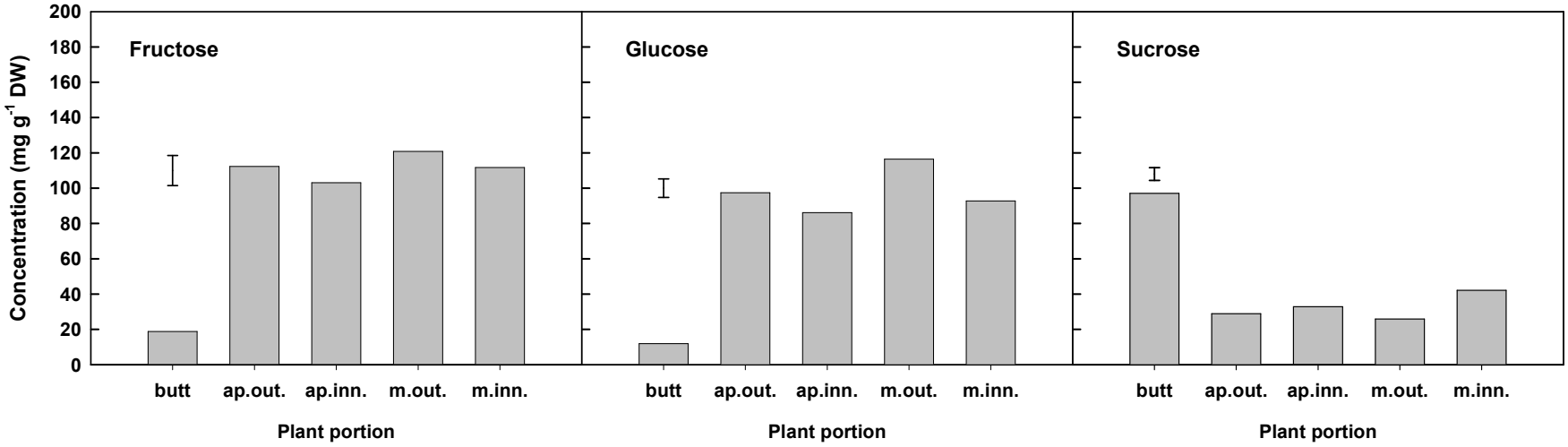


Figure 5.11 - Concentration of fructose, glucose and sucrose at different plant portions of celery grown in the late UK season and harvested at optimum maturity (HM3).

Fructose and glucose were found to be at very low concentration at butt ends of celery plants, while they were found to be at approximately 100 mg g^{-1} DW at apical outer, apical inner, middle outer and middle inner petioles (**Figure 5.11**). Sucrose followed the opposite pattern, being present at 100 mg g^{-1} DW in the butt ends and roughly at 30 mg g^{-1} DW in all the other portions. Fructose concentration was not significantly affected by continuous ethylene, air and 1-MCP treatments, remaining stable at about 20 mg g^{-1} DW at butt ends and roughly at 120 mg g^{-1} DW at apical outer petiole ends (**Figure 5.12**). Glucose sharply diminished in concentration by four-fold, from 32 mg g^{-1} DW to roughly 8 mg g^{-1} DW in all samples. In more detail, the sharp decrease occurred immediately at the beginning of storage in the ethylene and air treated samples, while it occurred after 3 days of storage in the 1-MCP treated. On the other hand, glucose concentration did not significantly change regardless of postharvest treatments (**Figure 5.13**). The concentration of sucrose steadily increased by a factor of two in the butt ends celery tissues over storage, while it did not significantly vary at apical petiole ends (**Figure 5.14**).

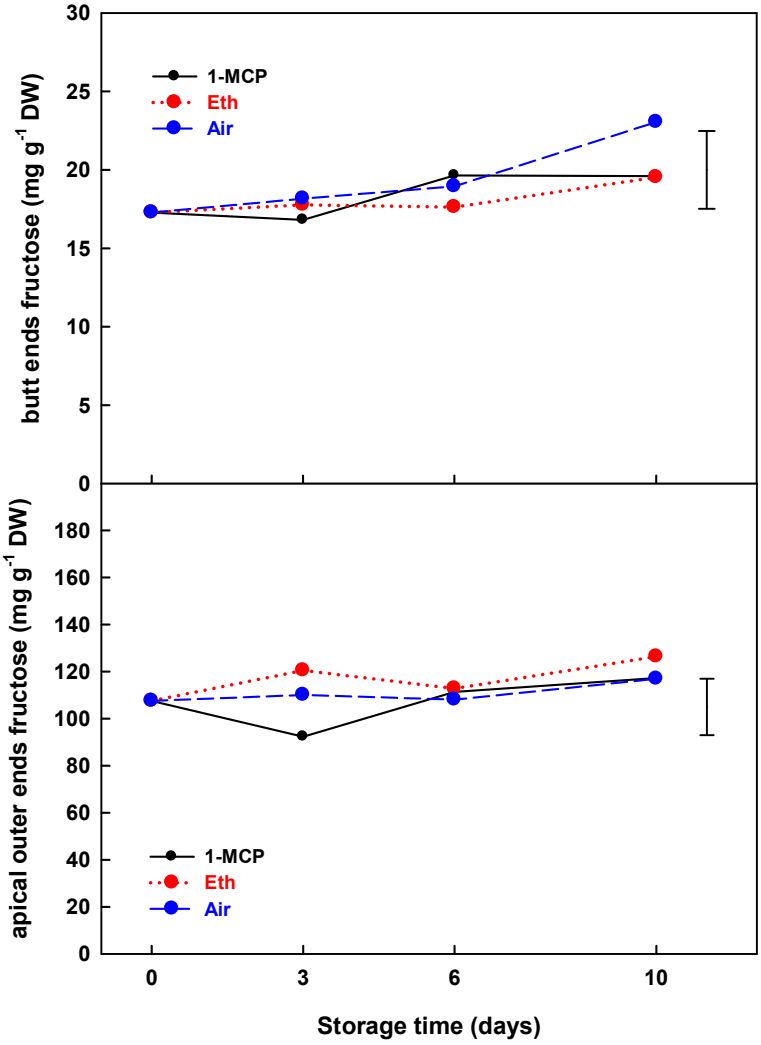


Figure 5.12 - Effect of 10 µL L^{-1} continuous ethylene and 1 µL L^{-1} 1-MCP treatments on the fructose concentration at cut petiole and butt ends of celery grown in the late UK season and harvested at optimum maturity (HM3). LSD bars are shown.

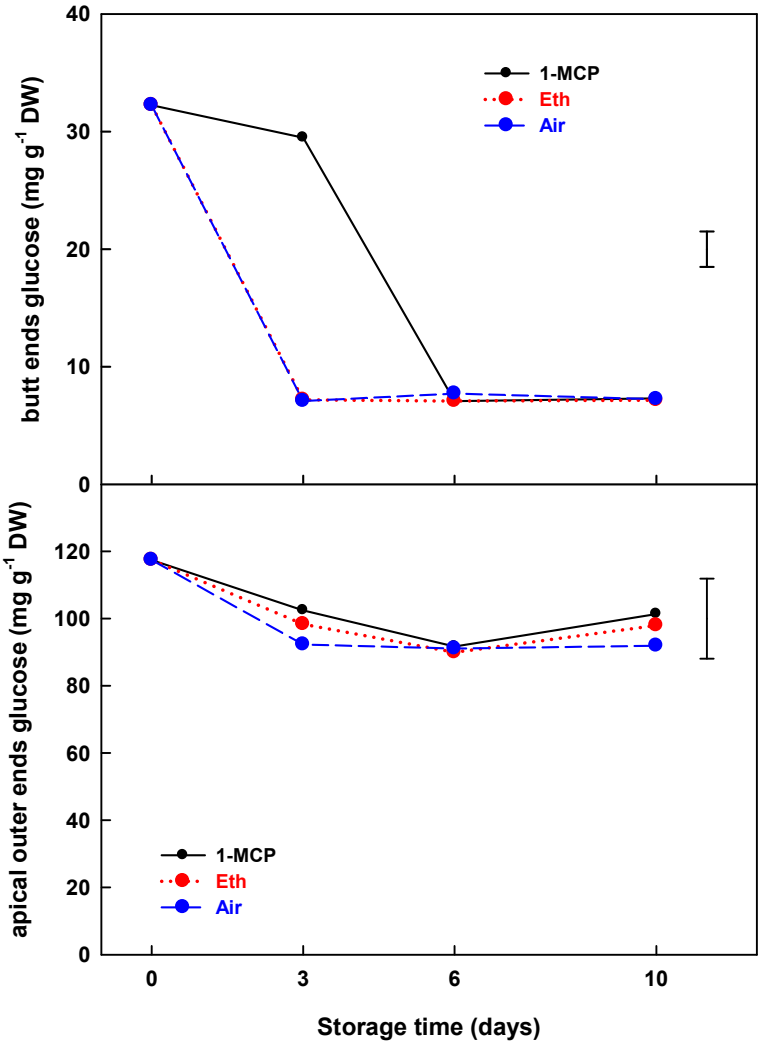


Figure 5.13 - Effect of 10 µL L^{-1} continuous ethylene and 1 µL L^{-1} 1-MCP treatments on the glucose concentration at cut petiole and butt ends of celery grown in the late UK season and harvested at optimum maturity (HM3). LSD bars are shown.

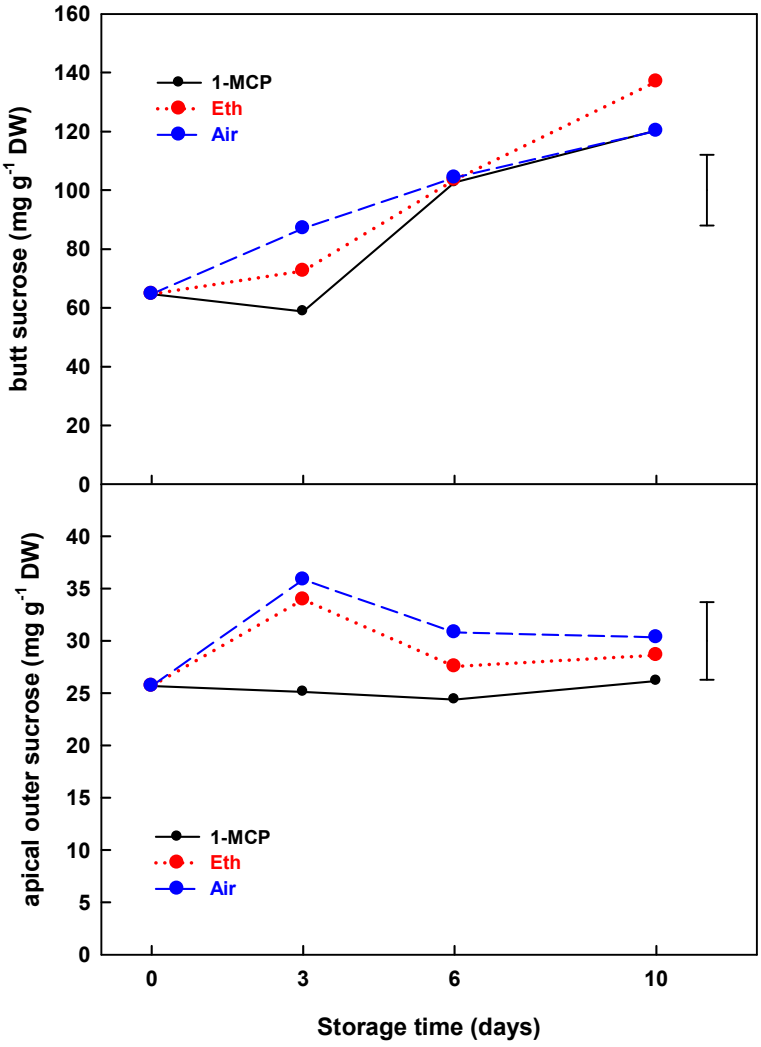


Figure 5.14 - Effect of 10 µL L^{-1} continuous ethylene and 1 µL L^{-1} 1-MCP treatments on the sucrose concentration at cut petiole and butt ends of celery grown in the late UK season and harvested at optimum maturity (HM3). LSD bars are shown.

5.10 Discussion

It is well known that ethylene stimulates many physiological processes in plants related to biotic and abiotic stresses as well as senescence and decay (Blankenship, 2001). During all these processes, a high amount of energy is released in the plant metabolism, consequently resulting in increased respiratory activity. The effect of 1-MCP has been extensively studied in several products and it showed to reduce the incidence of several physiological disorders and pathological diseases at postharvest level (Watkins, 2008).

In the present study, it was hypothesized that continuous ethylene supplementation and 1-MCP treatment has significant effect on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery. Yet, results arising from this experimental work showed that this assumption was not true. In effect, cut-end browning and pithiness were not significantly affected by ethylene and 1-MCP treatments. Current literature reports that ethylene applied using Ethephon directly to celery plants grown in protected environment significantly stimulated pithiness, especially at the basal part of the petioles (Pressman *et al*., 1984). Firstly, the celery cultivar used by the previous authors (cv. Florida 683) was different from that used in this study (Monterey and Victoria). Secondly, Pressman *et al*., (1984) does not specify the exact quantity of Ethephon applied to the samples. Furthermore, the authors dispensed ethylene only once pre-harvest while, in the present study, 10 μL L^{-1} of the exogenous phyto-hormone were applied postharvest in a continuous flow at 5 °C throughout the storage trial. As well as ethylene, 1-MCP treatment did not show any significant effect on pithiness and cut-end browning. Literature on the influence of exogenous ethylene in celery is scarce. It has only been reported that it stimulates the synthesis of phenolic compounds without any focus to stem-end discolouration (Heredia and Cisneros-Zevallos, 2009). Saltveit, (2004) found that exposing whole heads of Iceberg lettuce to 1 μL L^{-1} 1-MCP at 5 °C resulted in a decrease in the accumulation of phenolic compounds and subsequent tissue browning previously induced by continuous ethylene exposure at 10 μL L^{-1}. 1-MCP has never been used on celery, and Saltveit results contrast with what was found in this study, suggesting that, contrary to lettuce, the mechanism of celery browning is not promoted by endogenously produced ethylene.

Even though both postharvest treatments did not affect cut-end browning and pith development, a clear effect was observed on the respiration rate of celery. In more detail, exogenous ethylene had a stimulating effect while 1-MCP had an inhibitory one. These findings are in accordance with Blankenship (2003). It is well known that ethylene induces an increase in the metabolic activity of living tissues, yet such metabolic activity does not seem to be involved in the mechanisms of browning reaction. When ethylene binds to its receptors embedded in plant cells, it elicits a cascade of events that lead to various senescence processes like leaf yellowing, abscission of flowers and fruit ripening (Blankenship, 2001). On the other hand, 1-MCP

blocks ethylene action by binding to its receptors, consequently preventing senescence to occur. The detailed mechanisms of the cascade of physiological/biochemical events induced by ethylene and leading to product decay still remains unclear. However, such underlying processes require high amounts of energy to occur, resulting in consequent increased respiration rate. This could explain the fact that, in the present study, exogenous ethylene showed to stimulate celery respiration rate while 1-MCP has showed to inhibit it.

Biochemical analyses results showed that the sugar metabolism seems to be more active and variable at butt ends rather than apical cut petiole ends of celery. At bottom cut-ends, the concentration of glucose decreased over storage, whilst sucrose concentration followed the opposite trend, independently of postharvest gaseous treatments. This seems to suggest that glucose is consumed by the plants over time for obtaining energy. It is likely that this phenomenon, in parallel with the continuous accumulation of sucrose over storage, is due to the usual rise in metabolic activity of plants over storage, leading to quality loss.

5.11 Conclusions

The present experimental work has shown that the assumption that continuous ethylene supplementation and 1-MCP treatment having a significant effect on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery was not true. According to the findings arising from the present experimental work, postharvest browning of celery seems to be independent of ethylene. Furthermore, plant senescence processes may play a key role in stimulating the biochemical mechanisms that lead to the reduction of visual quality in this crop. Even though this study has elucidated some aspects, literature on celery still remains sparse, especially on the underlying physiological and biochemical mechanisms and the role that pre-harvest factors have on its postharvest visual quality. Hence, supplementary research needs to be conducted to better clarify the relative physiology and biochemistry.

6. EFFECT OF DEFICIT IRRIGATION ON THE VISUAL QUALITY, PHYSIOLOGY AND BIOCHEMISTRY OF FRESH-CUT CELERY

6.1 Introduction

Current literature reports scarce research on the influence of pre-harvest factors on cut-end browning in celery. Guerra *et al*., (2010) showed that browning potential and total quinone content was higher in over-mature plants of the self-blanching Golden Clause celery cv. In contrast, lettuce, another crop which suffers from postharvest discolouration, has been extensively studied. For instance, Luna *et al*., (2013) found that cut-edge browning was 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 despite the accumulation of phenolic compounds (Luna *et al*., 2012). The findings revealed in these two studies appear contradictory as higher production of phenolic compounds should result in higher oxidation, thus, more pronounced cut-edge browning. Further investigation is needed to better elucidate the links between irrigation management and cut-end discolouration with the relative biochemistry in celery.

6.2 Aim of the study

The aim of this study is to investigate the different degrees of deficit irrigation have on the visual quality, physiology and biochemistry of fresh-cut celery.

6.3 Specific objectives

- To determine the effects that water stress has on postharvest cut-end browning, pithiness and respiration rate of fresh-cut celery.
- To investigate variations in the metabolism of sugars and phenolic compounds as affected by water treatments.

6.4 Hypothesis

The experiment was carried out with the following hypotheses:

6.4.1 Null hypothesis

Water stress has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

6.4.2 Alternate hypothesis

Water stress has no significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

6.5 Plant material

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 6.1**) located at Cranfield University, Bedfordshire, UK, was used to carry out the trial, which started on 7th July 2015 and finished on 20th November 2015. Imperial cv. celery plants were grown in 12 L capacity plastic pots (diameter: 30cm, height: 23cm) filled with Fenland peat (**Figure 6.2**) 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 in 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® (2.5g in 5 L of water) and Movento® (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). This rate of nutrition was decided according to the standard fertilization practice adopted by

G's Fresh Ltd. Fertilization was applied once before commencement of water treatments. 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 otherwise could be detrimental to the crop. Optimum harvest date was adjudged by 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 6.1 - Garden polytunnel (Haygrove Ltd) used to carry out the deficit irrigation trial (photo taken by the author).

Figure 6.2 - Fenland peat used to grow celery plants (photo taken by the author).

Information on the physico-chemical characteristics of the Fenland peat soil collected from Dimmocks Cote Farm are not reported in this Thesis as it was not possible for G's Fresh Ltd to provide relative data.

6.6 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 6.3**). Three irrigation treatments were applied: 800 (control irrigation), 600 (light water stress) and 400 (heavy water stress) mL every alternate day. Water was administered at 11:00am. Control irrigation treatment was decided according to the standard celery growing practise adopted by G's Fresh Ltd. Control irrigation treatment (800 mL per alternate day) corresponded to the amount of water bringing the soil to field capacity (45% volumetric water content). The other two treatments were applied to induce two different levels of drought stress in celery plants. Field capacity was derived by watering soil to full saturation, waiting until excess water drained away and measuring moisture from the growing pots. Plants were divided into three main blocks, each of which was arranged in three main plots. Each block received a different irrigation treatment. Each main plot was composed by 15 plants (12 for postharvest analyses and 3 additional to spare). Plots were arranged in a completely randomized design as shown in (

Figure 6.4). 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 6.6**).

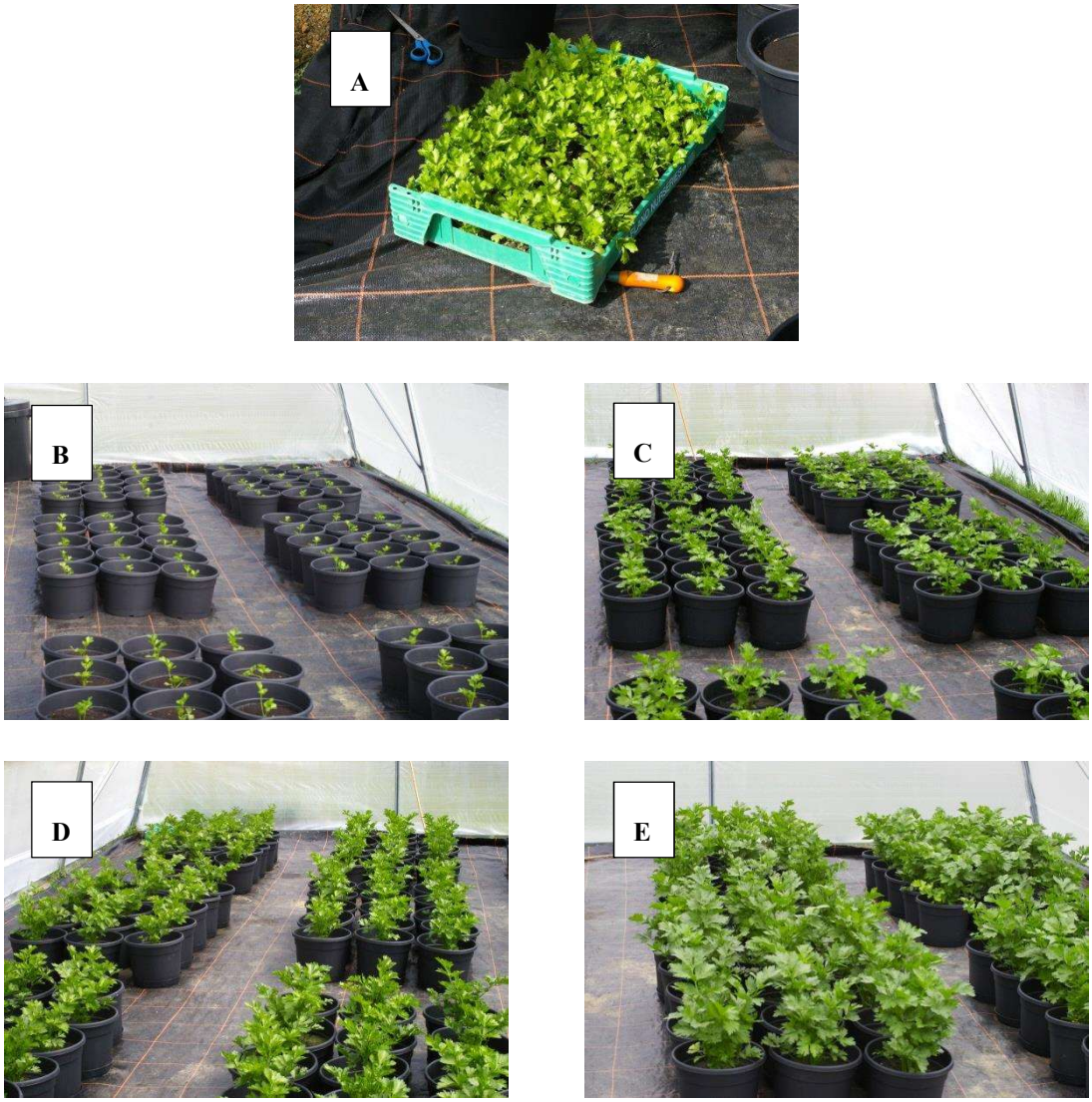


Figure 6.3 - Growing stages of celery before commencement of water treatments expressed in days after planting (DAP): plug plants (A); 5 DAP (B); 16 DAP (C); 27 DAP (D); 38 DAP (E) (photos taken by the author).

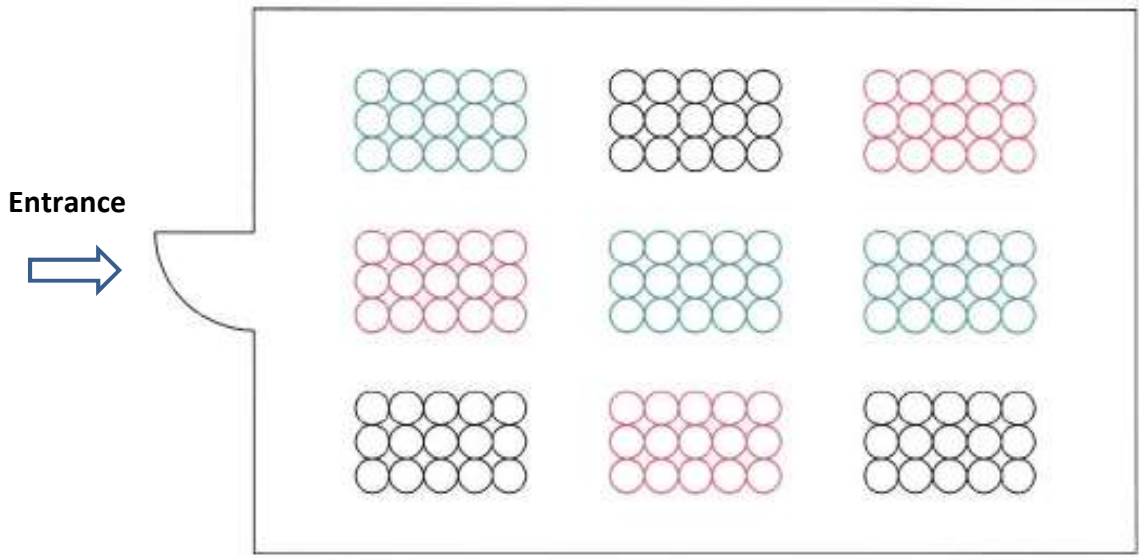


Figure 6.4 - Disposition of pots (circles) in the experimental polytunnel. Colours correspond to the application of different water treatments (black: 800; green: 600; red: 400 mL every alternate day).

Figure 6.5 - Harvested celery grown during the irrigation trial (photo taken by the author).

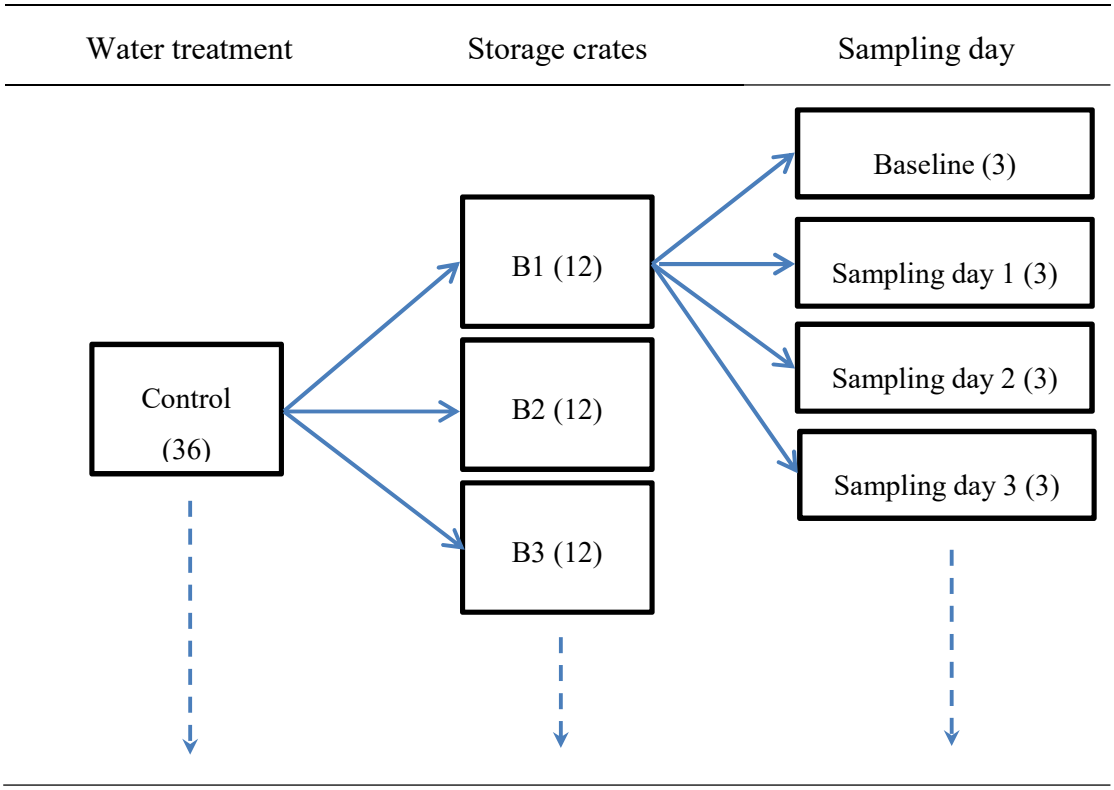


Figure 6.6 - Celery irrigation experiment layout (the number of samples are indicated in brackets).

6.7 Soil moisture measurement

Soil moisture content was measured periodically by impedance using a Thetaprobe meter (ThetaKit type ML2x, Delta-T Devices, Cambridge, UK) (**Figure 6.7**) to confirm irrigation treatments. 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 or % soil volume).

Figure 6.7 - Thetaprobe meter used to ascertain soil moisture (ThetaKit type ML2x) (photos taken by the author).

6.8 List of visual assessments, physiological attributes and biochemical analysis taken

In this experiment, the following analysis were taken: subjective colour (section **3.2.1**), pithiness (section **3.2.2**), bolting (section **3.2.3**), respiration rate (section **3.3.1**), objective colour (section **3.3.2**), dry matter content (section **3.5**), extraction and quantification of phenolic compounds (section **3.4.2**), extraction and quantification of non-structural carbohydrates (section **3.4.3**), extraction and quantification of plant growth regulators (PGRs) (section **3.4.4**).

6.9 Statistical analysis

Data analysis was done with the software STATISTICA, file version 13.2. (StatSoft, Inc. UK). Means were firstly checked for their residuals and secondly submitted to Analysis of variance (ANOVA) 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 treatments and storage time as factors. Respiration rate, subjective colour, objective colour, pithiness, dry matter content, phenolic compounds, non-structural carbohydrates and plant growth regulators were included in the design as variables. Blocks were taken as main statistical units. Least Significant Differences (LSDs; $P= 0.05$) were calculated from each analysis to compare the means.

6.10 Results and discussion

6.10.1 Soil water status

Volumetric water content of the growing medium (Fenland peat) was significantly affected by irrigation treatments (see Table D1 in Appendix D) (**Figure 6.8**). Values ranged from 13.5 to 47.0 m^3 m^{-3} of soil. Root development was also greatly influenced by water treatments. Volumetric water content fluctuated throughout the examined growing period (from commencement of water treatments to the 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.

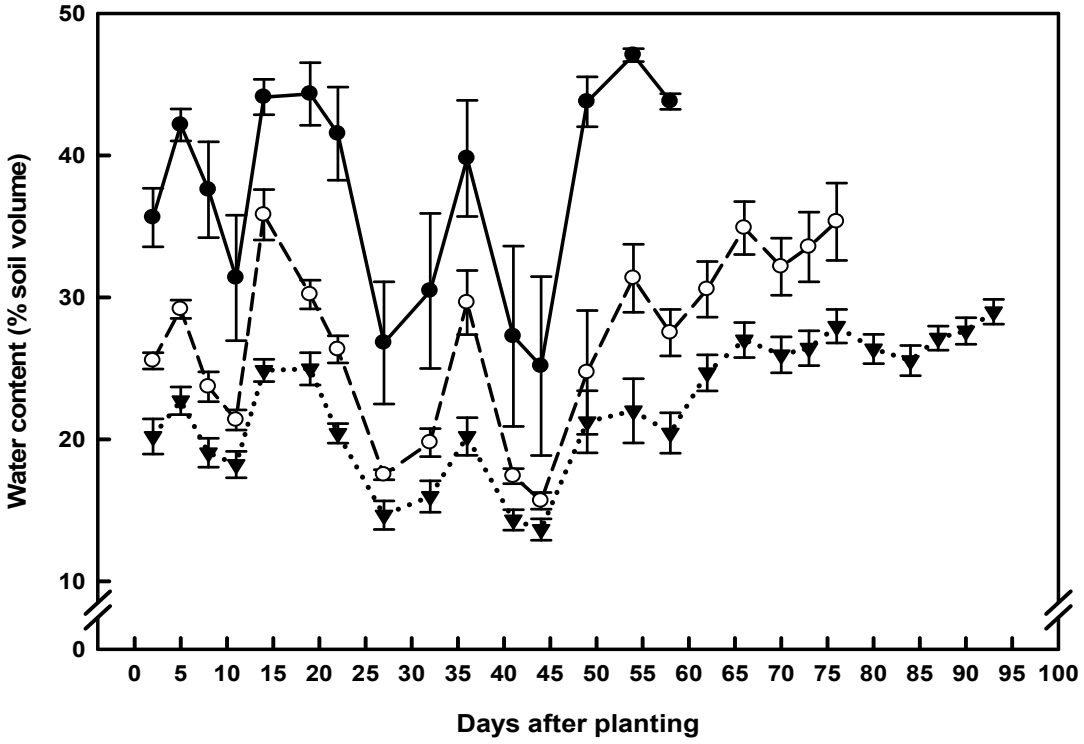


Figure 6.8 - Water volume of the growing substrate (Fenland peat) used in the celery irrigation trial. Different symbols correspond to each water treatment: 800 mL (●), 600 mL (○) and 400 mL (▼) every alternate day. Values represent the means every alternate day of soil moisture content in the pots. Standard error bars of the means are shown.

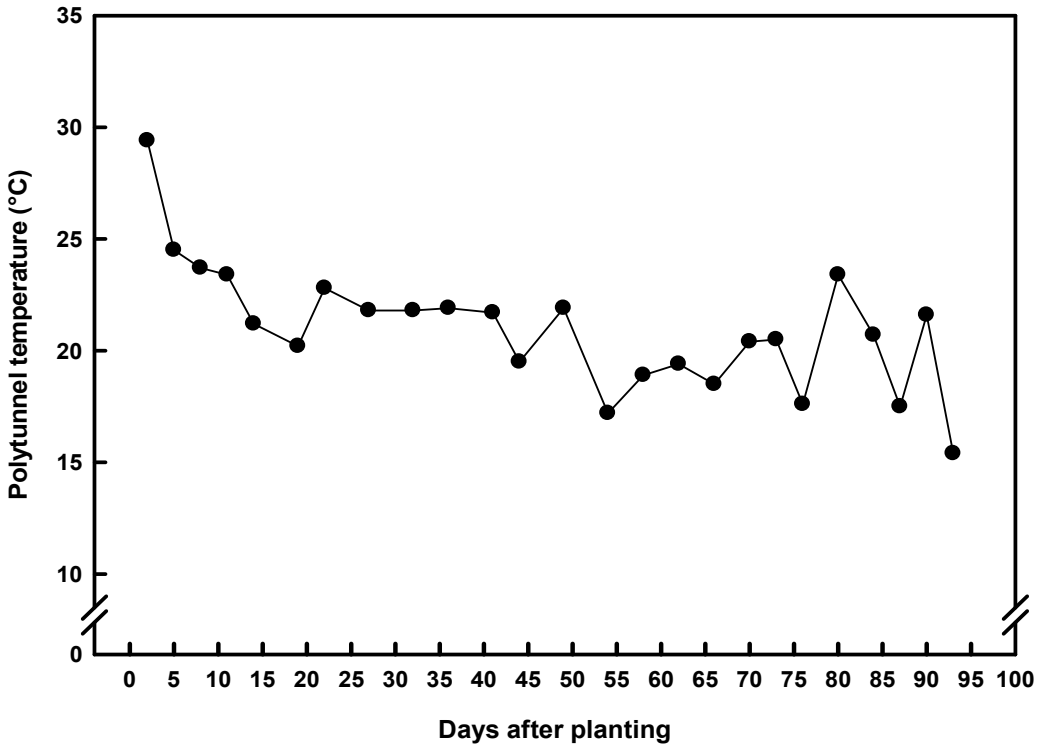


Figure 6.9 - Daily polytunnel temperatures recorded during the celery irrigation trial.

6.10.2 Respiration rate

Overall, postharvest respiration rate was significantly influenced by water treatments and time held in storage (see Table D2 in Appendix D) (**Figure 6.10**). Values ranged from 10.5 to 22.0 mL CO_2 kg^{-1} h^{-1}. 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 800 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 fluctuated with no apparent trend. 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.

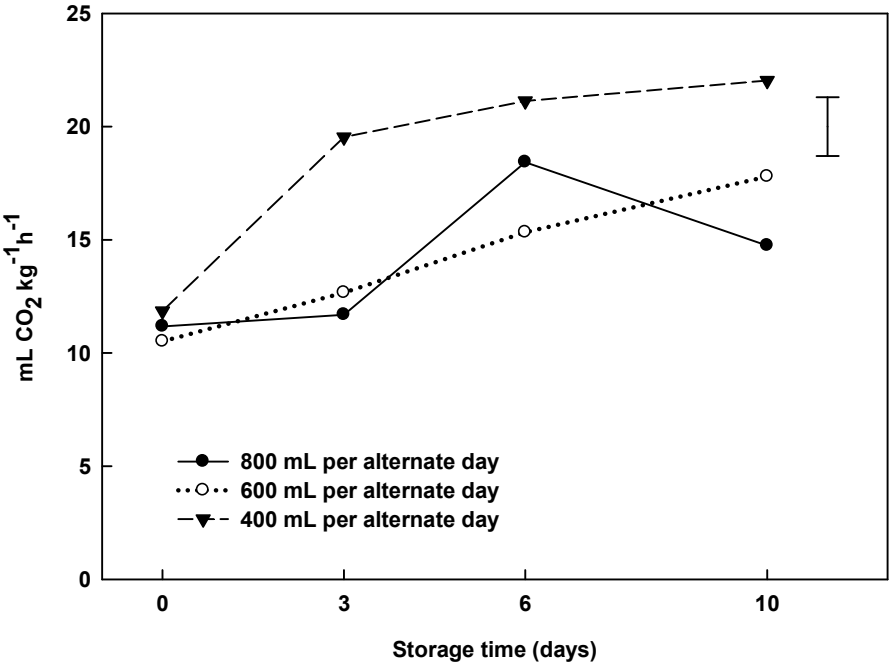


Figure 6.10 - Effect of water treatments on the respiration rate of celery. Lines indicate different water treatments: 800 mL every alternate day (continuous lines with ● symbol), 600 mL every alternate day (dotted lines with ○ symbol) and 400 mL every alternate day (point-dotted lines with ▼ symbol). LSD bars are shown.

6.10.3 Cut-end browning

The two above graphs show a clear pattern. There was a significant increase in browning over storage time. Additionally, browning severity significantly developed with the degree of water stress imposed (see Tables D3 and D4 in Appendix D) (**Figure 6.11**). In more detail, 800 mL per alternate day irrigated samples showed the lowest level of browning. Overall, most of the discolouration developed during the initial phase of storage. In general, increasing level of stress induced by reduced irrigation treatments promoted greater cut-end browning. These results are in contrast 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.

Hue angle significantly decreased over storage time and with water stress treatment applied to plants (**Figure 6.12**), confirming the subjective visual assessments reported in

Figure 6.11 (see Tables D7 and D8 in Appendix D). In other words, the intensity of browning was higher following the reduced irrigation regimes, particularly for celery butts.

Cut petiole ends browning was strongly and negatively correlated with cut petiole ends hue angle (R^2 = -0.84). There was also a strong and negative correlation between butt ends browning and butt ends hue angle (R^2 = -0.83) (results in Appendix E).

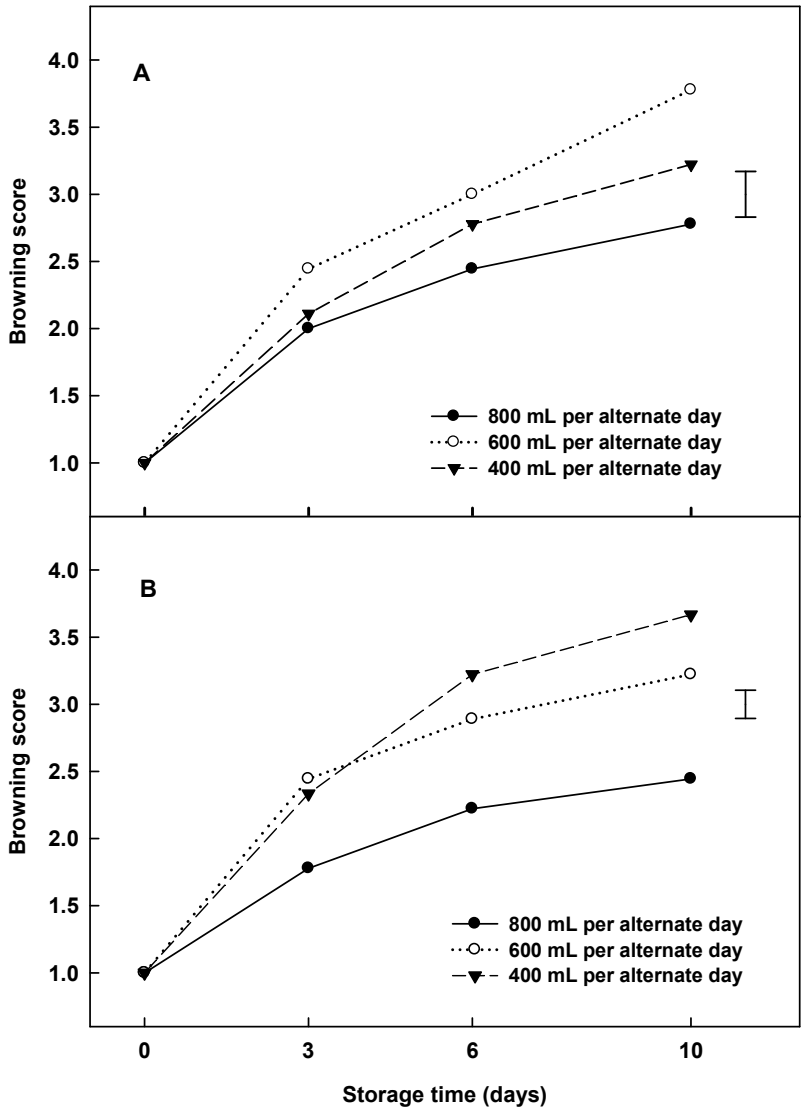


Figure 6.11 - Effect of water treatments on the cut-end browning at cut petiole ends (A) and butt ends (B) of celery. Lines indicate different water treatments: 800 mL every alternate day (continuous lines with ● symbol), 600 mL every alternate day (dotted lines

with ○ symbol) and 400 mL every alternate day (point-dotted lines with ▼ symbol). LSD bars are shown.

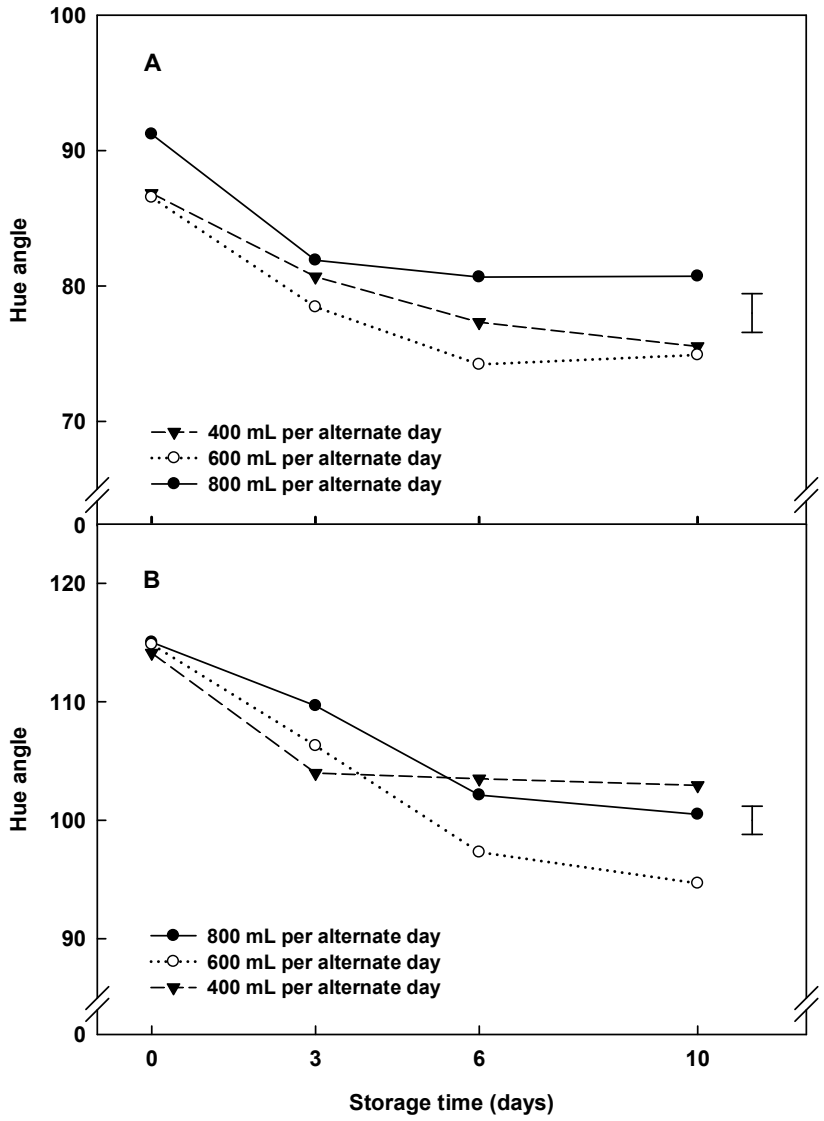


Figure 6.12 - Effect of water treatments on the hue angle at cut petiole ends (A) and butt ends (B) of celery. Lines indicate different water treatments: 800 mL every alternate day (continuous lines with ● symbol), 600 mL every alternate day (dotted lines with ○ symbol) and 400 mL every alternate day (point-dotted lines with ▼ symbol). LSD bars are shown.

6.10.4 Pithiness

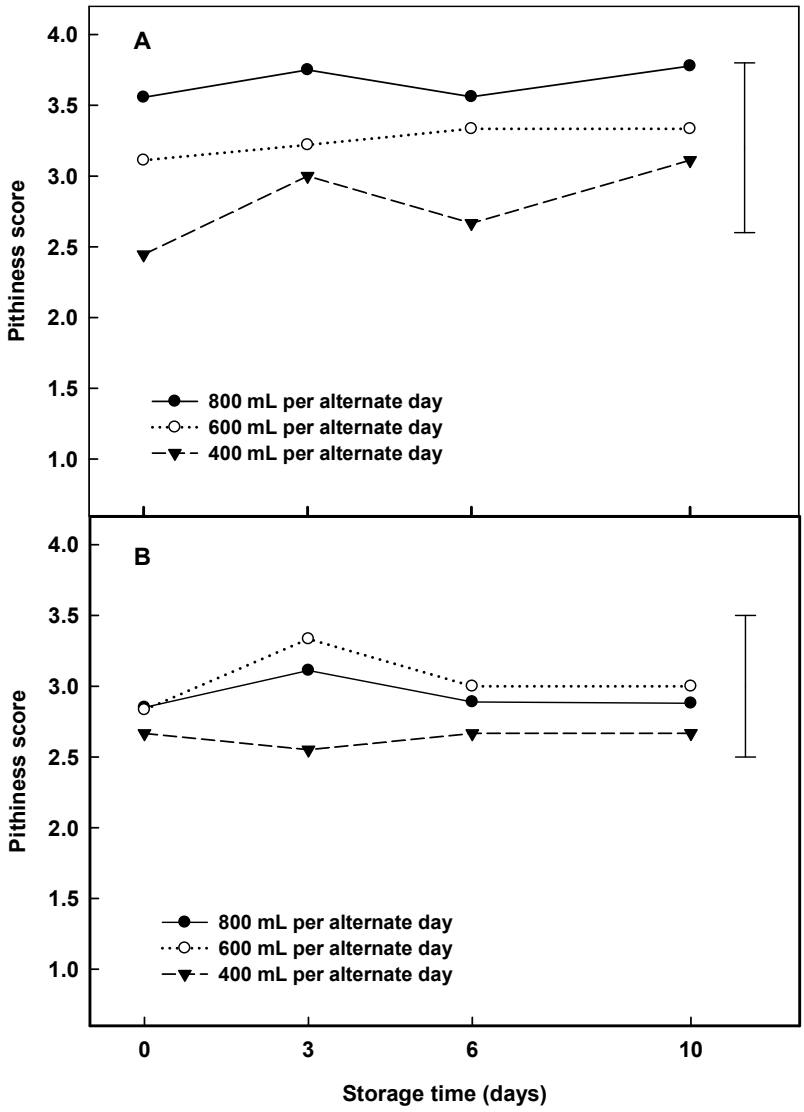


Figure 6.13 - Effect of water treatments on the pithiness at cut petiole ends (A) and butt ends (B) of celery. Lines indicate different water treatments: 800 mL every alternate day (continuous lines with ● symbol), 600 mL every alternate day (dotted lines with ○ symbol) and 400 mL every alternate day (point-dotted lines with ▼ symbol). LSD bars are shown.

Pithiness was generally high at both butt and cut petiole ends of celery, with scores ranging from 2.5 to 4.0 (**Figure 6.13**). However, No significant differences in pithiness were found between irrigation regimes (see Tables D5 and D6 in Appendix D). The

prominent pithiness could be probably the result of the infrequent irrigation applied throughout the trial, which was administered every alternate day. This statement finds confirmation in a previous study conducted by Breschini and Hartz (2002) on celery grown in the open field with a drip irrigation system. Celery grows best in soils with constant soil moisture. Thus, it is likely that lack of constant soil moisture may have triggered the stress response in plants, manifested by the collapse of parenchyma tissue.

6.10.5 Dry matter content

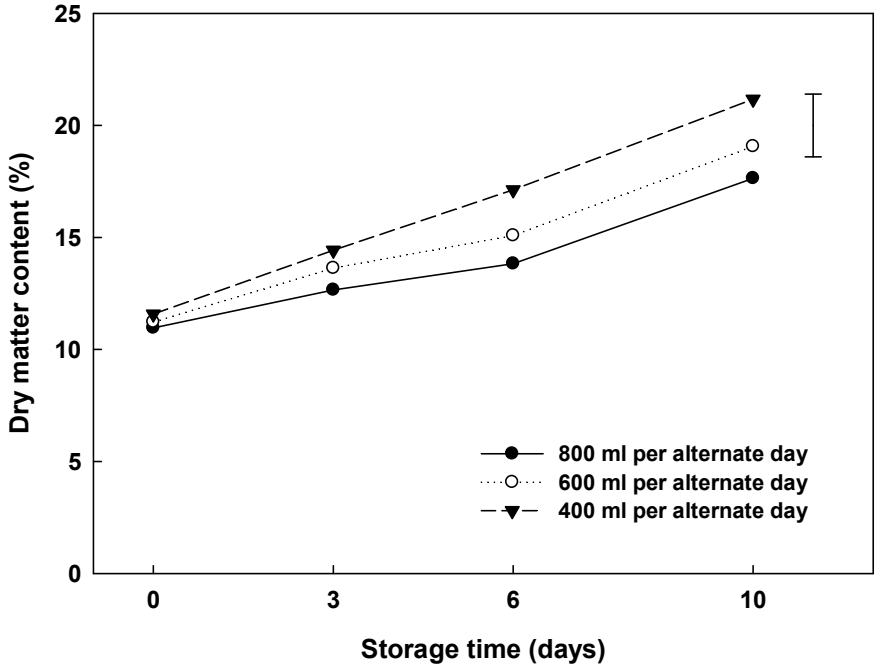


Figure 6.14 - Effect of water treatments on the dry matter content of celery. Lines indicate different water treatments: 800 mL every alternate day (continuous lines with ● symbol), 600 mL every alternate day (dotted lines with ○ symbol) and 400 mL every alternate day (point-dotted lines with ▼ symbol). LSD bar is shown.

Application of water treatments resulted in a significant increase in dry matter content of celery plants (see Table D9 in Appendix D) (**Figure 6.14**). The increase was the highest in the plants which received the least amount of irrigation (400 mL per alternate day), almost doubling linearly in their values after 10 storage days. On the other hand, the control-irrigated plants increase was the smoothest.

Dry matter content was highly and positively correlated with cut ends browning (R^2 = 0.80) (results in Appendix E).

6.10.6 Sugars

Fructose, glucose and sucrose content decreased over storage, especially in the celery samples irrigated with 800 and 600 mL every alternate day at cut petiole ends. On the other hand, concentration of all compounds was relatively stable over storage at the butt end region, with the exception for sucrose (**Figure 6.15**, **Figure 6.16** and **Figure 6.17**). Values of sugars ranged between 112 and 9 mg g^{-1} DW. Celery plants which received the most stressful irrigation treatment (400 mL every alternate day) had the most stable sugars concentration during storage overall. Fructose and glucose were generally higher at cut petiole ends rather than butt ends. This trend was opposite for sucrose, which was at minimum content at cut petiole ends. These findings correspond with Keller and Matile (1989) who reported that sucrose concentration was lower in the parenchyma tissue of celery petioles compared to other sugars. In general, the decreasing trend of fructose and glucose over storage and with higher levels of water stress applications could be due to the activation of plant defence mechanisms. Some examples include large root development, variations in stomatal conductance and production of secondary metabolites (Bolat *et al*., 2014). An important physiological mechanism is explained by Pereira and Chaves (1993). According to the authors, photoassimilates allocate in different plant portions when exposed to drought stress, as result of their major contribution as osmotic solutes. In effect, simple sugars attract water, thus, enhancing cell turgor. In the case of this experiment, simple sugars are more present in the control irrigated plants than in the stressed ones. This contradictory result has two possible explanations. Firstly, glucose and fructose could have been predominantly used to supply energy requirements of the water stress physiological mechanisms. Secondly, it is likely that other molecules are involved in the osmotic stress adaptation in celery. Two examples include sugar-alcohols and aminoacids (Bartels and Sunkar, 2005).

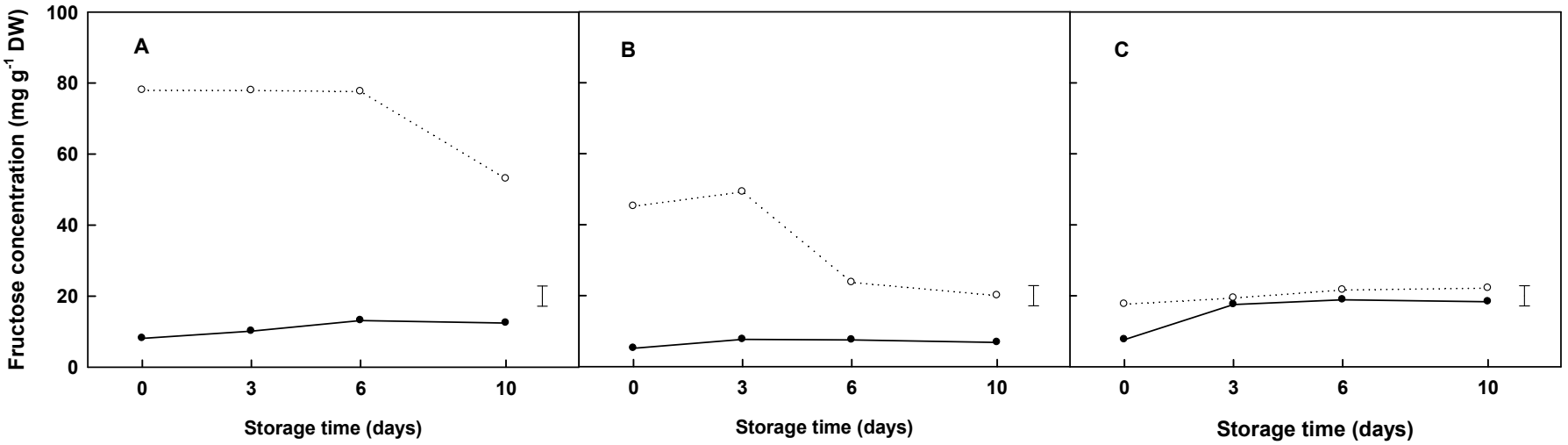


Figure 6.15 - Effect of water treatments on the fructose concentration at cut petiole ends (dotted lines with ○ symbol) and butt ends (continuous lines with ● symbol) of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) every alternate day.

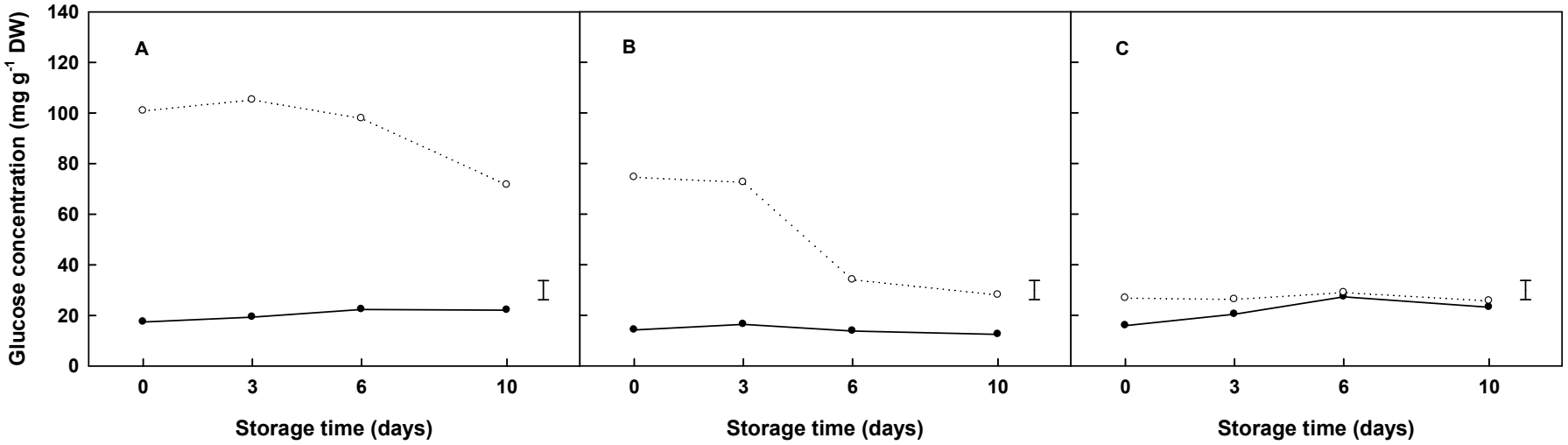


Figure 6.16 - Effect of water treatments on the glucose concentration at cut petiole ends (dotted lines with ○ symbol) and butt ends (continuous lines with ● symbol) of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) every alternate day.

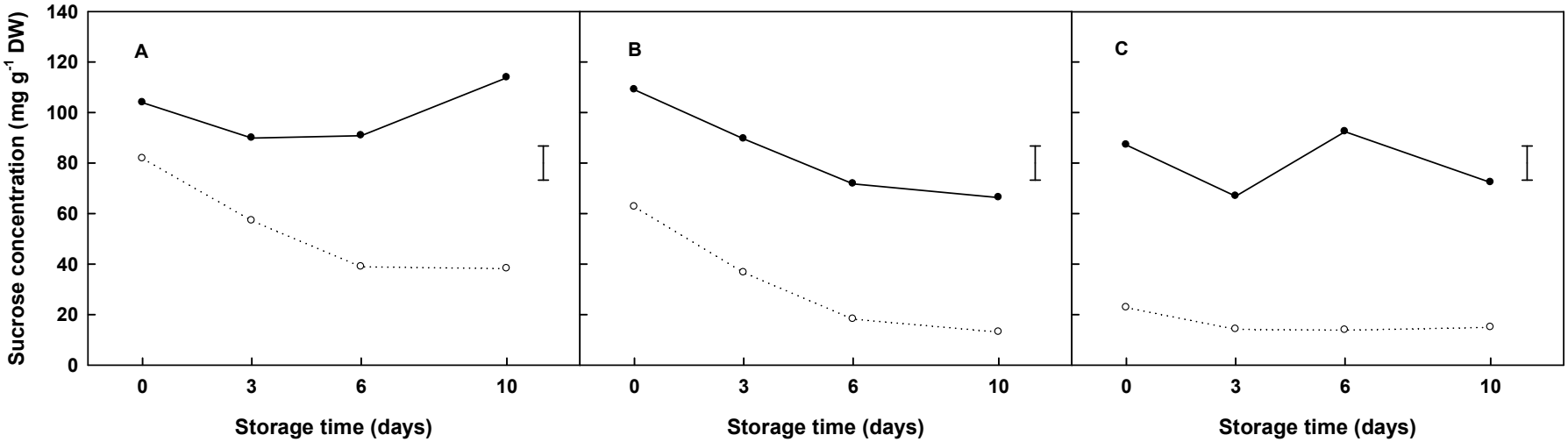


Figure 6.17 - Effect of water treatments on the sucrose concentration at cut petiole ends (dotted lines with ○ symbol) and butt ends (continuous lines with ● symbol) of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) every alternate day.

6.10.7 Chlorogenic acid

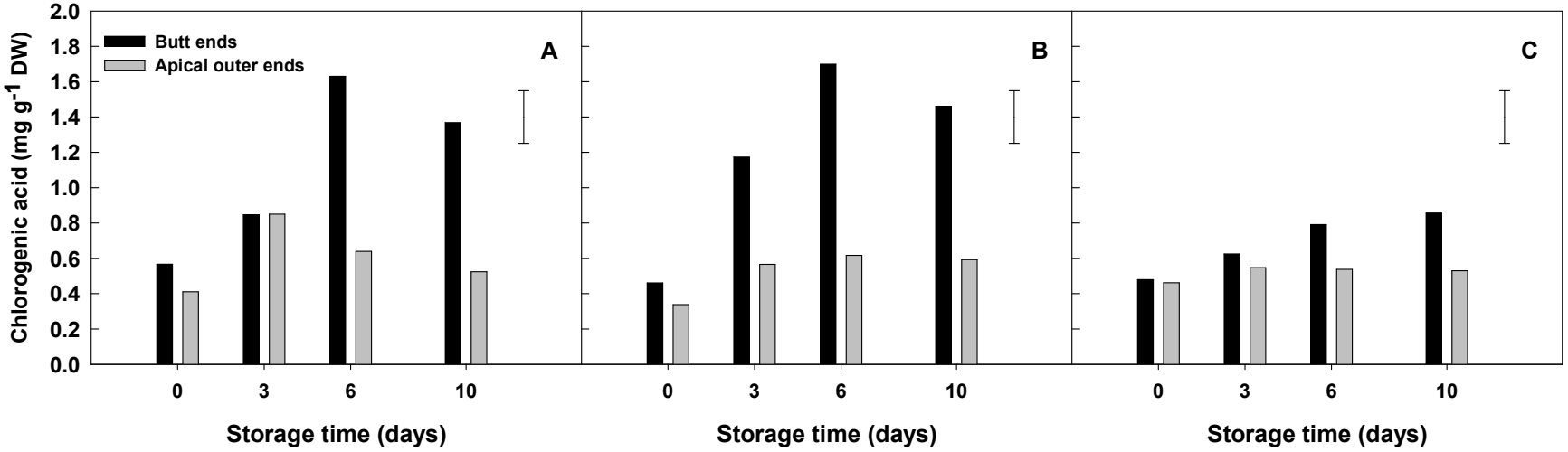


Figure 6.18 - Chlorogenic acid content at butt ends and apical outer ends of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) of water every alternate day. LSD bars are shown.

Results showed that chlorogenic acid content of celery ranged between 0.4 and 1.6 mg g^{-1} DW. Concentration of the phenolic compound was found to be very variable in literature. In previous studies, it was found to range from 0.5 to 30.0 nmol g^{-1} fresh tissue. In this experimental work, chlorogenic acid significantly increased at butt ends over storage in the 800 mL and 600 mL every alternate day water-treated samples, whilst it did not change in the less irrigated plants. Regarding apical cut-ends, it was relatively stable (**Figure 6.18**). These findings contrast with those of Vina and Chaves (2006) who reported chlorogenic acid content to decrease in pre-cut celery stored at 0, 4 and 10 °C, although the authors did not relate chlorogenic acid evolution with different celery tissues. Another study conducted by the same authors (Vina and Chaves, 2007) showed that levels of chlorogenic acid increased in the first week and subsequently decreasing until the end storage (21 days). Evolution of chlorogenic acid content during storage seems to depend on several factors like plant cv., postharvest treatments and tissue type. Loaiza Velarde *et al*. (1997) observed a higher content of the compound in lettuce stored after 3 days at 10 °C, while it was at low levels in the samples which received 50 °C heat shock treatment for 90 seconds.

A significant positive correlation was found between chlorogenic acid and browning at cut ends ($R^2 = 0.48$) (results in Appendix E).

6.10.8 Plant growth regulators (PGRs)

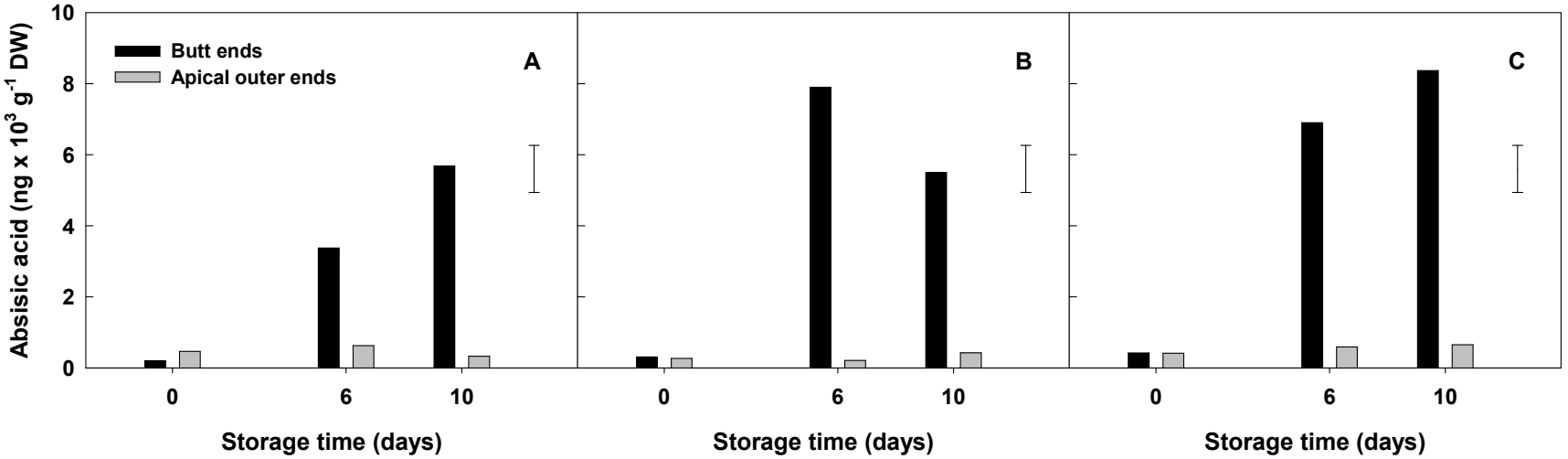


Figure 6.19 - Abscisic acid content at butt ends and apical outer ends of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) of water every alternate day. LSD bars are shown.

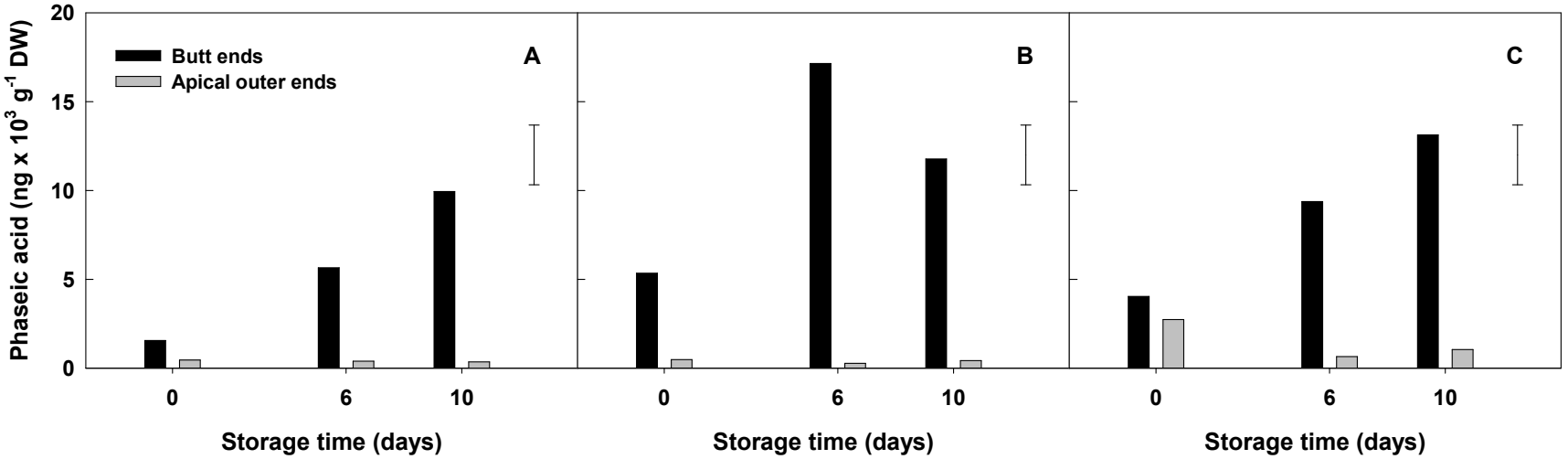


Figure 6.20 - Phaseic acid content at butt ends and apical outer ends of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) of water every alternate day. LSD bars are shown.

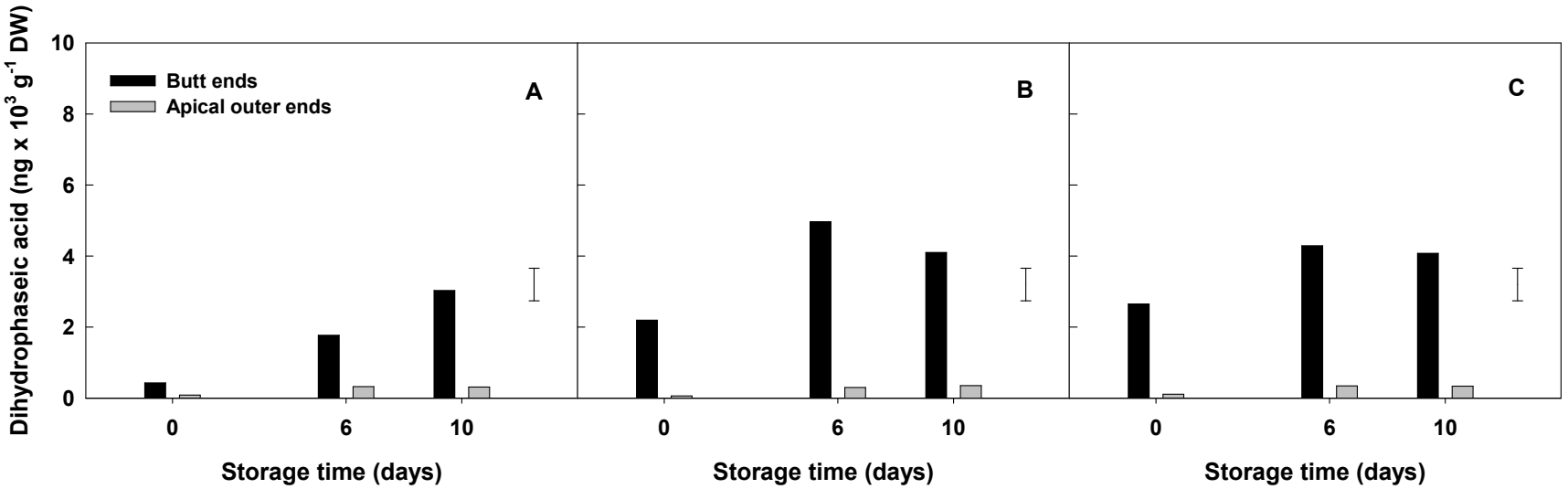


Figure 6.21 - Dihydrophaseic acid content at butt ends and apical outer ends of celery irrigated with 800 mL (A), 600 mL (B) and 400 mL (C) of water every alternate day. LSD bars are shown.

Three plant hormones were identified and quantified in this experiment: abscisic acid (ABA) (**Figure 6.19**), and two related catabolites: phaseic acid (PA) (**Figure 6.20**) and dihydrophaseic acid (DPA) (**Figure 6.21**). Overall concentration of the compounds was much higher at the basal part of the petioles rather than the upper part. ABA average content was 0.445 µg g^{-1} DW at apical cut ends, whilst it was 4.294 µg g^{-1} DW at butt ends. PA average concentration was 0.755 µg g^{-1} DW at apical cut ends, while it was 8.658 µg g^{-1} DW at butt ends. DPA mean content was 0.248 µg g^{-1} DW at apical cut ends and 3.057 µg g^{-1} DW at butt ends. At butt ends, average concentration of the three compounds was higher in water-stressed plants rather than the control-irrigated. This is an unsurprising finding as ABA is synthetized by plant roots in response to decreases in soil water potential (Gomez *et al*, 1988; Sharp, 2002; Steuer *et al*., 1988). Once produced, the phytohormone is translocated to the leaves, where it stimulates stomatal closure by altering guard cell ion transport, which attenuates transpiration rate, hence, water loss (Finkelstein, 2013). Additionally, ABA was found to induce expression of genes involved in the *de novo* synthesis of osmoprotectant molecules (Fujita *et al*., 2011). Results arising from this experiment showed that ABA was low at the beginning of storage and it increased over time at butt ends. This pattern could be due to the increasing desiccation effect that vegetables normally undergo over postharvest storage, which stimulates production of the phytohormone. This hypothesis is confirmed by evidence that environmental stresses related to water loss like hot temperatures, drought and soil salinity showed to promote ABA synthesis (Kim *et al*., 2010; Cutler *et al*., 2010). In addition, exogenous applications of ABA showed to lower postharvest fresh weight-loss by reducing transpiration of Romaine lettuce leaves stored at ambient temperature (Liu *et al*., 2015). ABA could be a mediator molecule of pithiness in celery. According with Aloni and Pressman (1979), water deprival, as well as both endogenous and exogenous ABA, induced pithiness development in celery petioles.

6.11 Conclusions

This experimental work was premised on the hypothesis that water stress has significant effect on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery. The results arising from this experimental work confirmed this assumption. The experiment in this chapter

investigated the effects of irrigation management on the visual quality, physiology and biochemistry of fresh-cut celery. The study showed that the application of water stress significantly decreased postharvest visual quality in terms of cut-end discolouration whilst metabolic activity was enhanced. These findings are in contradiction with those reported in previous works carried out on lettuce showing that increasing levels of drought stress resulted in less pronounced cut-edge browning despite the accumulation of phenolic compounds in affected midrib tissues.

7. GENERAL PROJECT CONCLUSIONS

The present study was based on the following hypotheses:

- Horticultural maturity has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.
- Continuous ethylene supplementation and 1-MCP treatment has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.
- Water stress has significant effects on the incidence of postharvest browning and other visual, physiological and biochemical characteristics in fresh-cut celery.

Results arising from this experimental work showed that celery postharvest browning: 1-) is more pronounced at late stages of crop maturity, 2-) is independent of the ethylene mechanisms, 3-) is more severe in drought-stressed plants. The three main points are discussed below:

1-) Both subjective and objective colour measurements showed that incidence of cut-end browning increased with plant developmental stage in both Spanish and UK celery (**Figure 4.17** and **Figure 4.18**). This suggests that plant senescence may play a pivotal role in boosting the biochemical mechanisms that lead to the physiological "disorder". Harvesting celery at an earlier date can potentially result in reduced discolouration, with positive consequences for growers, retailers and the UK celery industry. On the other hand, there might be undesirable side-effects deriving from adopting this practice. Reduced yield, alterations of aroma, flavour and texture are all factors that should be considered, as they can leverage on consumers' preferences. Changes in agronomic techniques and in field management should also be considered, as they can result in problems related to company rearrangements. The effects of crop maturity stage on the physiological and biochemical characteristics of celery have been sparsely investigated. Guerra *et al*., (2010) found that browning potential and total quinone content were higher in self-whitening celery (cv. Golden Clause) when harvested 124 days after planting rather than 93 days.

The experiment in chapter n.4 showed that browning incidence was higher in over-mature celery. The possible explanation of this finding is that plant senescence triggers physiological and biochemical processes leading to postharvest discolouration. Plant maturity is associated with the synthesis of phytohormones (especially, ethylene, abscisic acid, salicylic acid and methyl-jasmonate), which stimulate the phenylpropanoid metabolism. Phenylpropanoids are a family of organic compounds involved in plant defence against biotic and abiotic stresses (Davey *et al*., 2004). This class of compounds also includes hydroxycynnamic acids (i.e. chlorogenic acid and isochlorogenic acid) which are the substrates of the browning reaction. Hence, from a logical deduction, late maturity stages results in increased postharvest discolouration in celery.

The experiment in chapter n.4 also showed that chlorogenic acid content does not seem to be associated with late crop maturity stages, yet it seems to increase at the end of storage (**Figure 4.27**, **Figure 4.28** and **Figure 4.29**). Although chlorogenic acid is thought to be related to browning in vegetables, it is likely that other hydroxycinnamic acids are responsible of browning in celery tissues.

2-) Findings arising from the experiment in chapter n.5 indicated that applying 10 μL L^{-1} continuous ethylene and 1 μL L^{-1} 1-MCP for 24 h had no influence on postharvest discolouration in fresh-cut celery (**Figure 5.8** and **Figure 5.9**). This suggests that ethylene does not seem to be involved in the mechanism of browning in this crop. Unlike other leafy vegetables, the effects of the plant hormone on browning have never been investigated in celery. Ethylene treatment of 126 μmol·m^{-3} concentration showed to induce russet spotting in lettuce midrib tissues, whilst previous treatments with 1-MCP prevented the problem (Fan and Mattheis, 2000). Ethylene showed to stimulate the phenylpropanoid metabolism, while 1-MCP inhibited it. Saltveit (2004) found that exposing whole heads or leaves of iceberg lettuce to 1-MCP significantly decreased the accumulation of phenolic compounds and subsequent tissue discoloration induced by previous exposure to 1.0 μL L^{-1} ethylene in air at 5 °C.

It is well known that ethylene synthesis is stimulated by environmental stresses in higher plants (i.e. drought, mechanical damage and high temperatures). Such stresses induce a series of physiological and biochemical changes (i.e. increase in respiratory

activity and up-regulation of phenylpropanoid compounds). It is however still difficult to distinguish if such changes are caused directly by the stresses or by ethylene itself. In many vegetative tissues, intense stresses promote only temporary rises in ethylene production, which have minimal lasting effects.

Ethylene stimulates production of phenylpropanoids, many of which are the substrate of the browning reaction (hydroxycinnamic acids). Tomás-Barberán *et al*., (1997) reported that 10 μL L^{-1} exogenous ethylene applications caused accumulation of phenolic compounds responsible to tissue discolouration in Romaine, butterleaf and Iceberg lettuce. Saltveit (2004) found that exposing whole heads or leaves of Iceberg lettuce to 1 μL L^{-1} 1-MCP significantly reduced browning and phenolic compounds related to the "disorders". The present work showed that ethylene does not seem to be involved in postharvest browning in celery. This suggests that the browning mechanisms might work differently in this crop. In celery, phenylpropanoid metabolism, and consequent darkening of tissues, might be stimulated by another chemical instead of ethylene.

3-) Both subjective and objective colour data showed that incidence of cut-end browning was higher in drought-stressed plants (**Figure 6.11** and **Figure 6.12**). These findings can be advantageous information to the growers, as they can set adjust irrigation strategies accordingly. Additionally, the way water is distributed to celery plants is important. Experiment in chapter n.6 showed that when applying water, soil moisture should be maintained as constant as possible to prevent pithiness. This is confirmed by Breschini and Hartz (2002) who demonstrated that infrequent irrigation caused evident pithiness of celery petioles grown in the field.

The higher severity of cut-end browning in drought-stressed celery plants could be explained by the following mechanism: When plants withstand water stress, a series of oxidative reactions are activated in the metabolism. In response to oxidative stress, phenylpropanoids are synthesized, which are the substrate of the browning reaction. Hence, the more water stress-induced phenylpropanoids are synthesized, the more discolouration appears on celery cut surfaces. However, previous studies on lettuce showed opposite findings. Cut edge browning of both Iceberg (Luna *et al*., 2012) and Romaine (Luna *et al*., 2013) lettuce was reduced in the less irrigated midribs despite phenolic compounds production in affected tissues.

REFERENCES

Able, A.J., Wong, L.S., Prasad, A., O'Hare, T.J. (2002) - The effects of 1-Methylcyclopropene on the shelf-life of minimally processed leafy Asian vegetables. *Postharvest Biology and Technology* (in press).

ADAS Consulting Ltd (2012) - Irrigation Best Practice - A Guide for Vegetable Growers.

Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A., and Potapovitch, A.I. (1989) - Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology*, 38, 1763–1769.

Allende, A., McEvoy, J.L., Luo, Y., Artes, F. and Wang, C.Y. (2006) - Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending shelf-life of minimally processed "Red Oak Leaf" lettuce. *Food Microbiology* 23, 241-249.

Aloni, B. and Pressman, E. (1979) - Petiole pithiness in celery leaves: induction by environmental stresses and the involvement of abscisic acid. *Physiologia Plantarum*. Vol. 47, Issue 1. Pages 61-65.

Alonso, J.M., Hirayama, T., Roman, G., Nourizadeh, S. and Ecker, J.R. (1999) - EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis. Science*, 284: 2148-2152.

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., and Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, 84, 551–562.

An, F., Zhao, Q., Ji, Y., Li, W., Jiang, Z., Yu, X., Zhang, C., Han, Y., He, W. and Liu, Y. (2010) - Ethylene-induced stabilization of ETHYLENE INSENSITIVE3 and EIN3-LIKE1 is mediated by proteasomal degradation of EIN3 binding F-box 1 and 2 that requires EIN2 in *Arabidopsis. Plant Cell*, 22: 2384-2401.

Andreotti, C., Ravaglia, D., Ragaini, A. and Costa, G. (2008) - Phenolic compounds in peach (*Prunus persica*) cultivars at harvest and during fruit maturation. *Annals of Applied Biology*, Vol 153, Issue 1, pp. 11-23.

Atkinson, L.D., Hilton, H.W., Pink, D.A. (2013) - A study of variation in the tendency for postharvest discoloration in a lettuce (Lactuca sativa L.) diversity set. *International Journal of Food Science and Technology* 48, 801-807.

Balasundram, N., Sundram, K. and Samman, S. (2006) - Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence and potential uses. *Food Chemistry*, Vol. 99, Issue 1, pp. 191-203.

Barker, A.V and Pilbeam, D.J. (2015) - Handbook of plant nutrition. 2nd edition. ISBN-13: 978-1439881972 ISBN-10: 1439881979.

Barrett, D.M., Garcia, E. and Wayne, E.J. (1998) - Textural modifications of processing tomatoes. *Critical Reviews in Food Science and Nutrition*, 38 (3): 173-258.

Barry, C.S., Llop-Tous, M.I. and Grierson, D. (2000). The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiology* 123, 979–986.

Beltràn, D., Selma, M.V., Marìn, A. and Gil, M.I. (2005) - Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agricultural Food Chemistry* 53, 5654-5663.

Blankenship, S.M. and Dole, J.M. (2003) - 1-methylcyclopropene: a review. *Postharvest Biology and Technology* Vol. 28 (1): 1-25.

Blankenship, S. (2001) - Ethylene effects and the benefits of 1-MCP. Perishable Handling Quarterly. Issue no. 108.

Blankenship, S. (2000) - Ethylene: The ripening hormone. 16th Annual Postharvest Conference, Yakima, WA March 14-15.

Bleecker, A.B. and Kende, H. (2000) - Ethylene: a gaseous signal molecule in plants. *Annu. Rev. Cell Dev. Biol.* 16: 1-18.

Brecht, J.K. (1995) - Physiology of lightly processed fruits and vegetables. *HortScience* 30, 18-22.

Breschini, S.J. and Hartz T.K. (2002) - Drip irrigation management affects celery yield and quality. *HortScience*. 37 (6), pp. 894-897.

Boerjan, W., Ralph J., Baucher, M. (2003) - Lignin biosynthesis. *Annu. Rev. Plant Biol.* 54: 519-46.

Burda, S., Oleswek, W. and Lee, C.Y. (1990) - Phenolic compounds and their changes in apples during maturation and cold storage. *Journal of Agricultural and Food Chemistry*, 38 (4), pp. 945-948.

Burg, P.B. (1973) - Ethylene in plant growth. *Proc. Nat. Acad. Sci. USA* Vol. 70, No. 2, pp. 591-597.

Camm, E.D., and Towers, G.H.N. (1973) - Phenylalanine ammonia lyase. *Phytochemistry*. Vol. 12, pp. 961-963.

Campos-Vargas, R. and Saltveit, M.E. (2002) - Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum* 114, 73-84.

Campos-Vargas, R., Nonogaki, H., Suslow, T. and Saltveit, M.E. (2005) - Heat-shock treatments delay the increase in wound-induced phenylalanine ammonia-lyaise activity by altering its expression, not its induction in Romaine lettuce (*Lactuca sativa*) tissue. *Physiologia Plantarum* 123: 82-91.

Cantos, E., Espìn J.C. and Tomàs-Barberàn, F.A. (2001) - Effect of wounding on phenolic enzymes in six minimally processed lettuce cv. upon storage. *Journal of Agricultural Food Chemistry* 49, 322-330.

Chang, C. (2016) - How do plants respond to ethylene and what is its importance? *BMC Biology* 14:7, DOI**:** 10.1186/s12915-016-0230-0.

Chen, Y.F., Gao, Z., Kerris, R.J., Wang, W., Binder, B.M. and Schaller, G.E. (2010) - Ethylene receptors function as components of high molecular- mass protein complexes in *Arabidopsis. PLoS ONE*, 5: e8640.

Choi, Y.J., Tomàs-Barberàn, F.A. and Saltveit, M.E. (2005) - Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. *Postharvest Biology and Technology* 37, 47-55.

Chutichudet, B., Chutichudet, P. and Kaewsit, S. (2011) - Influence of developmental stage on activities of polyphenol oxidase, internal characteristics and colour of lettuce cv. Grand Rapids. *American Journal of Food Technology* 6 (3), pp. 215-225.

Chutichudet, B. and Chutichudet, P. (2011) - Shading application on controlling the activity of polyphenol oxidase and leaf browning od "Grand Rapids" lettuce. *International Journal of Agricultural Research* (6) 5: 400-409.

Chutichudet, P., Chutichudet, B. and Kaewsit, S. (2009) - Effect of gypsum application on enzymatic browning activity in lettuce. *Pakistan Journal of Biological Sciences* 12 (18): 1226-1236.

Cook, R. (2008) - The dynamic US fresh produce industry: an industry in transition.

Collings E.R., García Cas. A.G., Ordaz Ortiz J.J. and Terry L.A. (2013) - A new real time automated method for measuring in-situ respiration rates of fresh produce. *VII International Postharvest Symposium, Kuala Lumpur, Malaysia.*

Couture, R., Cantwell, M.I., Ke, D. and Saltveit, M.E. (1993) - Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *HortScience*, vol 28, n. 7. 723-725.

Craig, W.J. (1999) - Health promoting properties of common herbs. The American *Journal of Clinical Nutrition*, 70 (suppl.), 491S-9S.

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., and Abrams, S. R. (2010) - Abscisic acid: emergence of a core signaling network. Annu. Rev. Plant Biol. 61, 651–679.

Davey, M.P., Bryant, N.D., Cummins, I., Ashender, T.W., Gates, P., Baxter, R. and Edwards, R. (2004) - Effects of elevated CO2 on the vascular and phenolic secondary metabolism of Plantago maritima. *Phytochemistry*, Vol. 65, Issue 15. Pages 2197-2204.

DEFA (2005) - Atlantic Provinces Vegetable Crops Production Guide. Publ. No. 1400A, Agdex No. 250/600.

Darezzo, H.M, Benedetti, B.C., Deliza, R., Cenci, S. and Gonçalves, F.B. (2003) - Evaluation of quality attributes of fresh-cut Lettuce (*Lactuca sativa* L.) stored in different controlled atmospheres. *Acta Horticulturae* 600, pp. 213-219.

David Rodarte Castrejòn, A., Eichholz, I, Rohn, S., Kroh, L.W. and Huyschens-Keil, S. (2008) - Phenolic compounds and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chemistry*, Vol. 109, Issue 3, pp. 564-572.

D'Antuono, L.F. and Neri, R. (2001) - The evaluation of nitrogen effect on lettuce quality by means of descriptive sensory profiling. *Acta Horticulturae* 563: 217-222.

DEFRA (2006) - Council Regulation (EC) No 510/2006 on protected geographical indications and protected designations of origin - "Fenland Celery".

Degl'Innocenti, E., Pardossi, A., Tognoni, F. and Guidi, L. (2007) - Physiological basis of sensitivity to enzymatic browning in "lettuce", "escarole" and "rocket salad" when stored as fresh-cut products. *Food Chemistry* 104, pp. 209-215.

Dixon, R.A., and Paiva, N.L., (1995) - Stress-induced phenylpropanoid metabolism. *Plant Cell* 7: 1085-1097.

Dong, C.H., Rivarola, M., Resnick, J.S., Maggin, B.D. and Chang, C. (2008) - Subcellular co localization of Arabidopsis RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 ethylene signaling. *Plant Journal*, 53: 275-286.

Dong, X., Zhang, Y.L., Wang, F., Pang, M.X. and Qi, J.H. (2016) - Relationship between chemical oxidation and browning of flavanols. *2nd International Conference on Agricultural and Biological Sciences*. *IOP Conf. Series: Earth and Environmental Science 41*. DOI:10.1088/1755-1315/41/1/012012.

Downes K., Chope G. and Terry L.A. (2010) - Postharvest application of ethylene and 1-methylcyclopropene either before and after curing affects onion (*Allium cepa*

L.) bulb quality during long term cold storage. *Postharvest Biology and Technology* 55, pp. 36-44.

Du, H., Zhang, L., Liu, L., Tang, X.-F., Yang, W.-J., Wu, Y.-M., Huang, Y.-B., Tang, Y.-X.- (2009) - Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry* 74 (1), pp. 1-11.

DuPont, M.S., Mondin, Z., Williamson, G. and Price, K.R. (2000) - Effect of variety, processing and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of Agricultural Food Chemistry* 48: 3957-3964.

El Beltagy, A.S. and Hall, M.A. (1974) - Effect of water stress upon endogenous ethylene levels in *Vicia faba*. *New Phytologist* 73: 47-59.

European Commission (2013) - Crop Production Statistics. Available on site http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database [accessed: October 2013]

Fan, X. and Mattheis, J.P. (2000) - Reduction of ethylene-induced physiological disorders of carrots and iceberg lettuce by 1-Methylcyclopropene. *HortScience* 35, 1312-1314.

Fan, X. (2005) - Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. *Journal of the Science of Food in Agriculture* 85: 995-1000.

Finch-Savage, W.E. (1984) - The establishment of direct-sown germinating celery seeds in peat blocks. *Journal of Horticultural Science* 59, 87-93.

Finkelstein, R. (2013) - Abscisic acid synthesis and response. The Arabidopsis Book 11.

Fujita, Y., Fujita, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2011). ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res*. 124, 509–525.

Fujita, S., Tono, T., Kawahara, H. J., (1993) - *Sci. Food Agric.* 55, 643-651.

Gao, Z., Wen, C.K., Binder, B.M., Chen, Y.F., Chang, J., Chiang, Y.H., Kerris, R.J., Chang, C and Schaller, G.E. (2008) - Heteromeric interactions among ethylene

receptors mediate signaling in *Arabidopsis*. *Journal of Biological Chemistry*, 283: 23801-23810.

Garcia and Barrett (2000) - Preservative treatments for fresh cut fruits and vegetables. University of California, Davis, CA 95616-8631.

Gariepy *et al*., (1986) - Controlled atmosphere storage of celery with a silicone membrane system. *International Journal of Refrigeration* 9, pp. 234-239.

Ghasemnezhad, M., Sherafati, M. and Payvast, G.A. (2011) - Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicuum annum*) fruits at two different harvest times. *Journal of Functional Foods*, Vol 3, Issue 1, pp. 44-49.

Gil-Izquierdo, A., Gil, M. I., Conesa, M. A., & Ferreres, F. (2001). The effect of storage temperature on vitamin C and phenolics content of artichoke (*Cynara scolymus* L.) heads. *Innovative Food. Science and Emerging Technologies*, 2, 199–202.

Goeschl, J.D., Rappaport, L. and Pratt, H.K. (1966) - Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. *Plant Physiology* 41, 877-884.

Gòmez, J., Sanchez-Martinez, D., Stiefel, V., Rigau, J., Puigdomènech, P. and Pagès, M. (1988) - A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein. *Nature* 334, 262 - 264.

Gómez, P.A. and Artés, F. (2004) - Controlled atmospheres enhance postharvest green celery quality. *Postharvest Biology and Technology* 34: 203-209.

Gómez, P.A. and Artés, F. (2005) - Keeping quality of green celery as affected by modified atmosphere packaging. *European Journal of Horticultural Science* 69 (5): 215-219.

Guerra, N., Carrozzi, L., Goñi, M.G. Roura, S. and Yommi, A. (2010) - Quality characterization of celery (Apium graveolens L.) by plant zones and two harvest dates. *Journal of Food Science* 75 (6), pp. S327-S332.

He, Q. and Luo, Y. (2007) - Enzymatic browning and its control in fresh cut produce. *Stewart Postharvest Review* 3 (6), art no 16.

He, Q., Luo, Y. and Chen, P. (2008) - Elucidation of the mechanism of enzymatic browning inhibition by sodium chlorite. *Food Chemistry* 110, 847-851.

Harborne, J. B. (1989) - General procedures and measurement of total phenolics. *J. B. Harborne (Ed.). Methods in plant biochemistry: Volume 1 Plant Phenolics* (pp. 1–28). London: Academic Press.

Hartz, T. (1999) - Drip irrigation and fertigation management of celery. FREP Contract 97-0365 M97-02 (www.cdfa.ca.gov).

Hartz, F.K. and Breschini, S.J. (2001) - Pre-sidedress soil nitrate testing (PSNT) improves N management in lettuce production. Western Nutrient Management Conference, 20-26.

Harris, D.R., Seberry, J.A. Wills, R.B.H. and Spohr, L.J. (2000) - Effect of fruit maturity on efficiency of 1-methylcyclopropene to delay the ripening of banana. *Postharvest Biology and Technology* 20, 303-308.

Hayward, H.E. (1938) - The structure of economic plants. Macmillan Publishers, New York.

He, Q. and Luo,Y. (2007) - Enzymatic browning and its control in fresh-cut produce. *Stewart Postharvest Review* 3 (6), art. no. 16.

Heredia, J.B. and Cisneros-Zevallos, L. (2009) - The effects of exogenous ethylene and methyl jasmonate on the accumulation of phenolic antioxidants in selected whole and wounded fresh produce. *Food Chemistry* 115 (4), pp. 1500-1508.

Hilton, H. W., Clifford, S. C., Wurr, D. C. E. and Burton, Kerry S. (2009) - The influence of agronomic factors on the visual quality of field-grown minimally-processed lettuce. *Journal of Horticultural Science & Biotechnology*, Vol.84 (No.2). pp. 193-198.

Hirayama, T., Kieber, J.J., Hirayama, N., Kogan, M., Guzman, P., Nourizadeh, S., Alonso J.M., Dailey, W.P., Dancis, A. and Ecker, J.R. (1999) - RESPONSIVE-

TO-ANTAGONIST1, a Menkes/Wilson disease related copper transporter is required for ethylene signaling in *Arabidopsis. Cell*, 97: 383-393.

Honma, S. (1959) - A method for celery hybridization. *Proceedings of the American Society for Horticultural Science* 73, 345-348.

Hua, J. and Meyerowitz, E.M (1998) - Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. *Cell* 94: 261-271.

Iyidogan, N.F. and Bayiindirli, A. (2004) - Effect of L-Cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. *Journal of Food Engineering*, 62, 299-304.

Johnson, P.R., and Ecker, J.R. (1998). The ethylene gas signal transduction pathway: A molecular perspective. Annu. Rev. Genet. 32**,** 227–254.

Jones, D.H., (1984) - Phenylalanine ammonia lyase: regulation of its induction, and its role in plant development. *Phytochemistry*, vol 23, n. 7, pp. 1349-1359.

Ju, C. and Chang, C. (2012) - Advances in ethylene signalling: protein complexes at the endoplasmic reticulum membrane. *AoB Plants* pls031.

Kader, A.A., Zagory, D. and Kerbel, E.L. (1989) - Modified atmosphere packaging of fruits and vegetables. Crti. Rev. Food Sci. Nutr. 28: 1-/30.

Kader, A.A. (2002) - Postharvest biology and technology: an overview. In: Kader A.A. (Ed.), Postharvest Technology of Horticultural Crops, third ed. University of California Pub. 3311, USA, pp. 39-47.

Kang, Y.J., Choi, J.H., Jeong, M.C. and Kim, D.M. (2008) - Effect of maturity at harvest on the quality of head lettuce during storage. *Korean Journal of Horticultural Science and Technology* 26 (3), pp. 272-276

Kays, S.J. (1999) - Pre-harvest factors affecting appearance. *Postharvest Biology and Technology* 15: 233-247.

Ke, D. and Saltveit, M.E. (1988) - Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in Iceberg lettuce. *Plant Physiology* 88 (4): 1136-1140.

Ke, D. and Saltveit, M.E. (1989) - Wound-indiced ethylene production, phenolic metabolism and susceptibility to russet spotting in Iceberg lettuce. *Physiologia Plantarum* 76, 412-418.

Kende, H. (1993) - Ethylene Biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 283-307.

Khoddami, A., Wilkes, M.A. and Roberts, T.H. (2013) - Techniques for the analysis of Molecules 18 (2): 2328-2375.

Khumjing, C., Chutichudet, B., Chutichudet, P. and Boontiang, K. (2011) - Effects of different calcium applications for controlling browning appearance in lettuce. *International Journal of Agricultural Research* 6 (3): 238:254.

Kim, T.H., Böhmer, M., Hu, H., Nishimura, N., and Schroeder, J. I. (2010) - Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} Signaling. *Annu. Rev. Plant Biol*. 61, 561–591.

Klein, B.P. (1987) - Nutritional consequences of minimal processing of fruits and vegetables. *Journal of Food Quality*, Vol. 10 (3), pp. 179-193.

Komthong, P, Katoh, T, Igura, N and Shimoda, M. (2006) - Changes in odours of apple juice during enzymatic browning. *Food Quality and Reference* 17: 497-504.

Laminkanra O. (2002) - Fresh-cut fruits and vegetables: science, technology, and market. ISBN 9781587160301.

Landi, M., Degl'Innocenti, E., Guglielminetti, L. and Guidi, L. (2013) - Role of ascorbic acid in the inhibition of polyphenol oxidase and the prevention of browning in different browning-sensitive *Lactuca sativa* var. *capitata* (L.) and *Eruca sativa* (Mill.) stored as fresh-cut produce. *Journal of the Science of Food and Agriculture*, Vol. 93 (8), pp. 1814-1819.

Liu, S., Yang, M., Zhao, H., Li, H., Suo, B. and Wang, Y. (2015) - Exogenous abscisic acid inhibits the water-loss of postharvest Romaine lettuce during storage by inducing stomatal closure. *Food Sci. & Technology,* Vol. 35, n.4.

Loaiza-Velarde, J.G., Mangrich, M.E., Campos-Vargas, R. and Saltveit, M.E. (2003) - Heat shock reduces browning of fresh-cut celery petioles. *Postharvest Biology and Technology* 27: 305-311.

Lopez-Galvez, G., Saltveit, M., Cantwell, M. (1997) - Wound-induced phenylalanine amonia-lyaise activity: factors affecting its induction and correlation with the quality of minimally processed lettuce. *Postharvest Biology and Technology* 9, pp. 223-233.

Luna, M.C., Tudela, J.A., Martínez-Sánchez, A., Allende, A., and Gil, M.I. (2013) - Optimizing water management to control respiration rate and reduce browning and microbial load of fresh-cut romaine lettuce. *Postharvest Biology and Technology* 80, pp. 9-17.

Luna, M.C., Tudela, J.A., Martínez-Sánchez, A., Allende, A., Marín, A. and Gil, M.I. (2012) - Long-term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology* 73, pp. 37-45.

Mai, F. and Glomb, M.A. (2014) - Lettucenin sesquiterpenes contribute significantly to the browning of lettuce. *Journal of Agricultural and Food Chemistry*. 62 (20), pp. 4747-4753.

Mai, F. and Glomb, M.A. (2013) - Isolation of phenolic compounds from Iceberg lettuce and impact on enzymatic browning. *Journal of Agricultural and Food Chemistry* 61, 2868-2874.

Manzocco, L., Quarta, B. and Dri, A. (2009) - Polyphenol oxidase inactivation by light exposure in modelsystems and apple derivatives. *Innovative Food Science and Emerging Technologies*, 10: 509-511.

Mayer, A.M. and Harel, E., (1979) - Polyphenol oxidases in plants. *Phytochemistry*, 18, 193–215.

Merchante, C., Alonso, J.M. and Stepanova, A.N. (2013) - Ethylene signaling: simple ligand, complex regulation. *Current Opinion in Plant Biology* 16: 554-560.

Nagai, T. and Suzuki, N. (2001) - J. Sci. Food Chem. 49, 3922-3926.

Ordaz-Ortiz, J.J., Foukaraki, S. and Terry, L.A. (2015) - Assessing temporal flux of plant hormones in stored processing potatoes using high definition accurate mass spectrometry. *Horticultural Research* 2, 15002; doi:10.1038/hortres.2015.2; published online: 25 February 2015.

Paul, V., Pandey, R., Srivastava, G.C. (2012) - The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene - An overview. *Journal of Food Science and Technology* 49 (1), pp. 1-21.

Pereyra, L., Roura, S.I. and Del Valle, C.E. (2005) - Phenylalanine ammonia lyase activity in minimally processed Romaine lettuce. LWT - *Food Science and Technology* 38 (1), pp. 67-72.

Poulsen, N., Johansen, A.S. and Sorensen, J.N. (1995) - Influence of growth conditions on the value of crisphead lettuce 4 - Quality changes during storage. *Plant Foods for Human Nutrition* 47: 157-162.

Pressman, E., Negbi, M., Sachs, M. and Jacobsen, J.V. (1977) - Varietal differences in light requirements for germination of celery (*Apium graveolens* L.) seeds and the effects of thermal and solute stress. *Australian Journal of Plant Physiology* 4, 821-831.

Pressman, E and Negbi, M. (1980) - The effect of day length on the response of celery to vernalization. *Journal of Experimental Botany* 31 (5): 1291-1296.

Pressman, E., Huberman, M., Aloni, B. and Jaffe, M.J. (1984) - Pithiness in plants: I. The effect of mechanical perturbation and the involvement of ethylene in petiole pithiness in celery. *Plant Cell Physiology* 25 (6): 891-897.

Pressman, E. and Negbi, M., (1987) - Interaction of day length and applied gibberellins on stem growth and leaf production in three varieties of celery. *Journal of Experimental Botany* 38, 968-971.

Queiroza, C, Lopeza, M.L.M., Fialhoa, E. and Valente-Mesquita, V.L. (2008) - Polyphenols Oxidase: characteristics and mechanisms of browning control. Food Reviews International, 24 (4): 361-375.

RB209 Fertilizer Manual, 8th edition (2010).

Reinhold, V.N., (1989) - Vegetable Production Ontario Canada ISBN 0-442-26721-5.

Resnick, J.S., Rivarola, M. and Chang, C. (2008) - Involvement of RTE1 in conformational changes promoting ETR1 ethylene receptor signaling in *Arabidopsis*. *Plant Journal*, 56: 423-431.

Rizzo, V. and Muratore, G. (2009) - Effects of packaging on shelf-life of fresh celery. *Journal of Food Engineering* 90: 124-128.

Roelofse, E.W., Hand, D.W, and Hall, R.L. (1990) - The effects of temperature and "night break" lighting on the development of glasshouse celery. *Journal of Horticultural Science* 65, 297-307.

Rosen, J.C. and Kader, A.A. (1989) - Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. *J. Food Sci.* 54:656-659.

Rubatzky, V.E., Quiros, C.F. and Simon, P.W. (1999) - Carrots and related vegetable Umbelliferae. *Crop Production Science in Horticulture*. ISBN 0-85199-129-7.

Saltveit, M.E., and Mangrich M.E. (1996) - Using density measurement to study the effect of excision, storage, abscisic acid, and ethylene on pithiness in celery petioles. *Journal of the American Society of Horticultural Science* 121:137-141.

Saltveit, M.E. (1997) - Physical and physiological changes in minimally processed fruits and vegetables. In: Tomàs-Barberàn, F.A. (Ed), Phytochemistry of Fruit and Vegetables. Oxford University Press, pp. 205-220.

Saltveit, M.E. (2000) - Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biology and Technology* 21, 61-69.

Saltveit, M.E. (2004) - Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut “iceberg” lettuce. *Postharvest Biology and Technology* 34: 75-80.

Saltveit, M.E., Choi, Y.J., Tomàs-Barberàn, A. (2005) - Mono-carboxylic acids and their salts inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa*) leaf tissue. *Physiologia Plantarum* 125: 454-463.

Saltveit, M.E. and Qin, L. (2008) - Heating the ends of leaves during coring of whole heads of lettuce reduces subsequent phenolic accumulation and tissue browning. *Postharvest Biology and Technology* 47: 255-259.

Salunkhe, D.K. and Kadam, S.S. (1998) - Handbook of vegetable science and technology: production, composition, storage and processing. Marcel Dekker Inc. New York.

Sharp, R.E., (2002) - Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant, Cell & Environment*. Vol. 25, Issue 2: 211-222.

Silva, E. (2008) - Respiration and ethylene and their relationship to postharvest handling In Wholesale success: a farmer's guide to selling, postharvest handling, and packing produce (Midwest edition). Available online at: http://www.familyfarmed.org/retail.html (verified 3 March 2010).

Steuer, B., Stuhlfauth, T., Heinrich, P.F. (1988). The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure. *Photosynthesis Research*. 18 (3): 327–336.

Stoop, J.M.H., Williamson, J.D. and Mason-Pharr, D. (1996) - Mannitol metabolism in plants: a method for coping with stress. *Trends in Plant Science*. Volume 1, Issue 5: 139-144.

Subramanian, N., Venkatesh, P., Ganguli, S. and Sinkar, V.P. (1999) - Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. *Journal of Agricultural and Food Chemistry:* 47(7): 2571-2578.

Sudheer, K.P. and Indira, V. (2007) - Post harvest technology of horticultural crops. *Horticultural Science*, Vol. 7.

Terry, L.A., Ilkenhans T., Poulston S., Rowsell L. ad Smith A.W.J. (2007) - Development of a new palladium-promoted ethylene scavenger. *Postharvest Biology and Technology*, 45, 214-220.

Giné Bordonaba, J., Terry, L.A. (2008) - Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *Journal of Agriculture and Food Chemistry* 56, 7422-7430.

Tomàs-Barberàn, F., and Espìn, J.C. (2001) - Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, 81, 853–876.

Thomas, T.H. (1989) - Gibberellin involvement in dormancy-break and germination of seeds of celery (*Apium graveolens* L.). *Plant Growth Regulation* 8, 255-261.

University of California, Davis (2012) - Small-scale Processing Technologies for Horticultural Products, Chapter 12: Fresh-cut Products.

USDA (2014) - Vegetables 2013 Summary. National Agricultural Statistics Service. ISSN: 0884-6413.

USDA (2013) - Nutrient Database, available at http://ndb.nal.usda.gov/ndb/foods/show/2962 [accessed: 02/11/2013].

Varoquaux, P. and Wiley, R.C. (1994) - Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In Minimally Processed Refrigerated Fruits and Vegetables ed. Wiley, R.C. pp. 226–268. London, UK: Chapman and Hall.

Varzakas, T. and Manolopolou, E. (2017) - Comparison of HACCP and ISO 22000 in the ready-to-eat fruit and vegetable industry in conjunction with application of failure mode and effect analysis (FMEA) and Ishikawa diagrams. *Minimally Processed Refrigerated Fruits and Vegetables. Food Engineering Series*, pp. 685-721.

Vicuna, D. (Thesis) (2005) - The role of peroxidases in the development of plants and their responses to abiotic stresses. Doctoral. Paper 15.

Viña, S.Z. and Chaves, A.R. (2006) - Antioxidant responses in minimally processed celery during refrigerated storage. *Food Chemistry* 94, 68-74.

Viña, S.Z. and Chaves, A.R. (2008) - Effect of heat treatment and refrigerated storage on antioxidant properties of pre-cut celery (*Apium graveolens* L.). *International Journal of Food Science and Technology* 43: 44-51.

Vogt, T. (2010) - Phenylpropanoid biosynthesis. *Mol. Plant.*: 3, 2-20.

Wang, K.L.C., Li, H. and Hecker, J.R. (2002) - Ethylene biosynthesis and signaling networks. *The Plant Cell*, 14 (1): S131-S151.

Wang, S.W. and Lin, H.S. (2000) - Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry*, 48 (2), pp.140-146.

Watkins, C.B. (2008) - Overview of 1-methylcyclopropene trials and uses for edible horticultural crops. *HortScience*, 43 (1), pp. 86-94.

Welbaum G.E. (2015) - Vegetable production and practices. ISBN-13: 978-1845938024.

Whitaker, J.R. & Lee, C.Y (1995) - Recent advances in chemistry of enzymatic browning. ACS Symposium Series; American Chemical Society: Washington DC.

Whitaker, J.R and Lee, C.Y. (1995) - Recent advances in chemistry of enzymatic browning. Lee, C.Y.; Whitaker, J.R.; eds. In *Enzymatic Browning and Its Prevention*; American Chemical Society: Washington, DC; ACS Symposium Series; 2-7.

Whitlock, A. (1979) - Growing guide N.6 (Celery). Grower Books 49.

WRAP (2011) - Fruit and vegetable resource maps (RSC-008).

Wurr, D., Parr, A., Feuerhelm, S., Kennedy, S., Pennings, H., Oost, E., Cornai, I., Harriman, M., Sawday, J. and Tucker, A. (2003) - Improving the quality and

shelf-life of cut salad products. Department for Environment, Food and Rural Affairs final report for project number HLO142.

Yagar, H. (2004) - Some biochemical properties of polyphenol oxidase from celery, preparative biochemistry and biotechnology, 34:4, 387-397.

Yao, Y., Sang, W., Zhou, M. and Ren, G. (2010) - Phenolic composition and antioxidant activities of 11 celery cultivars. *Journal of Food Science* 75 (1): C9-C13.

Yommi, A., Di Gerònimo, N., Carrozzi, L., Goni, G. and Roura, S. (2012) - Quality indices changes in petioles of celery (*Apium graveolens* L.) harvested at different physiological stages. *Acta Horticulturae* 934, pp. 1213-1218.

Yommi, A., Di Gerònimo, N., Carrozzi, L., Goni, G. and Roura, S. (2013) - Morphological, physicochemical and sensory evaluation of celery harvested from early to late maturity. *Horticultura Brasileira* 31(2), pp. 236-241.

Zhan, L., Li, Y., Hu, J., Pang, L. and Fan, H. (2012) - Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. *Innovative Food Science and Emerging Technologies* 14: 70-76.

Zhan, L., Hu, J., Lim, L.T., Pang, L., Li, Y. and Shao, J. (2013) - Light exposure inhibiting tissue browning and improving capacity of fresh-cut celery (*Apium graveolens* var. dulce). *Food Chemistry* 141: 2473-2478.

Zhang, L., Lu, Z. and Yu, Z. and Gao, X. (2005) - Preservation of fresh-cut celery by treatment of ozonated water. *Food Control* 16: 279-283.

Zhong, S., Lin, Z. and Grierson, D. (2008) - Tomato ethylene receptor–CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum. *Journal of Experimental Botany*, 59: 965-972.

Appendix A - Normality tests

Table A1. Normality tests for Early Spanish season datasets (**chapter 4**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	2.34500	2 (adjusted)	0.30959
Cut petiole ends browning	Normal	3.20826	3 (adjusted)	0.36062
Butt ends browning	Normal	7.20769	2 (adjusted)	0.12722
Cut petiole ends pithiness	Normal	3.86366	2 (adjusted)	0.14488
Butt ends pithiness	Normal	15.60253	2 (adjusted)	0.11041
Cut petiole ends hue angle	Normal	5.42185	2 (adjusted)	0.08648
Butt ends hue angle	Normal	9.73387	3 (adjusted)	0.11097
Chlorogenic acid	Normal	10.35588	2 (adjusted)	0.12188

Table A2. Normality tests for Late Spanish season datasets (**chapter 4**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	1.09026	3 (adjusted)	0.77943
Cut petiole ends browning	Normal	6.17245	3 (adjusted)	0.14665
Butt ends browning	Normal	8.54425	3 (adjusted)	0.09034
Cut petiole ends pithiness	Normal	7.36621	4 (adjusted)	0.11574
Butt ends pithiness	Normal	4.36926	3 (adjusted)	0.23817

Cut petiole ends hue angle	Normal	2.59664	4 (adjusted)	0.21342
Butt ends hue angle	Normal	5.90233	4 (adjusted)	0.14211
Chlorogenic acid	Normal	4.42889	2 (adjusted)	0.11527

Table A3. Normality tests for Late UK season datasets (**chapter 4**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	5.96776	3 (adjusted)	0.22673
Cut petiole ends browning	Normal	3.91615	4 (adjusted)	0.29185
Butt ends browning	Normal	2.01513	3 (adjusted)	0.37844
Cut petiole ends pithiness	Normal	7.82839	4 (adjusted)	0.14363
Butt ends pithiness	Normal	4.84537	3 (adjusted)	0.18729
Cut petiole ends hue angle	Normal	3.36505	4 (adjusted)	0.13962
Butt ends hue angle	Normal	5.44271	3 (adjusted)	0.13770
Chlorogenic acid	Normal	8.86387	2 (adjusted)	0.09532

Table A4. Normality tests for Early Spanish season datasets (**chapter 5**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	1.55193	5 (adjusted)	0.90700
Cut petiole ends browning	Normal	3.20036	4 (adjusted)	0.39142
Butt ends	Normal	8.38033	4 (adjusted)	0.08434

browning				
Cut petiole ends pithiness	Normal	3.00538	6 (adjusted)	0.26492
Butt ends pithiness	Normal	7.47311	5 (adjusted)	0.21551
Cut petiole ends hue angle	Normal	5.53160	6 (adjusted)	0.47765
Butt ends hue angle	Normal	5.00247	5 (adjusted)	0.41558

Table A5. Normality tests for Late Spanish season datasets (**chapter 5**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	4.63770	5 (adjusted)	0.21203
Cut petiole ends browning	Normal	7.20656	6 (adjusted)	0.12765
Butt ends browning	Normal	7.32943	6 (adjusted)	0.09834
Cut petiole ends pithiness	Normal	4.23812	5 (adjusted)	0.15257
Butt ends pithiness	Normal	2.32695	6 (adjusted)	0.54126
Cut petiole ends hue angle	Normal	2.69660	5 (adjusted)	0.74664
Butt ends hue angle	Normal	8.02471	6 (adjusted)	0.23630

Table A6. Normality tests for Late UK season datasets (**chapter 5**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	6.77737	2 (adjusted)	0.11629
Cut petiole ends browning	Normal	5.90283	5 (adjusted)	0.16673

Butt ends browning	Normal	9.69726	3 (adjusted)	0.08973
Cut petiole ends pithiness	Normal	7.37380	6 (adjusted)	0.17794
Butt ends pithiness	Normal	8.92817	9 (adjusted)	0.14675
Cut petiole ends hue angle	Normal	2.86767	7 (adjusted)	0.35797
Butt ends hue angle	Normal	9.94922	6 (adjusted)	0.12681
Fructose	Normal	12.59060	11 (adjusted)	0.09816
Glucose	Normal	9.68996	11 (adjusted)	0.26843
Sucrose	Normal	6.53398	8 (adjusted)	0.17627

Table A7. Normality tests for deficit irrigation trial datasets (**chapter 6**)

Variable	Distribution	Chi-Square	df	*p*
Respiration rate	Normal	6.87799	2 (adjusted)	0.07210
Cut petiole ends browning	Normal	5.96830	2 (adjusted)	0.13558
Butt ends browning	Normal	4.47600	2 (adjusted)	0.10667
Cut petiole ends pithiness	Normal	6.45944	2 (adjusted)	0.12027
Butt ends pithiness	Normal	4.55537	2 (adjusted)	0.10252
Cut petiole ends hue angle	Normal	9.61955	2 (adjusted)	0.12815
Butt ends hue angle	Normal	3.72353	2 (adjusted)	0.15540
Dry matter content	Normal	2.66226	2 (adjusted)	0.26418

Soil moisture content	Normal	3.50953	6 (adjusted)	0.22836
Fructose	Normal	6.50299	8 (adjusted)	0.14971
Glucose	Normal	3.54396	6 (adjusted)	0.23539
Sucrose	Normal	11.60846	6 (adjusted)	0.09130
Chlorogenic acid	Normal	3.34987	4 (adjusted)	0.29512
Abscisic acid	Normal	5.03884	5 (adjusted)	0.21344
Phaseic acid	Normal	7.82053	4 (adjusted)	0.16752
Dihydrophaseic acid	Normal	6.54553	4 (adjusted)	0.13297

Appendix B - ANOVA tables for chapter 4

Table B1. ANOVA table for the effect of crop maturity stage on the respiration rate of celery grown in the Early Spanish season (**Figure 4.15**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	3095.212	1	3095.212	2674.341	0.000000
Harvest maturity	33.305	3	11.102	9.592	0.000344
Block	4.195	2	2.097	1.812	0.187919
Storage days	42.389	2	21.194	18.312	0.000025
Harvest maturity*Storage days	58.895	6	9.816	8.481	0.000095
Error	24.305	21	1.157		

Table B2. ANOVA table for the effect of crop maturity stage on the cut petiole end browning of celery grown in the Early Spanish season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	240.1467	1	240.1467	6453.628	0.000000
Harvest maturity	3.0741	3	1.0247	27.538	0.000000
Block	0.2222	2	0.1111	2.986	0.091236
Storage days	21.9732	2	10.9866	295.251	0.000000
Harvest maturity*Storage days	0.5343	6	0.0891	2.393	0.062247
Error	0.8186	22	0.0372		

Table B3. ANOVA table for the effect of crop maturity stage on the butt end browning of celery grown in the Early Spanish season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	146.6117	1	146.6117	1845.304	0.000000
Harvest maturity	1.0142	3	0.3381	4.255	0.016310
Block	0.0433	2	0.0216	0.272	0.764141

Storage days	14.5731	2	7.2865	91.711	0.000000
Harvest maturity*Storage days	0.1810	6	0.0302	0.380	0.883983
Error	1.7479	22	0.0795		

Table B4. ANOVA table for the effect of crop maturity stage on the cut petiole pithiness of celery grown in the Early Spanish season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept		0			
Harvest maturity	3.958956	2	1.979478	61.73183	0.000000
Block	0.027618	2	0.013809	0.43065	0.655974
Storage days	0.041667	1	0.041667	1.29941	0.026779
Harvest maturity*Storage days	0.420911	5	0.084182	2.62530	0.055564
Error	0.641315	20	0.032066		

Table B5. ANOVA table for the effect of crop maturity stage on the butt pithiness of celery grown in the Early Spanish season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept		0			
Harvest maturity	16.38067	2	8.190337	153.8731	0.000000
Block	0.04884	2	0.024421	0.4588	0.638527
Storage days	0.04167	1	0.041667	0.7828	0.386800
Harvest maturity*Storage days	0.37670	5	0.075341	1.4154	0.261429
Error	1.06456	20	0.053228		

Table B6. ANOVA table for the effect of crop maturity stage on the bolting of celery grown in the Early Spanish season (**Table 4.5**).

Effect	SS	Degree of freedom	MS	F	*p*

Intercept		0			
Harvest maturity	0.317541	2	0.158770	5.098103	0.016247
Block	0.186806	2	0.093403	2.999163	0.072585
Storage days	0.018150	1	0.018150	0.582795	0.454131
Harvest maturity*Storage days	0.104331	5	0.020866	0.670015	0.650727
Error	0.622861	20	0.031143		

Table B7. ANOVA table for the effect of crop maturity stage on the cut petiole end hue angle of celery grown in the Early Spanish season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	370309.0	1	370309.0	48145.11	0.000000
Harvest maturity	284.3	3	94.8	12.32	0.000061
Block	16.6	2	8.3	1.08	0.357279
Storage days	2395.4	2	1197.7	155.72	0.000000
Harvest maturity*Storage days	78.2	6	13.0	1.70	0.169293
Error	169.2	22	7.7		

Table B8. ANOVA table for the effect of crop maturity stage on the butt end hue angle of celery grown in the Early Spanish season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	241365.2	1	241365.2	69028.49	0.000000
Harvest maturity	31.5	3	10.5	3.01	0.050154
Block	2.5	2	1.2	0.36	0.704035
Storage days	548.5	2	274.3	78.44	0.000000
Harvest maturity*Storage days	11.1	6	1.8	0.53	0.781129
Error	76.9	22	3.5		

Table B9. ANOVA table for the effect of crop maturity stage on the respiration rate of celery grown in the Late Spanish season (**Figure 4.15**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept		0			
Harvest maturity	3.7129	3	1.2376	0.18714	0.019414
Block	71.6898	2	35.8449	5.42003	0.112083
Storage days	104.9778	1	104.9778	15.87348	0.000516
Harvest maturity*Storage days	108.6671	7	15.5239	2.34733	0.054771
Error	165.3351	25	6.6134		

Table B10. ANOVA table for the effect of crop maturity stage on the cut petiole end browning of celery grown in the Late Spanish season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept		0			
Harvest maturity	1.08372	1	1.08372	4.52495	0.045419
Block	0.10917	2	0.05458	0.22790	0.798144
Storage days	13.05500	1	13.05500	54.50989	0.000000
Harvest maturity*Storage days	2.96821	5	0.59364	2.47870	0.064872
Error	5.02945	21	0.23950		

Table B11. ANOVA table for the effect of crop maturity stage on the butt end browning of celery grown in the Late Spanish season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	270.5210	1	270.5210	4036.458	0.000000
Harvest maturity	3.8864	4	0.9716	14.497	0.000002
Block	0.1235	2	0.0617	0.921	0.409826
Storage days	5.3531	2	2.6765	39.937	0.000000
Harvest maturity*Storage	1.2395	8	0.1549	2.312	0.048216

days					
Error	1.8765	28	0.0670		

Table B12. ANOVA table for the effect of crop maturity stage on the cut petiole end pithiness of celery grown in the Late Spanish season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	169.4914	1	169.4914	2361.219	0.000000
Harvest maturity	5.5704	4	1.3926	19.400	0.000000
Block	0.0642	2	0.0321	0.447	0.643920
Storage days	10.9086	2	5.4543	75.985	0.000000
Harvest maturity*Storage days	0.1778	8	0.02222	0.310	0.955961
Error	2.0099	28	0.0718		

Table B13. ANOVA table for the effect of crop maturity stage on the butt end pithiness of celery grown in the Late Spanish season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	180.0000	1	180.0000	3738.462	0.000000
Harvest maturity	5.6296	4	1.4074	29.231	0.000000
Block	0.2815	2	0.1407	2.923	0.070320
Storage days	8.1037	2	4.0519	84.154	0.000000
Harvest maturity*Storage days	0.6370	8	0.0796	1.654	0.154457
Error	1.3481	28	0.0481		

Table B14. ANOVA table for the effect of crop maturity stage on the cut petiole end hue angle of celery grown in the Late Spanish season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	427660.4	1	427660.4	154838.3	0.000000
Harvest maturity	674.2	4	168.5	61.0	0.000000

Block	2.2	2	1.1	0.4	0.676969
Storage days	2908.1	2	1454.1	526.5	0.000000
Harvest maturity*Storage days	104.5	8	13.1	4.7	0.000951
Error	77.3	28	2.8		

Table B15. ANOVA table for the effect of crop maturity stage on the butt end hue angle of celery grown in the Late Spanish season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	296459.3	1	296459.3	109243.2	0.000000
Harvest maturity	254.0	4	63.5	23.4	0.000000
Block	21.8	2	10.9	4.0	0.092215
Storage days	556.4	2	278.2	102.5	0.000000
Harvest maturity*Storage days	53.6	8	6.7	2.5	0.036718
Error	76.0	28	2.7		

Table B16. ANOVA table for the effect of crop maturity stage on the respiration rate of celery grown in the Late UK season (**Figure 4.15**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	2609.903	1	2609.903	237.9096	0.000000
Harvest maturity	142.811	4	35.703	3.2545	0.029664
Block	48.893	2	24.446	2.2284	0.130430
Storage days	13.476	2	6.738	0.6142	0.549707
Harvest maturity*Storage days	114.980	8	14.372	1.3101	0.287368
Error	252.313	23	10.970		

Table B17. ANOVA table for the effect of crop maturity stage on the cut petiole end browning of celery grown in the Late UK season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	240.9165	1	240.9165	3431.673	0.000000
Harvest maturity	3.6680	4	0.9170	13.062	0.000005
Block	0.1045	2	0.0522	0.744	0.484590
Storage days	9.8756	2	4.9378	70.335	0.000000
Harvest maturity*Storage days	1.0894	8	0.1362	1.940	0.094773
Error	1.8955	27	0.0702		

Table B18. ANOVA table for the effect of crop maturity stage on the butt end browning of celery grown in the Late UK season (**Figure 4.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	173.5174	1	173.5174	1559.456	0.000000
Harvest maturity	0.4229	4	0.1057	0.950	0.040538
Block	0.1810	2	0.0905	0.813	0.454031
Storage days	7.4580	2	3.7290	33.514	0.000000
Harvest maturity*Storage days	0.8854	8	0.1107	0.995	0.462237
Error	3.0042	27	0.1113		

Table B19. ANOVA table for the effect of crop maturity stage on the cut petiole end pithiness of celery grown in the Late UK season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	94.98547	1	94.98547	1749.543	0.000000
Harvest maturity	7.29184	4	1.82296	33.577	0.000000
Block	0.16376	2	0.08188	1.508	0.239387
Storage days	7.34894	2	3.67447	67.680	0.000000
Harvest maturity*Storage days	2.31513	8	0.28939	5.330	0.000451
Error	1.46587	27	0.05429		

Table B20. ANOVA table for the effect of crop maturity stage on the butt end pithiness of celery grown in the Late UK season (**Figure 4.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	170.6352	1	170.6352	3547.571	0.000000
Harvest maturity	16.0791	4	4.0198	83.573	0.000000
Block	0.2569	2	0.1284	2.670	0.087461
Storage days	9.4650	2	4.7325	98.391	0.000000
Harvest maturity*Storage days	2.0642	8	0.2580	5.364	0.000431
Error	1.2987	27	0.0481		

Table B21. ANOVA table for the effect of crop maturity stage on the cut petiole end hue angle of celery grown in the Late UK season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	410801.9	1	410801.9	81762.46	0.000000
Harvest maturity	446.4	4	111.6	22.21	0.000000
Block	18.1	2	9.1	1.80	0.184146
Storage days	4336.7	2	2168.3	431.57	0.000000
Harvest maturity*Storage days	139.7	8	17.5	3.48	0.006996
Error	135.7	27	5.0		

Table B22. ANOVA table for the effect of crop maturity stage on the butt end hue angle of celery grown in the Late UK season (**Figure 4.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	295666.8	1	295666.8	125625.2	0.000000
Harvest maturity	51.2	4	12.8	5.4	0.002422
Block	3.5	2	1.8	0.8	0.481348
Storage days	815.0	2	407.5	173.1	0.000000
Harvest maturity*Storage	32.0	8	4.0	1.7	0.143831

days					
Error	63.5	27	2.4		

Table B23. ANOVA table for the effect of crop maturity stage on the chlorogenic acid concentration at butt and cut petiole ends of celery grown in the Early Spanish season (**Figure 4.27**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	69.87936	1	69.87936	363.7065	0.000000
Portion	0.02605	1	0.02605	0.1356	0.716211
Block	0.23684	2	0.11842	0.6163	0.548982
Harvest maturity(portion)	5.24933	4	1.31233	6.8304	0.000987
Storage days(portion)	11.35820	2	5.67910	29.5585	0.000001
Harvest maturity*Storage days(portion)	3.09588	4	0.77397	4.0283	0.013393
Error	4.22689	22	0.19213		

Table B24. ANOVA table for the effect of crop maturity stage on the chlorogenic acid concentration at butt and cut petiole ends of celery grown in the Late Spanish season (**Figure 4.28**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	89.37248	1	89.37248	1108.789	0.000000
Portion	3.74302	1	3.74302	46.437	0.000001
Block	0.62759	2	0.31380	3.893	0.435685
Harvest maturity(portion)	6.53555	4	1.63389	20.271	0.000000
Storage days(portion)	25.50791	2	12.75395	158.230	0.000000
Harvest maturity*Storage days(portion)	7.07142	4	1.76786	21.933	0.000000
Error	1.77328	22	0.08060		

Table B25. ANOVA table for the effect of crop maturity stage on the chlorogenic acid concentration at butt and cut petiole ends of celery grown in the Late UK season (**Figure 4.29**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	39.94345	1	39.94345	489.1079	0.000000
Portion	3.43856	1	3.43856	42.1051	0.000002
Block	0.10283	2	0.05142	0.6296	0.542136
Harvest maturity(portion)	0.39673	4	0.09918	1.2145	0.333020
Storage days(portion)	6.32188	2	3.16094	38.7057	0.000000
Harvest maturity*Storage days(portion)	1.88383	4	0.47096	5.7669	0.002487
Error	1.79665	22	0.08167		

Appendix C - ANOVA tables for chapter 5

Table C1. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the respiration rate of celery grown in the Early Spanish season (**Figure 5.4**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	1642.356	1	1642.356	1682.779	0.000000
Harvest maturity	5.423	1	5.423	5.556	0.023671
Block	0.123	2	0.062	0.063	0.938984
Baseline	10.168	1	10.168	10.418	0.002571
Harvest maturity(Baseline)	1.551	1	1.551	1.589	0.215134
Treatment(Baseline)	27.864	2	13.932	14.275	0.000024
Storage days(Baseline)	5.207	2	2.603	2.667	0.082408
Treatment*Storage days(Baseline)	3.208	4	0.802	0.822	0.519515
Treatment(Harvest maturity*Baseline)	10.536	2	5.268	5.397	0.008645
Storage days(Harvest maturity*Baseline)	9.614	2	4.807	4.925	0.012531
Treatments*Storage days(Harvest maturity*Baseline)	13.579	4	3.395	3.478	0.016234
Error	37.087	38	0.976		

Table C2. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end browning of celery grown in the Early Spanish season (**Figure 5.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	160.3941	1	160.3941	1414.772	0.000000
Harvest maturity	0.0314	1	0.0314	0.277	0.016097
Block	0.0913	2	0.0457	0.403	0.671348
Baseline	2.7080	1	2.7080	23.886	0.000019
Harvest maturity(Baseline)	0.2224	1	0.2224	1.962	0.169400

Treatment(Baseline)	1.0878	2	0.5439	4.798	0.053874
Storage days(Baseline)	0.1358	2	0.0679	0.599	0.554447
Treatment*Storage days(Baseline)	0.2280	4	0.0570	0.503	0.733821
Treatment(Harvest maturity*Baseline)	0.7357	2	0.3679	3.245	0.050004
Storage days(Harvest maturity*Baseline)	0.2227	2	0.1114	0.982	0.383742
Treatments*Storage days(Harvest maturity*Baseline)	0.3292	4	0.0823	0.726	0.579687
Error	4.3081	38	0.1134		

Table C3. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end browning of celery grown in the Early Spanish season (**Figure 5.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	121.7882	1	121.7882	1095.149	0.000000
Harvest maturity	1.3329	1	1.3329	11.986	0.001341
Block	0.1418	2	0.0709	0.638	0.534138
Baseline	2.9437	1	2.9437	26.471	0.000008
Harvest maturity(Baseline)	0.0184	1	0.0184	0.165	0.686664
Treatment(Baseline)	0.7210	2	0.3605	3.242	0.052131
Storage days(Baseline)	1.3635	2	0.6818	6.131	0.004925
Treatment*Storage days(Baseline)	1.3594	4	0.3399	3.056	0.028092
Treatment(Harvest maturity*Baseline)	0.1783	2	0.0892	0.802	0.456025
Storage days(Harvest maturity*Baseline)	1.6693	2	0.8347	7.506	0.001790
Treatments*Storage days(Harvest maturity*Baseline)	1.3522	4	0.3380	3.040	0.028702
Error	4.2259	38	0.1112		

Table C4. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end pithiness of celery grown in the Early Spanish season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	117.9799	1	117.9799	3494.773	0.000000
Harvest maturity	13.5180	1	13.5180	400.427	0.000000
Block	0.0869	2	0.0434	1.287	0.287868
Baseline	2.0560	1	2.0560	60.901	0.000000
Harvest maturity(Baseline)	1.0071	1	1.0071	29.831	0.000003
Treatment(Baseline)	0.4978	2	0.2489	7.373	0.121969
Storage days(Baseline)	2.4897	2	1.2449	36.875	0.000000
Treatment*Storage days(Baseline)	0.4673	4	0.1168	3.460	0.016612
Treatment(Harvest maturity*Baseline)	0.0700	2	0.0350	1.037	0.364281
Storage days(Harvest maturity*Baseline)	1.5008	2	0.7504	22.229	0.000000
Treatments*Storage days(Harvest maturity*Baseline)	1.2728	4	0.3182	9.426	0.000021
Error	1.2828	38	0.0338		

Table C5. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end pithiness of celery grown in the Early Spanish season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	192.2589	1	192.2589	1530.904	0.000000
Harvest maturity	40.0210	1	40.0210	318.676	0.000000
Block	0.2480	2	0.1240	0.987	0.381960
Baseline	0.9400	1	0.9400	7.485	0.009405
Harvest maturity(Baseline)	0.1242	1	0.1242	0.989	0.326256
Treatment(Baseline)	0.0597	2	0.0299	0.238	0.789613
Storage days(Baseline)	4.3795	2	2.1898	17.436	0.000004

Treatment*Storage days(Baseline)	0.3249	4	0.0812	0.647	0.632609
Treatment(Harvest maturity*Baseline)	0.0487	2	0.0243	0.194	0.824629
Storage days(Harvest maturity*Baseline)	0.5239	2	0.2620	2.086	0.138190
Treatments*Storage days(Harvest maturity*Baseline)	0.3601	4	0.0900	0.717	0.585668
Error	4.7722	38	0.1256		

Table C6. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end hue angle of celery grown in the Early Spanish season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	192.2589	1	192.2589	1530.904	0.000000
Harvest maturity	40.0210	1	40.0210	318.676	0.000000
Block	0.2480	2	0.1240	0.987	0.381960
Baseline	0.9400	1	0.9400	7.485	0.009405
Harvest maturity(Baseline)	0.1242	1	0.1242	0.989	0.326256
Treatment(Baseline)	0.0597	2	0.0299	0.238	0.789613
Storage days(Baseline)	4.3795	2	2.1898	17.436	0.000004
Treatment*Storage days(Baseline)	0.3249	4	0.0812	0.647	0.632609
Treatment(Harvest maturity*Baseline)	0.0487	2	0.0243	0.194	0.824629
Storage days(Harvest maturity*Baseline)	0.5239	2	0.2620	2.086	0.138190
Treatments*Storage days(Harvest maturity*Baseline)	0.3601	4	0.0900	0.717	0.585668
Error	4.7722	38	0.1256		

Table C7. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end hue angle of celery grown in the Early Spanish season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	289601.2	1	289601.2	60743.23	0.000000
Harvest maturity	14.0	1	14.0	2.95	0.014209
Block	3.3	2	1.7	0.35	0.708640
Baseline	53.5	1	53.5	11.22	0.001839
Harvest maturity(Baseline)	11.8	1	11.8	2.47	0.124610
Treatment(Baseline)	4.4	2	2.2	0.46	0.633475
Storage days(Baseline)	13.2	2	6.6	1.38	0.263956
Treatment*Storage days(Baseline)	5.7	4	1.4	0.30	0.877940
Treatment(Harvest maturity*Baseline)	0.5	2	0.2	0.05	0.950114
Storage days(Harvest maturity*Baseline)	40.4	2	20.2	4.23	0.021908
Treatments*Storage days(Harvest maturity*Baseline)	5.6	4	1.4	0.29	0.881051
Error	181.2	38	4.8		

Table C8. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the respiration rate of celery grown in the Late Spanish season (**Figure 5.5**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	3458.245	1	3458.245	1094.014	0.000000
Harvest maturity	60.174	2	30.087	9.518	0.000307
Block	16.794	2	8.397	2.656	0.379904
Baseline	2.000	1	2.000	0.633	0.430031
Harvest maturity(Baseline)	48.151	2	24.075	7.616	0.001276
Treatment(Baseline)	468.777	2	234.388	74.149	0.000000
Storage days(Baseline)	63.859	2	31.929	10.101	0.000202
Treatment*Storage days(Baseline)	66.540	4	16.635	5.262	0.001260

Treatment(Harvest maturity*Baseline)	95.415	3	31.805	10.061	0.000026
Storage days(Harvest maturity*Baseline)	68.470	4	17.117	5.415	0.001034
Treatments*Storage days(Harvest maturity*Baseline)	61.837	6	10.306	3.260	0.008639
Error	161.214	51	3.161		

Table C9. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end browning of celery grown in the Late Spanish season (**Figure 5.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	229.1634	1	229.1634	4880.409	0.000000
Harvest maturity	1.7750	2	0.8875	18.901	0.000001
Block	0.2990	2	0.1495	3.184	0.089595
Baseline	0.2239	1	0.2239	4.769	0.033511
Harvest maturity(Baseline)	0.3460	2	0.1730	3.685	0.031877
Treatment(Baseline)	0.2253	2	0.1127	2.399	0.100778
Storage days(Baseline)	0.0090	2	0.0045	0.096	0.908564
Treatment*Storage days(Baseline)	0.1296	4	0.0324	0.690	0.602076
Treatment(Harvest maturity*Baseline)	0.2027	3	0.0676	1.439	0.242090
Storage days(Harvest maturity*Baseline)	0.1173	4	0.0293	0.624	0.647161
Treatments*Storage days(Harvest maturity*Baseline)	0.0473	6	0.0079	0.168	0.984208
Error	2.4417	52	0.0470		

Table C10. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end browning of celery grown in the Late Spanish season (**Figure 5.8**).

Effect	SS	Degree of	MS	F	*p*

		freedom			
Intercept	210.8808	1	210.8808	4078.607	0.000000
Harvest maturity	0.9453	2	0.4727	9.142	0.000396
Block	0.0521	2	0.0261	0.504	0.606981
Baseline	0.3590	1	0.3590	6.944	0.011059
Harvest maturity(Baseline)	0.2522	2	0.1261	2.439	0.097178
Treatment(Baseline)	0.0514	2	0.0257	0.497	0.610944
Storage days(Baseline)	0.0812	2	0.0406	0.785	0.461358
Treatment*Storage days(Baseline)	0.1276	4	0.0319	0.617	0.652471
Treatment(Harvest maturity*Baseline)	0.3169	3	0.1056	2.043	0.119231
Storage days(Harvest maturity*Baseline)	0.2593	4	0.0648	1.254	0.300099
Treatments*Storage days(Harvest maturity*Baseline)	0.2881	6	0.0480	0.929	0.482421
Error	2.6886	52	0.0517		

Table C11. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end pithiness of celery grown in the Late Spanish season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	92.43133	1	92.43133	1556.606	0.000000
Harvest maturity	7.86391	2	3.93196	66.217	0.000000
Block	0.23148	2	0.11574	1.949	0.153977
Baseline	0.19914	1	0.19914	3.354	0.073538
Harvest maturity(Baseline)	0.18684	2	0.09342	1.573	0.218318
Treatment(Baseline)	0.02898	2	0.01449	0.244	0.784506
Storage days(Baseline)	0.11557	2	0.05779	0.973	0.385528
Treatment*Storage days(Baseline)	0.28035	4	0.07009	1.180	0.331979
Treatment(Harvest	0.05401	3	0.01800	0.303	0.822914

maturity*Baseline)					
Storage days(Harvest maturity*Baseline)	0.38735	3	0.12912	2.174	0.103845
Treatments*Storage days(Harvest maturity*Baseline)	0.25463	4	0.06366	1.072	0.381238
Error	2.73148	46	0.05938		

Table C12. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end pithiness of celery grown in the Late Spanish season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	136.6392	1	136.6392	1417.168	0.000000
Harvest maturity	5.9637	2	2.9819	30.927	0.000000
Block	0.4537	2	0.2269	2.353	0.106448
Baseline	1.2886	1	1.2886	13.364	0.000657
Harvest maturity(Baseline)	0.4374	2	0.2187	2.268	0.114929
Treatment(Baseline)	0.7985	2	0.3993	4.141	0.220200
Storage days(Baseline)	0.8165	2	0.4082	4.234	0.020519
Treatment*Storage days(Baseline)	1.1296	4	0.2824	2.929	0.030697
Treatment(Harvest maturity*Baseline)	0.3210	3	0.1070	1.110	0.354858
Storage days(Harvest maturity*Baseline)	0.3117	3	0.1039	1.078	0.367896
Treatments*Storage days(Harvest maturity*Baseline)	0.2037	4	0.0509	0.528	0.715551
Error	4.4352	46	0.0964		

Table C13. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end hue angle of celery grown in the Late Spanish season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*

Intercept	651528.0	1	651528.0	253670.4	0.000000
Harvest maturity	467.5	2	233.8	91.0	0.000000
Block	13.9	2	7.0	2.7	0.375834
Baseline	135.3	1	135.3	52.7	0.000000
Harvest maturity(Baseline)	9.3	2	4.6	1.8	0.174115
Treatment(Baseline)	3.4	2	1.7	0.7	0.523381
Storage days(Baseline)	95.0	2	47.5	18.5	0.000001
Treatment*Storage days(Baseline)	16.2	4	4.0	1.6	0.195589
Treatment(Harvest maturity*Baseline)	16.9	3	5.6	2.2	0.099169
Storage days(Harvest maturity*Baseline)	21.4	4	5.3	2.1	0.096538
Treatments*Storage days(Harvest maturity*Baseline)	14.2	6	2.4	0.9	0.486081
Error	133.6	52	2.6		

Table C14. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end hue angle of celery grown in the Late Spanish season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	419517.7	1	419517.7	158708.3	0.000000
Harvest maturity	81.5	2	40.8	15.4	0.000006
Block	22.7	2	11.4	4.3	0.118696
Baseline	122.5	1	122.5	46.4	0.000000
Harvest maturity(Baseline)	20.4	2	10.2	3.9	0.027426
Treatment(Baseline)	0.5	2	0.3	0.1	0.079748
Storage days(Baseline)	10.1	2	5.1	1.9	0.157716
Treatment*Storage days(Baseline)	8.9	4	2.2	0.8	0.505639
Treatment(Harvest maturity*Baseline)	36.5	3	12.2	4.6	0.006210

Storage days(Harvest maturity*Baseline)	12.9	4	3.2	1.2	0.312014
Treatments*Storage days(Harvest maturity*Baseline)	15.4	6	2.6	1.0	0.454403
Error	137.5	52	2.6		

Table C15. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the respiration rate of celery grown in the Late UK season (**Figure 5.6**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	4181.129	1	4181.129	1738.799	0.000000
Harvest maturity	111.496	2	55.748	23.184	0.000000
Block	42.923	2	21.462	8.925	0.230447
Baseline	5.443	1	5.443	2.263	0.138186
Harvest maturity(Baseline)	20.474	2	10.237	4.257	0.019097
Treatment(Baseline)	650.915	2	325.457	135.347	0.000000
Storage days(Baseline)	28.894	2	14.447	6.008	0.004365
Treatment*Storage days(Baseline)	7.039	4	1.760	0.732	0.574139
Treatment(Harvest maturity*Baseline)	36.037	4	9.009	3.747	0.009124
Storage days(Harvest maturity*Baseline)	9.067	4	2.267	0.943	0.446309
Treatments*Storage days(Harvest maturity*Baseline)	25.697	8	3.212	1.336	0.245728
Error	132.253	55	2.405		

Table C16. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end browning of celery grown in the Late UK season (**Figure 5.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	223.6686	1	223.6686	4517.607	0.000000

Harvest maturity	5.7373	2	2.8686	57.940	0.000000
Block	0.0173	2	0.0086	0.175	0.840276
Baseline	0.5273	1	0.5273	10.650	0.001848
Harvest maturity(Baseline)	0.3204	2	0.1602	3.236	0.046518
Treatment(Baseline)	0.4472	2	0.2236	4.516	0.075038
Storage days(Baseline)	1.0892	2	0.5446	10.999	0.000089
Treatment*Storage days(Baseline)	0.1043	4	0.0261	0.526	0.716712
Treatment(Harvest maturity*Baseline)	0.1866	4	0.0466	0.942	0.446271
Storage days(Harvest maturity*Baseline)	0.3100	4	0.0775	1.565	0.195675
Treatments*Storage days(Harvest maturity*Baseline)	0.4225	8	0.0528	1.067	0.398869
Error	2.8716	58	0.0495		

Table C17. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end browning of celery grown in the Late UK season (**Figure 5.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	208.0005	1	208.0005	4739.022	0.000000
Harvest maturity	1.1862	2	0.5931	13.513	0.000015
Block	0.0469	2	0.0235	0.534	0.588859
Baseline	2.7660	1	2.7660	63.019	0.000000
Harvest maturity(Baseline)	0.2225	2	0.1112	2.535	0.088042
Treatment(Baseline)	0.2743	2	0.1372	3.125	0.051400
Storage days(Baseline)	0.7599	2	0.3800	8.657	0.000513
Treatment*Storage days(Baseline)	0.2936	4	0.0734	1.672	0.168796
Treatment(Harvest maturity*Baseline)	0.1866	4	0.0466	1.063	0.383276
Storage	0.3182	4	0.0796	1.813	0.138675

days(Harvest maturity*Baseline)					
Treatments*Storage days(Harvest maturity*Baseline)	0.2085	8	0.0261	0.594	0.778998
Error	2.5457	58	0.0439		

Table C18. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end pithiness of celery grown in the Late UK season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	104.4938	1	104.4938	1085.608	0.000000
Harvest maturity	11.7343	2	5.8672	60.955	0.000000
Block	0.1210	2	0.0605	0.628	0.536993
Baseline	0.4457	1	0.4457	4.630	0.035588
Harvest maturity(Baseline)	0.8914	2	0.4457	4.630	0.013626
Treatment(Baseline)	0.1317	2	0.0658	0.684	0.598587
Storage days(Baseline)	0.1564	2	0.0782	0.812	0.448810
Treatment*Storage days(Baseline)	0.0823	4	0.0206	0.214	0.929762
Treatment(Harvest maturity*Baseline)	0.2634	4	0.0658	0.684	0.605859
Storage days(Harvest maturity*Baseline)	0.3128	4	0.0782	0.812	0.522456
Treatments*Storage days(Harvest maturity*Baseline)	0.1646	8	0.0206	0.214	0.987241
Error	5.5827	58	0.0963		

Table C19. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end pithiness of celery grown in the Late UK season (**Figure 5.7**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	172.4844	1	172.4844	2285.200	0.000000
Harvest maturity	46.2696	2	23.1348	306.507	0.000000

Block	0.2889	2	0.1444	1.914	0.156733
Baseline	0.1405	1	0.1405	1.861	0.177780
Harvest maturity(Baseline)	0.0702	2	0.0351	0.465	0.630301
Treatment(Baseline)	0.1015	2	0.0508	0.672	0.514400
Storage days(Baseline)	0.4966	2	0.2483	3.289	0.044338
Treatment*Storage days(Baseline)	0.0466	4	0.0117	0.154	0.960229
Treatment(Harvest maturity*Baseline)	0.2112	4	0.0528	0.700	0.595310
Storage days(Harvest maturity*Baseline)	0.3347	4	0.0837	1.109	0.361241
Treatments*Storage days(Harvest maturity*Baseline)	0.4801	8	0.0600	0.795	0.609111
Error	4.3778	58	0.0755		

Table C20. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the cut petiole end hue angle of celery grown in the Late UK season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	691821.0	1	691821.0	147691.1	0.000000
Harvest maturity	1054.8	2	527.4	112.6	0.000000
Block	4.5	2	2.3	0.5	0.620615
Baseline	204.6	1	204.6	43.7	0.000000
Harvest maturity(Baseline)	39.1	2	19.6	4.2	0.020255
Treatment(Baseline)	152.7	2	76.3	16.3	0.238344
Storage days(Baseline)	191.7	2	95.8	20.5	0.000000
Treatment*Storage days(Baseline)	27.9	4	7.0	1.5	0.216790
Treatment(Harvest maturity*Baseline)	5.5	4	1.4	0.3	0.879462
Storage days(Harvest maturity*Baseline)	28.0	4	7.0	1.5	0.215993

Treatments*Storage days(Harvest maturity*Baseline)	63.8	8	8.0	1.7	0.117165
Error	271.7	58	4.7		

Table C21. ANOVA table for the effect of crop maturity stage, ethylene and 1-MCP on the butt end hue angle of celery grown in the Late UK season (**Figure 5.9**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	444125.4	1	444125.4	159731.2	0.000000
Harvest maturity	4.0	2	2.0	0.7	0.049126
Block	5.9	2	2.9	1.1	0.353441
Baseline	234.6	1	234.6	84.4	0.000000
Harvest maturity(Baseline)	8.9	2	4.5	1.6	0.208832
Treatment(Baseline)	20.7	2	10.4	3.7	0.089941
Storage days(Baseline)	13.8	2	6.9	2.5	0.092547
Treatment*Storage days(Baseline)	20.3	4	5.1	1.8	0.136666
Treatment(Harvest maturity*Baseline)	6.4	4	1.6	0.6	0.680746
Storage days(Harvest maturity*Baseline)	14.6	4	3.6	1.3	0.277261
Treatments*Storage days(Harvest maturity*Baseline)	10.6	8	1.3	0.5	0.869211
Error	161.3	58	2.8		

Table C22. ANOVA table for the effect of ethylene and 1-MCP on the concentration of fructose at cut petiole ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	250796.6	1	250796.6	1274.088	0.000000
Baseline	76.7	1	76.7	0.389	0.540417
Block	4578.5	2	2289.2	11.630	0.157572

Treatment(Baseline)	762.9	2	381.5	1.938	0.172890
Storage days(Baseline)	771.2	2	385.6	1.959	0.169920
Treatment*Storage days(Baseline)	656.0	4	164.0	0.833	0.521621
Error	3543.2	18	196.8		

Table C23. ANOVA table for the effect of ethylene and 1-MCP on the concentration of glucose at cut petiole ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	211265.8	1	211265.8	1095.632	0.000000
Baseline	1334.4	1	1334.4	6.920	0.016969
Block	2931.7	2	1465.9	7.602	0.144043
Treatment(Baseline)	205.5	2	102.8	0.533	0.595891
Storage days(Baseline)	254.0	2	127.0	0.659	0.529588
Treatment*Storage days(Baseline)	94.1	4	23.5	0.122	0.972749
Error	3470.9	18	192.8		

Table C24. ANOVA table for the effect of ethylene and 1-MCP on the concentration of sucrose at cut petiole ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	16039.31	1	16039.31	850.4655	0.000000
Baseline	33.25	1	33.25	1.7631	0.200838
Block	188.98	2	94.49	5.0103	0.078627
Treatment(Baseline)	236.62	2	118.31	6.2733	0.008566
Storage days(Baseline)	83.25	2	41.63	2.2072	0.138898
Treatment*Storage days(Baseline)	47.94	4	11.98	0.6354	0.643789
Error	339.47	18	18.86		

Table C25. ANOVA table for the effect of ethylene and 1-MCP on the concentration of fructose at butt ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	6922.756	1	6922.756	827.2586	0.000000
Baseline	8.147	1	8.147	0.9735	0.336882
Block	85.339	2	42.670	5.0990	0.087599
Treatment(Baseline)	15.225	2	7.613	0.9097	0.420380
Storage days(Baseline)	45.490	2	22.745	2.7180	0.093003
Treatment*Storage days(Baseline)	18.273	4	4.568	0.5459	0.704269
Error	150.630	18	8.368		

Table C26. ANOVA table for the effect of ethylene and 1-MCP on the concentration of glucose at butt ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	5613.072	1	5613.072	5948.200	0.000000
Baseline	1370.342	1	1370.342	1452.158	0.000000
Block	8.460	2	4.230	4.482	0.206320
Treatment(Baseline)	327.364	2	163.682	173.455	0.000000
Storage days(Baseline)	321.605	2	160.802	170.403	0.000000
Treatment*Storage days(Baseline)	672.674	4	168.168	178.209	0.000000
Error	16.986	18	0.944		

Table C27. ANOVA table for the effect of ethylene and 1-MCP on the concentration of sucrose at butt ends of celery harvested at optimum date (HM3) and grown in the Late UK season (**Figure 5.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	162697.4	1	162697.4	824.1785	0.000000
Baseline	3492.4	1	3492.4	17.6913	0.000531
Block	575.2	2	287.6	1.4570	0.259125

Treatment(Baseline)	617.1	2	308.6	1.5630	0.236636
Storage days(Baseline)	12764.3	2	6382.2	32.3302	0.000001
Treatment*Storage days(Baseline)	1137.0	4	284.2	1.4399	0.261549
Error	3553.3	18	197.4		

Appendix D - ANOVA tables for chapter 6

Table D1. ANOVA table for the effect of water treatments on the soil moisture of pots filled with Fenland peat and used to grow celery (**Figure 6.8**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	100747.1	1	100747.1	7148.504	0.000000
Water Treatment	7523.0	2	3761.5	266.897	0.000000
Block	365.4	2	182.7	12.965	0.095667
Days after commencement of water treatments	3753.9	14	268.1	19.026	0.000000
Water treatments*Days after commencement of water treatments	507.5	28	18.1	1.286	0.187824
Error	1240.2	88	14.1		

Table D2. ANOVA table for the effect of water treatments on the respiration rate of celery (**Figure 6.10**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	8729.788	1	8729.788	1572.339	0.000000
Water Treatment	168.970	2	84.485	15.217	0.000071
Storage days	310.285	3	103.428	18.629	0.000003
Block	8.115	2	4.057	0.731	0.492863
Water treatments*Storage days	74.349	6	12.392	2.232	0.078251
Error	122.146	22	5.552		

Table D3. ANOVA table for the effect of water treatments on the cut petiole end browning of celery (**Figure 6.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	185.2623	1	185.2623	11191.10	0.000000
Water Treatment	3.1543	2	1.5772	95.27	0.000000

Storage days	23.2685	3	7.7562	468.53	0.000000
Block	0.1543	2	0.0772	4.66	0.120526
Water treatments*Storage days	1.4630	6	0.2438	14.73	0.000001
Error	0.3642	22	0.0166		

Table D4. ANOVA table for the effect of water treatments on the butt end browning of celery (**Figure 6.11**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	189.8272	1	189.8272	4540.564	0.000000
Water Treatment	1.5062	2	0.7531	18.013	0.000023
Storage days	25.3580	3	8.4527	202.183	0.000000
Block	0.2654	2	0.1327	3.174	0.091480
Water treatments*Storage days	0.7901	6	0.1317	3.150	0.022009
Error	0.9198	22	0.0418		

Table D5. ANOVA table for the effect of water treatments on the cut petiole end pithiness of celery (**Figure 6.13**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	314.0575	1	314.0575	2948.158	0.000000
Water Treatment	0.5979	2	0.2990	2.806	0.082121
Storage days	0.7265	3	0.2422	2.273	0.108260
Block	0.1735	2	0.0867	0.814	0.455924
Water treatments*Storage days	0.6344	6	0.1057	0.993	0.454451
Error	2.3436	22	0.1065		

Table D6. ANOVA table for the effect of water treatments on the butt end pithiness of celery (**Figure 6.13**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	393.3831	1	393.3831	2434.307	0.000000
Water Treatment	6.0033	2	3.0017	18.575	0.110019
Storage days	1.0198	3	0.3399	2.104	0.128845
Block	0.5196	2	0.2598	1.608	0.222997
Water treatments*Storage days	0.3211	6	0.0535	0.331	0.913216
Error	3.5552	22	0.1616		

Table D7. ANOVA table for the effect of water treatments on the cut petiole end hue angle of celery (**Figure 6.12**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	399999.0	1	399999.0	200808.8	0.000000
Water Treatment	85.0	2	42.5	21.3	0.000007
Storage days	1289.1	3	429.7	215.7	0.000000
Block	1.7	2	0.9	0.4	0.651490
Water treatments*Storage days	137.5	6	22.9	11.5	0.000008
Error	43.8	22	2.0		

Table D8. ANOVA table for the effect of water treatments on the butt end hue angle of celery (**Figure 6.12**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	234694.2	1	234694.2	81952.29	0.000000
Water Treatment	163.8	2	81.9	28.60	0.000001
Storage days	725.1	3	241.7	84.40	0.000000
Block	1.1	2	0.6	0.20	0.821159
Water treatments*Storage	19.5	6	3.3	1.13	0.375240

days					
Error	63.0	22	2.9		

Table D9. ANOVA table for the effect of water treatments on the dry matter content of celery (**Figure 6.14**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	8132.132	1	8132.132	2756.691	0.000000
Block	7.261	2	10.492	0.675	0.551768
Water Treatment	32.653	2	16.326	5.534	0.010555
Storage days	270.445	3	90.148	30.559	0.000000
Water treatments*Storage days	15.780	6	2.630	0.892	0.516614
Error	70.799	24	2.950		

Table D10. ANOVA table for the effect of water treatments on the fructose concentration at butt and cut petiole ends of celery (**Figure 6.15**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	50962.41	1	50962.41	4211.205	0.000000
Block	24.35	2	12.17	1.006	0.373616
Portion	17302.96	1	17302.96	1429.805	0.000000
Water treatment	7847.49	2	3923.74	324.233	0.000000
Storage days	613.69	3	204.56	16.904	0.000000
Water treatment*Portion	9525.17	2	4762.58	393.549	0.000000
Storage days*Portion	1196.96	3	398.99	32.970	0.000000
Water treatment*Storage days	1176.99	6	196.16	16.210	0.000000
Water treatments*Storage days*Portion	721.21	6	120.20	9.933	0.000001
Error	556.67	46	12.10		

Table D11. ANOVA table for the effect of water treatments on the glucose concentration at butt and cut petiole ends of celery (**Figure 6.16**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	105053.8	1	105053.8	4946.664	0.000000
Block	122.5	2	61.3	2.885	0.186012
Portion	27277.1	1	27277.1	1284.395	0.000000
Water treatment	13770.0	2	6885.0	324.195	0.000000
Storage days	1773.3	3	591.1	27.834	0.000000
Water treatment*Portion	14020.0	2	7010.0	330.079	0.000000
Storage days*Portion	2599.0	3	866.3	40.794	0.000000
Water treatment*Storage days	2288.6	6	381.4	17.961	0.000000
Water treatments*Storage days*Portion	1199.2	6	199.9	9.411	0.000001
Error	976.9	46	21.2		

Table D12. ANOVA table for the effect of water treatments on the sucrose concentration at butt and cut petiole ends of celery (**Figure 6.17**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	268609.1	1	268609.1	3972.696	0.000000
Block	271.1	2	135.6	2.005	0.146277
Portion	51499.6	1	51499.6	761.673	0.000000
Water treatment	10186.7	2	5093.3	75.330	0.000000
Storage days	7124.7	3	2374.9	35.125	0.000000
Water treatment*Portion	969.2	2	484.6	7.167	0.001952
Storage days*Portion	1233.9	3	411.3	6.083	0.001415
Water treatment*Storage days	4077.1	6	679.5	10.050	0.000000
Water	1881.0	6	313.5	4.637	0.000921

treatments*Storage days*Portion					
Error	3110.2	46	67.6		

Table D13. ANOVA table for the effect of water treatments on the concentration of chlorogenic acid at butt and cut petiole ends of celery (**Figure 6.18**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	43.10115	1	43.10115	1309.206	0.000000
Portion	3.57089	1	3.57089	108.467	0.155374
Block	0.11181	2	0.05590	1.698	0.194303
Water treatment(portion)	1.82185	4	0.45546	13.835	0.000000
Storage days(portion)	4.39297	6	0.73216	22.240	0.000000
Water treatments*Storage days(portion)	1.04430	12	0.08702	2.643	0.008894
Error	1.51439	46	0.03292		

Table D14. ANOVA table for the effect of water treatments on the concentration of abscisic acid at butt and cut petiole ends of celery (**Figure 6.19**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	18949415	1	18949415	474.8134	0.000000
Block	12501344	1	12501344	313.2448	0.266701
Portion	86636	2	43318	1.0854	0.349180
Treatment(portion)	1376316	4	344079	8.6216	0.000065
Storage days(portion)	13464340	4	3366085	84.3436	0.000000
Treatment*Storage days(portion)	1748145	8	218518	5.4754	0.000179
Error	1356912	34	39909		

Table D15. ANOVA table for the effect of water treatments on the concentration of phaseic acid at butt and cut petiole ends of celery (**Figure 6.20**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	9218544	1	9218544	482.8697	0.000000
Block	29257	2	14629	0.7663	0.472609
Portion	6657026	1	6657026	348.6968	0.000000
Treatment(portion)	1456134	4	364034	19.0682	0.000000
Storage days(portion)	1443158	4	360789	18.8982	0.000000
Treatment*Storage days(portion)	263853	8	32982	1.7276	0.127584
Error	649099	34	19091		

Table D16. ANOVA table for the effect of water treatments on the concentration of dihydrophaseic acid at butt and cut petiole ends of celery (**Figure 6.21**).

Effect	SS	Degree of freedom	MS	F	*p*
Intercept	74763631	1	74763631	292.3143	0.000000
Block	52696156	1	52696156	206.0339	0.118502
Portion	768885	2	384442	1.5031	0.236849
Treatment(portion)	9621644	4	2405411	9.4048	0.000031
Storage days(portion)	21636379	4	5409095	21.1487	0.000000
Treatment*Storage days(portion)	6330420	8	791303	3.0939	0.009871
Error	8695994	34	255765		

Appendix E - List of correlations

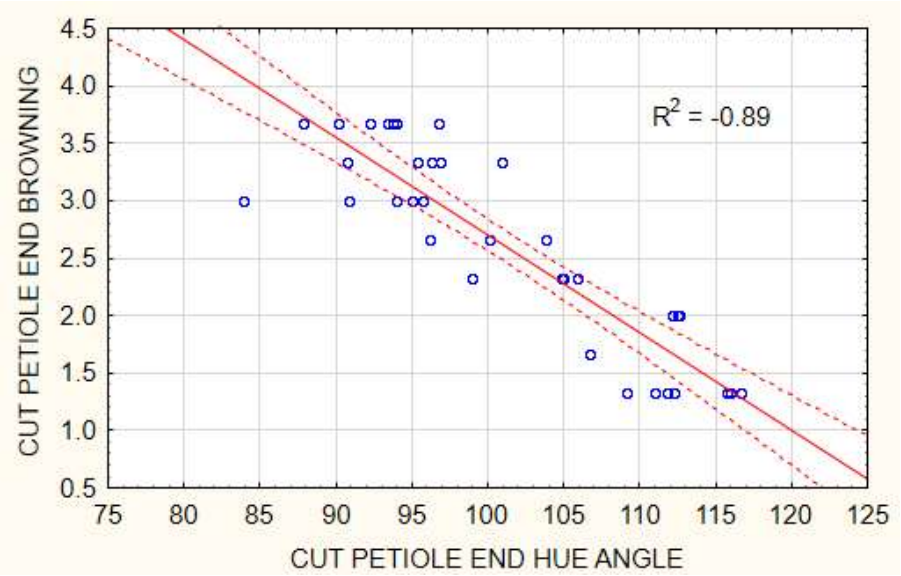


Figure E1 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Early Spanish season (chapter 4).

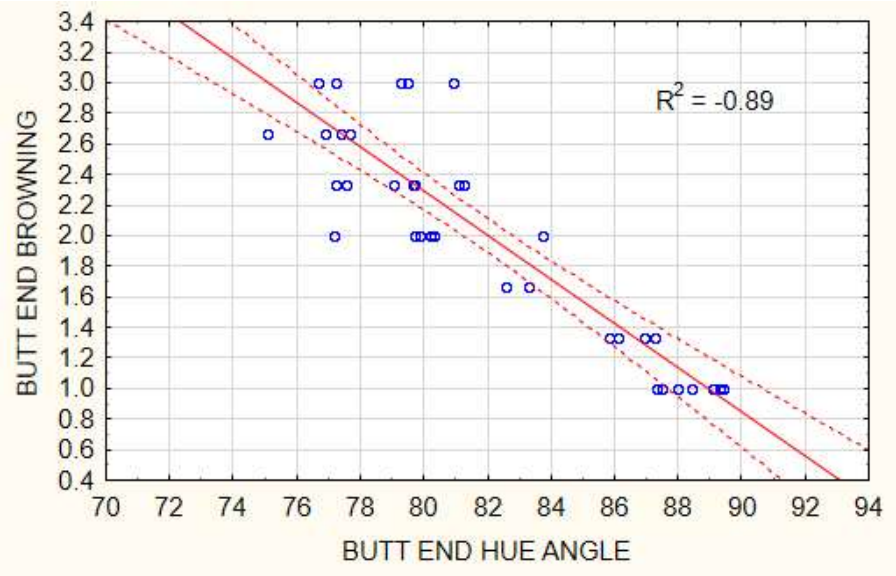


Figure E2 - Correlation between butt end browning and butt end hue angle of celery grown in the Early Spanish season (chapter 4).

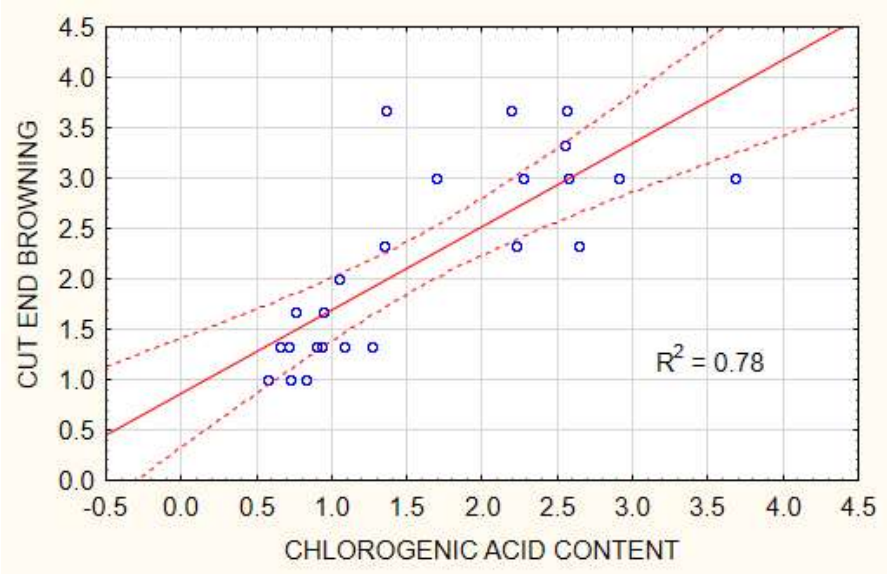


Figure E3 - Correlation between chlorogenic acid content and cut end browning of celery grown in the Early Spanish season (chapter 4).

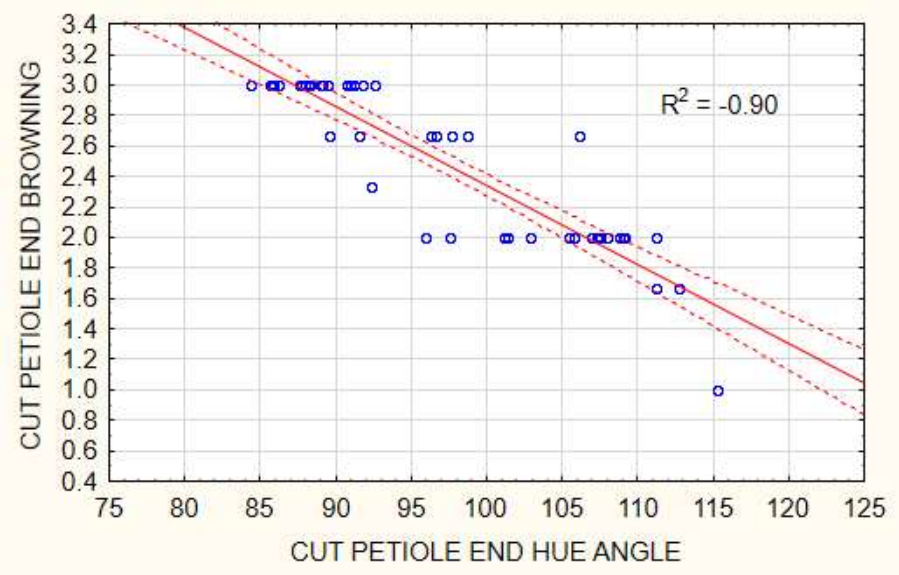


Figure E4 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Late Spanish season (chapter 4).

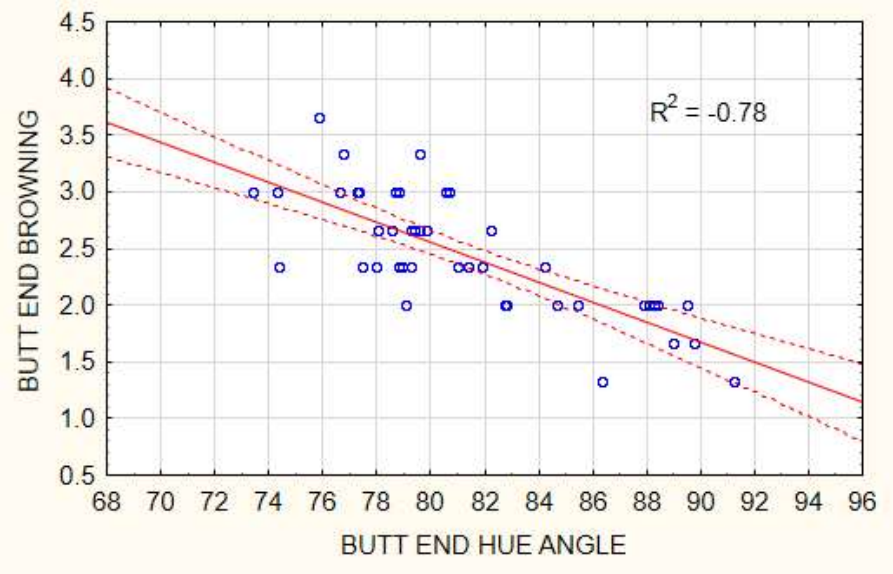


Figure E5 - Correlation between butt end browning and butt end hue angle of celery grown in the Late Spanish season (chapter 4).

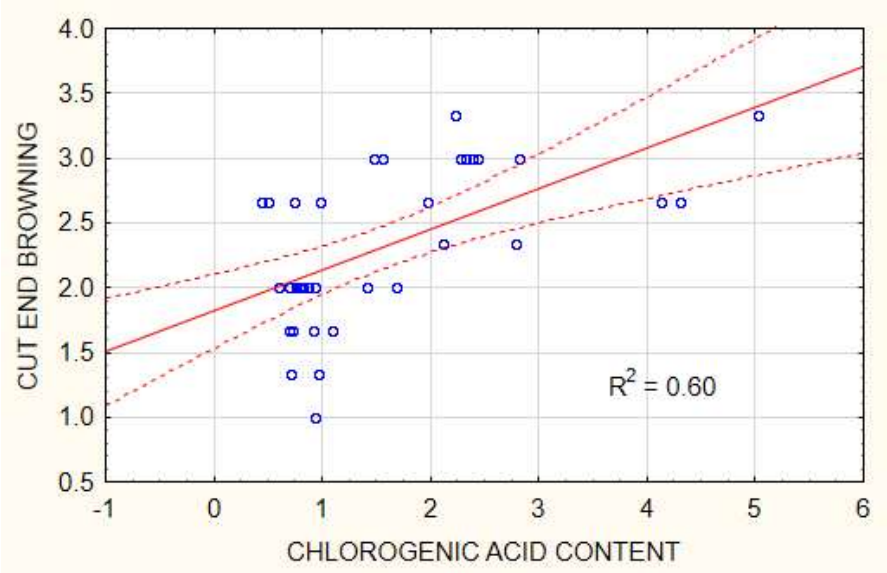


Figure E6 - Correlation between chlorogenic acid and browning of celery grown in the Late Spanish season (chapter 4).

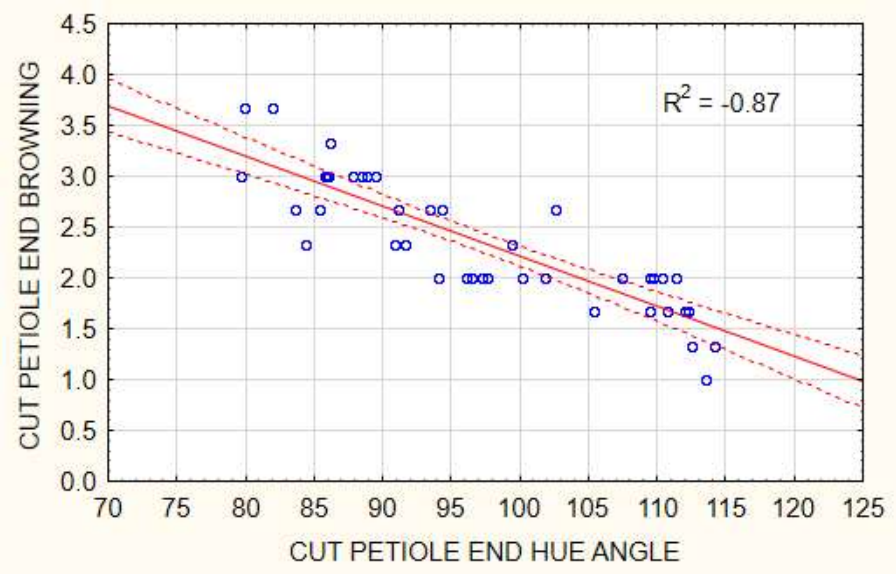


Figure E7 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Late UK season (chapter 4).

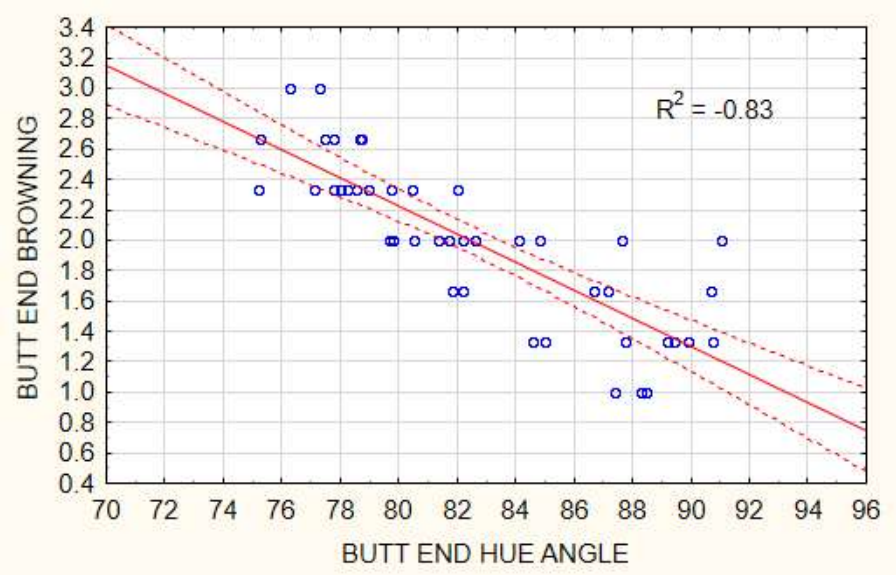


Figure E8 - Correlation between butt end browning and butt end hue angle of celery grown in the Late UK season (chapter 4).

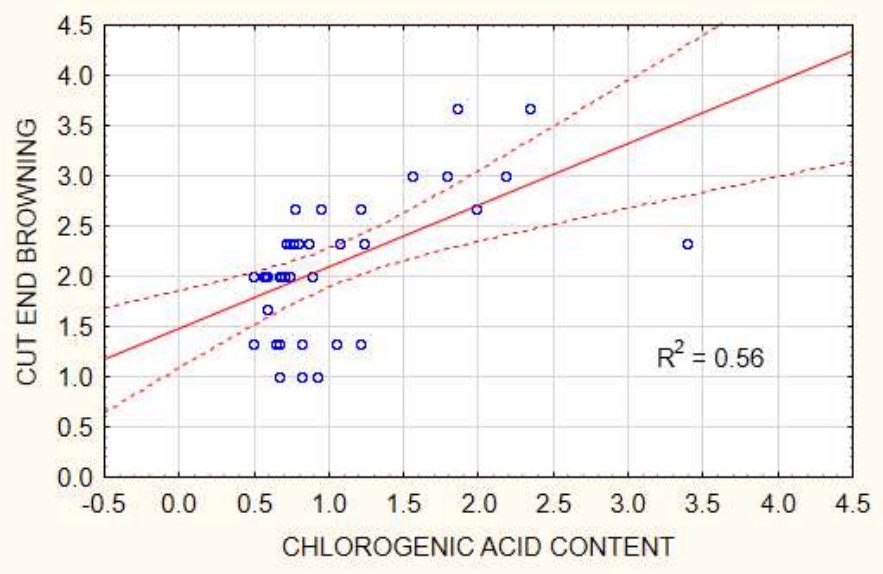


Figure E9 - Correlation between chlorogenic acid and browning of celery grown in the Late UK season (chapter 4).

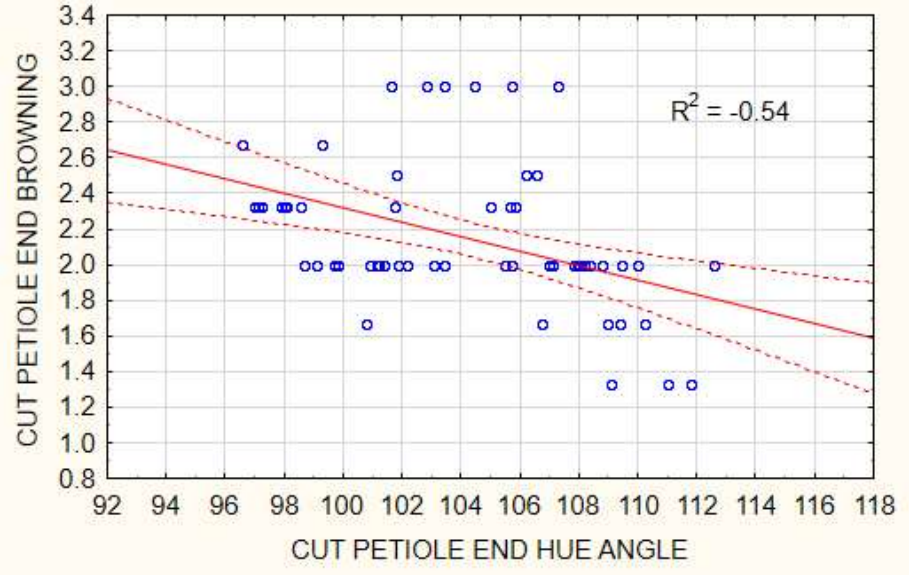


Figure E10 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Early Spanish season (chapter 5).

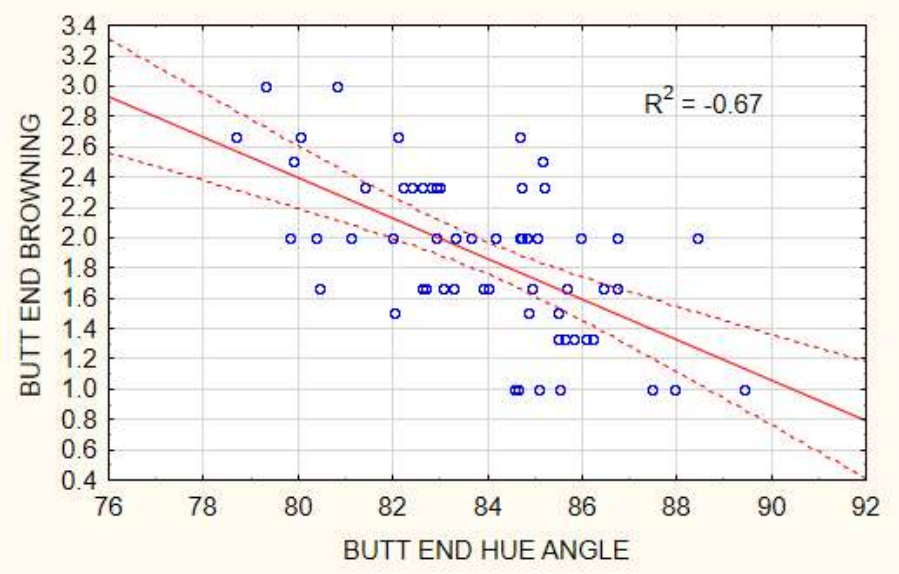


Figure E11 - Correlation between butt end browning and butt end hue angle of celery grown in the Early Spanish season (chapter 5).

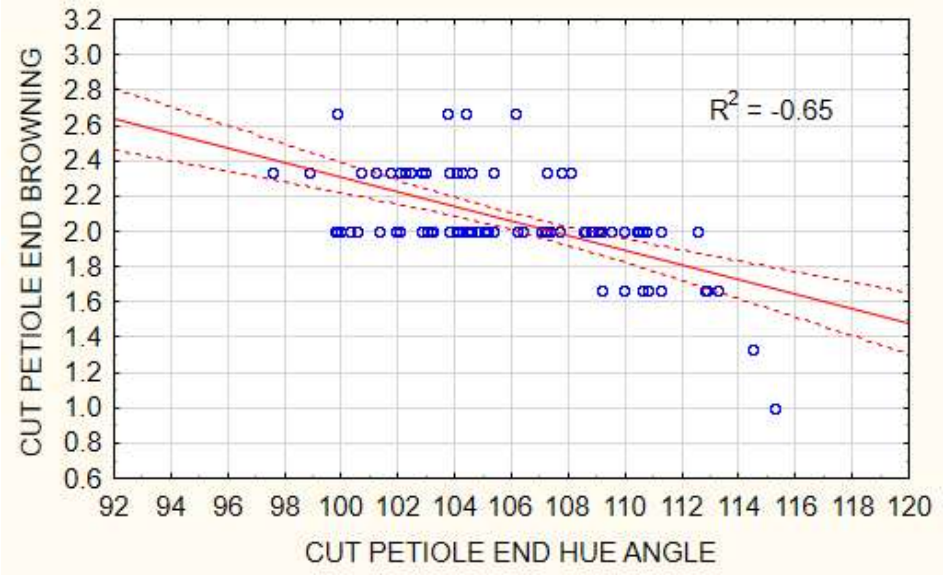


Figure E12 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Late Spanish season (chapter 5).

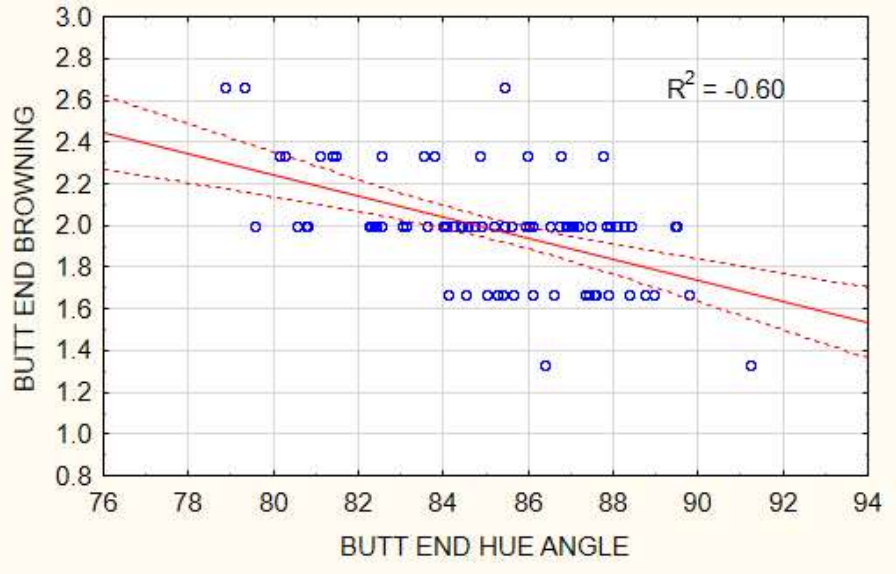


Figure E13 - Correlation between butt end browning and butt end hue angle of celery grown in the Late Spanish season (chapter 5).

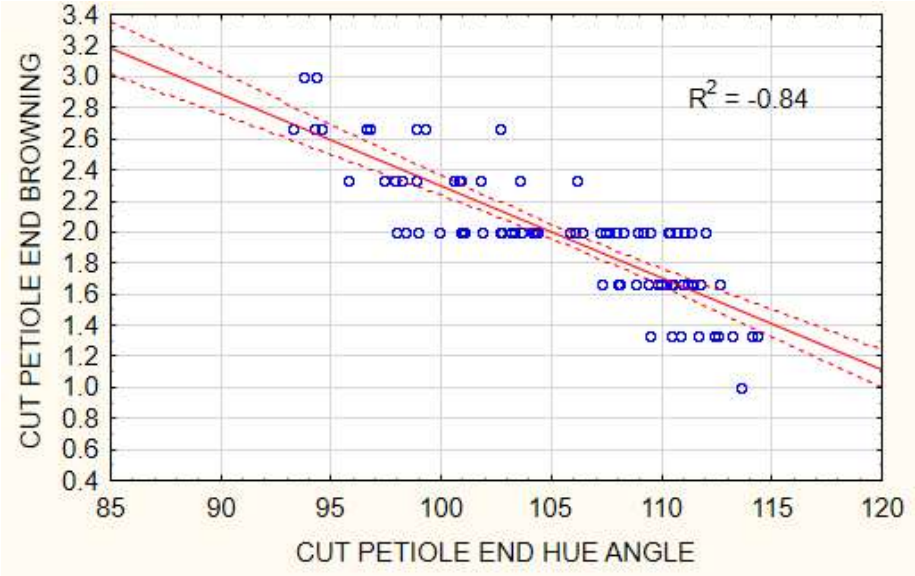


Figure E14 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the Late UK season (chapter 5).

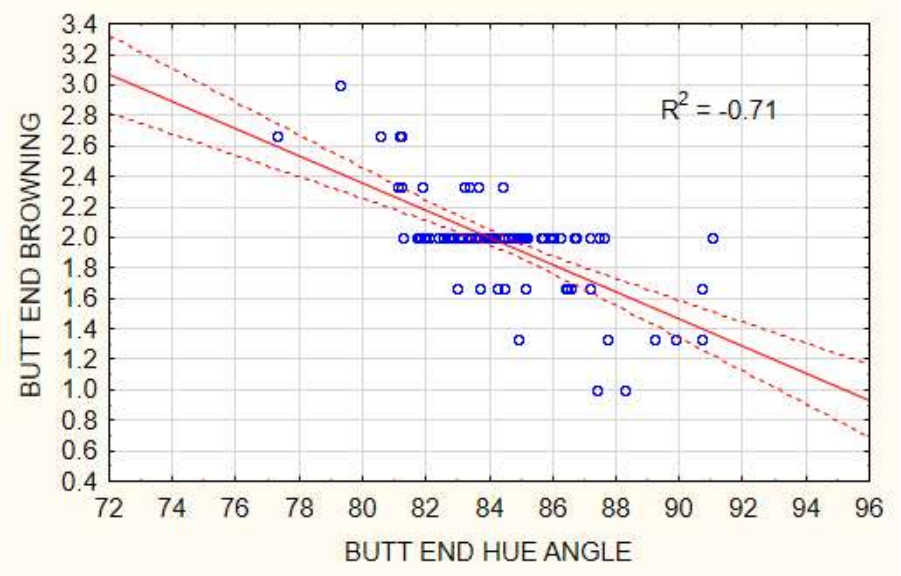


Figure E15 - Correlation between butt end browning and butt end hue angle of celery grown in the Late UK season (chapter 5).

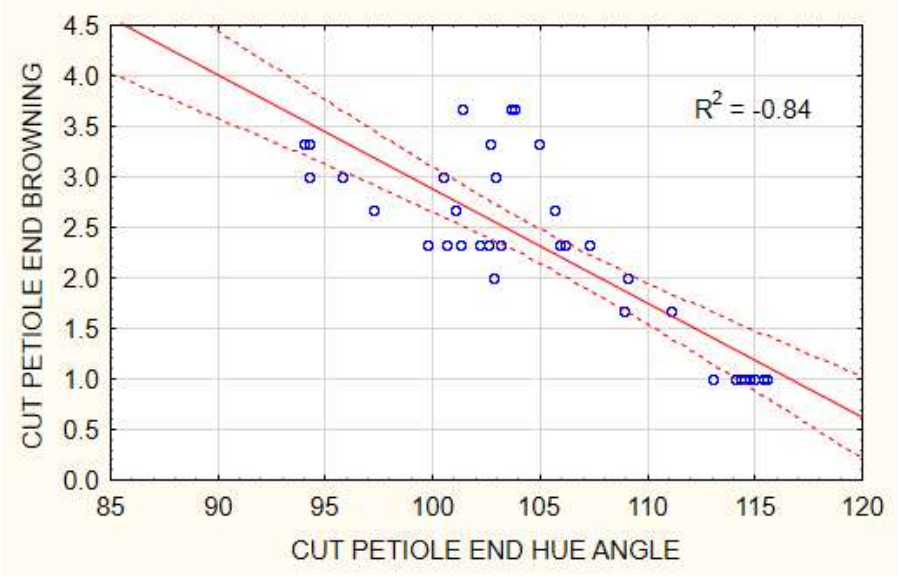


Figure E16 - Correlation between cut petiole end browning and cut petiole end hue angle of celery grown in the deficit irrigation trial (chapter 6).

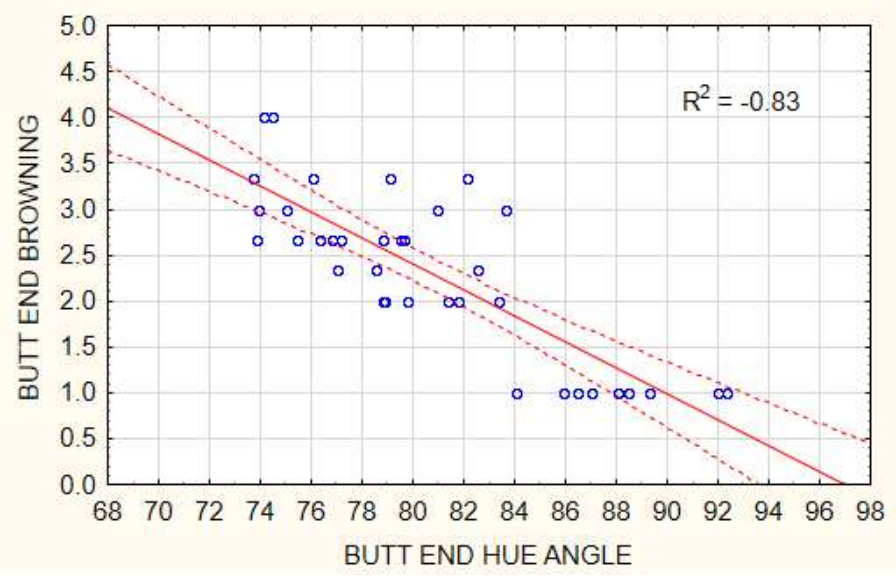


Figure E17 - Correlation between butt end browning and butt end hue angle of celery grown in the deficit irrigation trial (chapter 6).

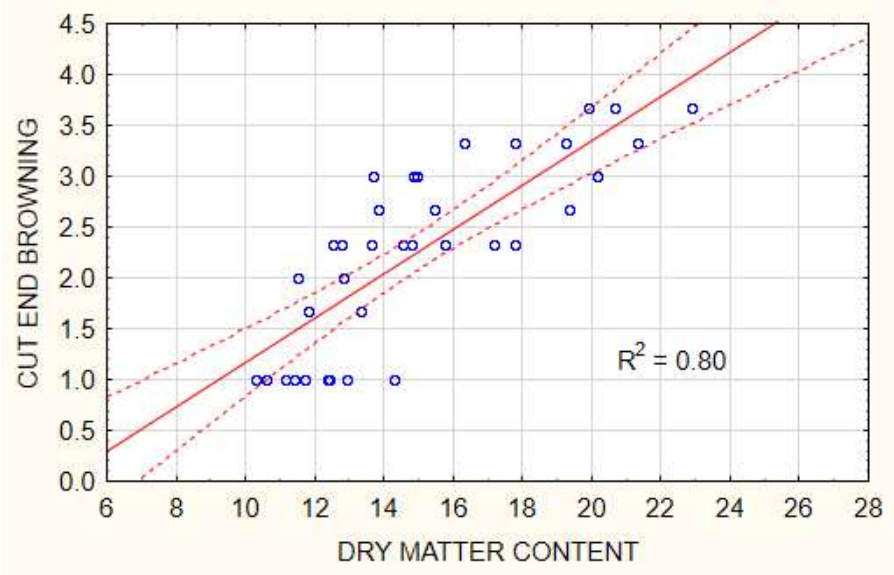


Figure E18 - Correlation between cut end browning and dry matter content of celery grown in the deficit irrigation trial (chapter 6).

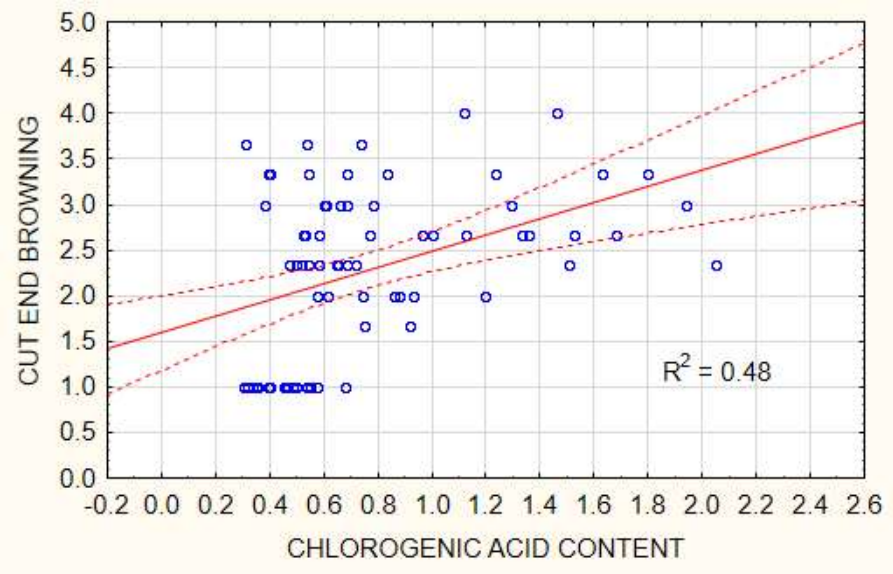


Figure E19 - Correlation between cut end browning and chlorogenic acid content of celery grown in the deficit irrigation trial (chapter 6).

Appendix F - Eucarpia Leafy Vegetables Symposium

San Pedro del Pinatar, Murcia, Spain, 14-17 April 2015 (Oral presentation)

TITLE: The influence of pre-harvest and postharvest factors on cut-end browning in celery

AUTHORS' NAMES: Simone Rossi, Katherine Cools and Leon A. Terry

AFFILATION: Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK

KEYWORDS: Celery, ethylene, browning, crop maturity

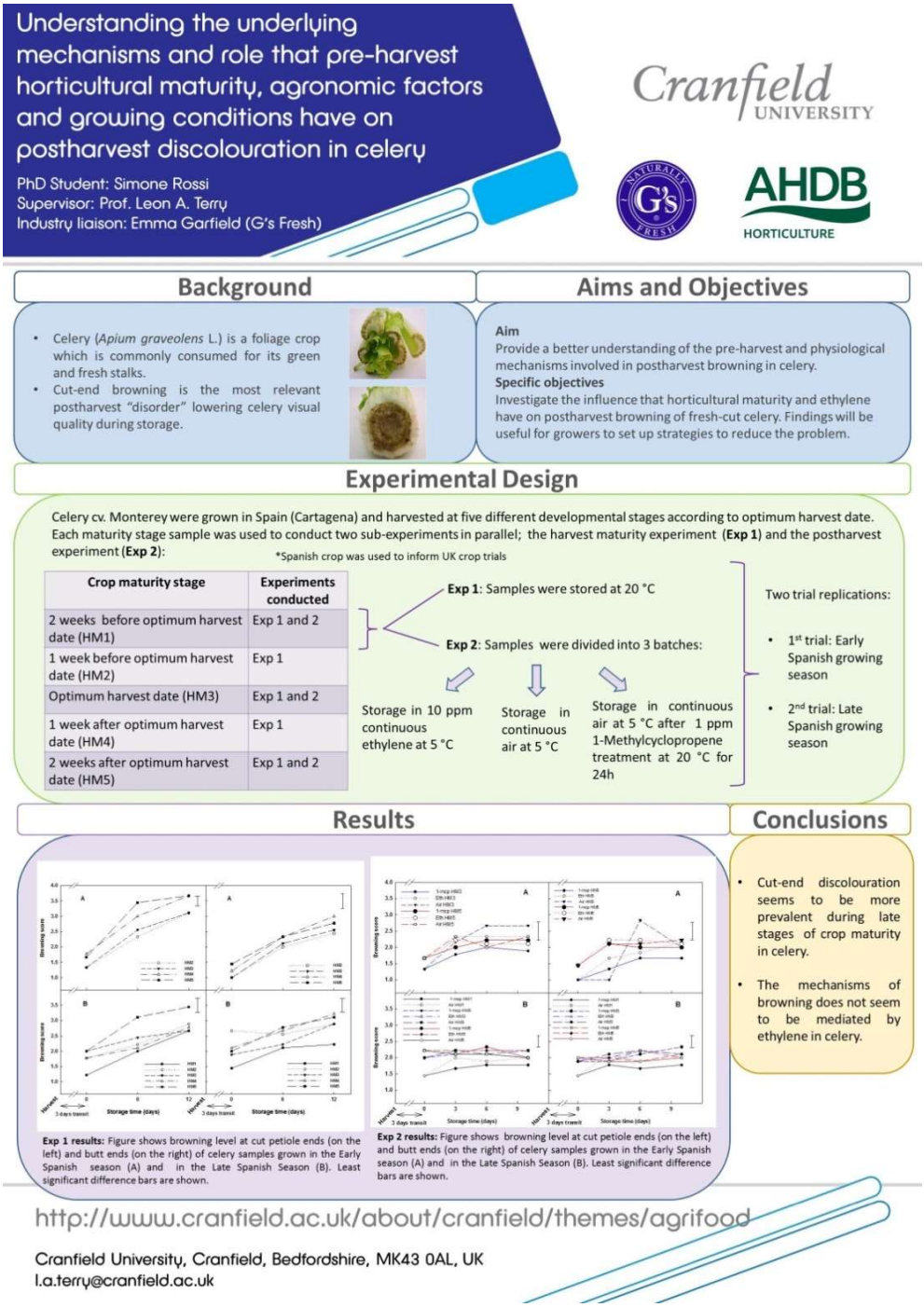
ABSTRACT

Celery (*Apium graveolens* L.) is a foliage crop which is commonly consumed for its long and thick petioles. Colour is a key quality parameter used by consumers to determine stalk freshness. Postharvest browning, which manifests as black/brown stains at the cut ends, is a physiological "disorder" that occurs during storage. It is believed to be due to the activity of polyphenol-oxidases (PPOs) on phenolic compounds to produce brown pigments. It represents an economic problem for the fresh produce industry as customers tend to reject affected petioles. For this reason, the effect of crop maturity and ethylene treatment on postharvest browning of cut ends was investigated. The experiment was conducted in Spain (Murcia) and replicated across two growing seasons. In each trial, celery cv. Monterey plants were harvested every week in order to obtain immature, mature and over-mature plants. Subjective and objective colour analyses were performed at regular time intervals during storage at cut petiole and butt ends. Cut-browning increased with storage time and crop maturity, with the blackening/darkening developing sharply in the first 6 days of storage. On the other hand, continuous ethylene supplementation did not influence browning. In summary, these results indicate that crop maturity impacts on postharvest discolouration of celery, whilst the mechanism of browning does not seem to be promoted by exogenous ethylene.

Appendix G - AHDB Annual Studentship Conference

Chateau Impney, Droitwich Spa, Worcestershire, 16-17 September 2015

(Poster presentation)



Appendix H - VIII International Postharvest Symposium

El Batel Conference Centre, Cartagena, Spain, 21-24 June 2016 (Oral presentation)

TITLE: The role of crop maturity and ethylene on postharvest browning in fresh-cut celery

AUTHORS' NAMES: Simone Rossi, Katherine Cools and Leon A. Terry

AFFILATION: Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK

KEYWORDS: Apium graveolens, physiological disorders, stage of maturity, plant growth regulators.

ABSTRACT

Celery (*Apium graveolens* L.) is a leafy vegetable which is commonly eaten raw and appreciated by consumers for the bright green and fresh petioles. Cut-end browning is one of the most relevant postharvest "disorders" negatively affecting visual quality and it renders products unmarketable. Cut-end browning is thought to be due to the enzymatic activity of polyphenol oxidases (PPOs) which oxidase phenolic compounds to produce dark pigments on damaged tissues. Given the relevance of this postharvest problem, in the present study we investigated the influence of crop developmental stage and the plant hormone ethylene on cut-end browning in celery. The experiment was replicated across two growing seasons; early and late. The crop reached commercial maturity 19 and 12 weeks after planting respectively. The cv. Monterey was used and grown in Spain (Murcia). In each replicate trial, celery heads were trimmed every week to obtain immature, commercially mature and over-mature plants. After harvest, samples were divided into two batches to run two experiments in parallel. The first batch was stored at 20 °C temperature and 55.5% relative humidity and the second was further divided into three sub-batches for postharvest treatments (continuous air, continuous ethylene and continuous air after 1-methylcyclopropene treatment) at 5 °C temperature and 95% relative humidity. During the storage period (12 days) samples were periodically assessed for subjective and objective colour at both apical petiole and butt ends surfaces. Results showed that browning developed over storage in general and intensified as crop maturity increased. Conversely, ethylene did not have any significant effect on cut-end discolouration. In conclusion, findings suggest that cut-end browning seems to be more prevalent in later stages of crop maturity, whilst ethylene does not seem to be involved in its underlying mechanism.

Appendix I - List of courses attended during the PhD

- **14 November 2013** - *Introduction to statistical treatment of experimental data*. Doctoral Training Centre, Cranfield University, Cranfield, UK.
- **9 October 2013** - *Refworks Training*. Kings Norton Library. Cranfield University, Cranfield UK.
- **17 December 2013** - *Personal development planning*. Doctoral Training Centre, Cranfield University, Cranfield, UK.
- **20 May 2014** - *Communicating your progress at the nine month review*. Doctoral Training Centre, Cranfield University, Cranfield, UK.
- **15-16 July 2014** - *Technical Writing Skills Workshop*. Doctoral Training Centre, Cranfield University, Cranfield, UK.
- **8 January 2015** - Safety Awareness Training Workshop. Liquid nitrogen practical decanting.
- **21-23 January 2015** - *7th European Short Course on Quality and Safety of Fresh-Cut Produce*. Cardiff University, Cardiff, Wales.
- **27 March 2015** - *Why publications are important for your research and your career*. Doctoral Training Centre, Cranfield University, Cranfield, UK.
- **5-7 May 2015** - *Modelling climate impacts on water and energy use in European irrigated agriculture*. Cranfield University, Cranfield, UK.
- **21-22 September 2015** - *Dealing with combined stresses for crops: approaches to improve water and nutrient use efficiency in cropping systems*. St. Leonard's Hall, University of Edinburgh, Edinburgh, UK.
- **17 March 2015** - *How to prepare for the Viva*. Doctoral Training Centre. Cranfield University, UK.
- **17 February 2016** - *Using the Thesis Template*. Information Technology Department.
- **2 April 2016 -** *Environmental Awareness*. Cranfield University Training Environment (CUTE).
- **2 April 2016** - *Fire Safety Essential*. Cranfield University Training Environment (CUTE).

- **3 April 2016** - *Manual Handling*. Cranfield University Training Environment (CUTE).
- **3 April 2016** - *Health and Safety Induction*. Cranfield University Training Environment (CUTE).
- **4 April 2016** - *Slips, trips and fall*s. Cranfield University Training Environment (CUTE).
- **5 April 2016** - *ErgoWize*. Cranfield University Training Environment (CUTE).
- **26 October 2016** - *Dealing with Pressure*. Cranfield Learning & Development
- **3 November 2016** - *Building Personal Resilience*. Cranfield Learning & Development.
- **1 February 2017 -** *Cranfield in Context* - *Welcome Event*. Cranfield Learning & Development.
- **21 February 2017** - *Unconscious Bias*. Cranfield Learning & Development