CRANFIELD UNIVERSITY

Angela Navarro-Calderon

Discovering Biomarkers of Postharvest Resilience and Flavour Life in Imported Citrus and Table Grapes

School of Water, Energy, and Environment
Plant Science Laboratory

Doctor of Philosophy (PhD)
Academic Year: 2016 - 2022

Supervisor: Dr M. Carmen Alamar
Associate Supervisor: Prof. Leon A. Terry
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ABSTRACT

Clementines and table grapes, which are the main fruit crops consumed in the UK after bananas and apples, are considered non-climacteric fruit, not showing an increase in respiration rate and ethylene production during ripening. Previous research has suggested that a different ripening hormone, abscisic acid (ABA), has a more crucial role in the ripening of this kind of produce.

The study presented herein aimed to identify biomarkers of postharvest resilience and flavour life of imported clementines and table grapes. For these studies two experiments were designed with the common objectives of determining: 1) the pre- or postharvest factors influencing the postharvest produce quality – both physiological and biochemical, and 2) the role of ABA and ABA catabolites on fruit senescence.

The main findings from these studies were that the canopy position of clementines significantly affected fruit postharvest quality and hormonal content. Fruit located on the inside canopy showed higher RR and lower sugar content than outside fruit at the end of postharvest storage, resulting in a shorter shelf-life. At the same time, inside fruit showed a higher content of ABA and ABA catabolites than outside fruit, coinciding with a lower consumer preference score for external appearance, aroma and flavour. This is the first study that determined the ABA and ABA catabolite contents in the pulp of clementines from different canopy positions during senescence, and related this to consumer acceptance.

The use of an ethylene inhibitor, 1-methylcyclopropane (1-MCP), during the postharvest storage of table grapes was investigated. The treatment did not have a positive effect on their postharvest quality; in fact, grapes were significantly affected by mould incidence at the end of the shelf-life. The hormonal content in different berry sections was also evaluated; the distal section, which showed a higher mould incidence than the proximal, had three times more ABA and ABA catabolites than the proximal section. This is the first time that the spatial distribution of ABA during the senescence of table grapes was profiled.

Despite being different products, similar novel results were observed for both clementines and table grapes. This study indicated that senescence processes in these non-climacteric produce was initiated after a significant increase in RR, and that ABA could be considered a biomarker for clementines and table grapes senescence since an ABA peak during postharvest storage preceded an increase in RR, mould incidence, organic acids, and sucrose hydrolysis. This coincided with a decrease in berry firmness. These findings are of significant importance for the industry. Understanding how ABA regulates senescence processes and the quality changes taking place during postharvest cold storage of clementines and tables grapes improves the consistency in fruit quality and reduces waste and consumer complaints.

Although clear beneficial findings have been identified, the results of this study were limited by time, resources, climatic conditions, and other factors. Therefore, recommendations for future work are: to perform molecular studies on genes regulating the ABA pathway from field to postharvest storage; to investigate the crosstalk between ABA, ethylene, and sucrose from ripening to senescence; and to further investigate the use of shade nets and harvesting by canopy position on fruit quality consistency and consumer acceptance.

Keywords: senescence, abscisic acid, ethylene, 1-methylcyclopropane, canopy, postharvest, clementines, table grapes, consumer, fresh produce supply chain.

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TABLE OF CONTENTS

ABSTRACT i
ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS iv
LIST OF FIGURES vii
LIST OF TABLES x
LIST OF ABBREVIATIONS xi
1 Chapter One: General Introduction 13
1.1 Project background 13
1.1.1 Citrus fruit 14
1.1.2 Table grapes 18
1.1.3 Hormonal regulation of ripening in non-climacteric produce 22
1.2 Aim and Objectives 25
1.3 Declaration 25
2 Chapter Two: General Materials and Methods 26
2.1 Introduction 26
2.2 Physiological analysis 26
2.2.1 External colour 26
2.2.2 Respiration rate 26
2.2.3 Total Soluble Solids and Titratable Acidity 27
2.3 Biochemical analysis 27
2.3.1 Sample preparation 27
2.3.2 Chemicals 28
2.3.3 Individual sugars 28
2.3.4 Organic acids 28
2.3.5 Abscisic acid and ABA catabolites 29
2.4 Statistical analysis 29
3 Chapter Three: Clementines 31
3.1 Introduction 31
3.2 Materials and Methods 32
3.2.1 Plant material 32
3.2.2 Experimental design 33
3.2.3 Physiological analysis 33
3.2.4 Biochemical analysis 34
3.2.5 Consumer tasting evaluation 35
3.2.6 Survey on consumer buying decisions 35
3.2.7 Statistical analysis 35
3.3 Results 35
3.3.1 Respiration rate and rind colour 35
3.3.2 Juice content, Total Soluble Solids and Titratable Acidity 37
3.3.3 Individual sugars 39

3.3.4 Organic acids 40
3.3.5 ABA and catabolites 41
3.3.6 Consumer tasting evaluation 43
3.3.7 Survey on consumer buying decisions 44
3.4 Discussion 45
3.4.1 The effect of harvest time and canopy position on the postharvest quality and consumer eating experience of 'Nadorcott' clementines 45
3.4.2 The role of ABA and ABA catabolites on clementine senescence 48
3.5 Conclusion 50
4 Chapter Four: Table grapes 52
4.1 Introduction 52
4.2 Materials and Methods 53
4.2.1 Plant material 53
4.2.2 Experimental design 53
4.2.3 Physiological analysis 54
4.2.4 Biochemical analysis 55
4.2.5 Statistical analysis 56
4.3 Results 56
4.3.1 Mould incidence, berry firmness, and berry skin colour 56
4.3.2 Respiration rate 59
4.3.3 Total Soluble Solids and Titratable Acidity 60
4.3.4 Individual sugars 61
4.3.5 Organic acids 62
4.3.6 ABA and catabolites 64
4.4 Discussion 66
4.4.1 The effect of 1-MCP and storage temperature on the postharvest quality of 'Krissy' table grapes 66
4.4.2 The role of ABA and ABA catabolites on grape berry senescence 67
4.5 Conclusions 70
5 Chapter Five: General Discussion and Conclusions 71
5.1 Introduction 71
5.2 Main findings and contribution to knowledge 71
5.2.1 Senescence processes in clementines and table grapes start after a significant increase in respiration rate 71
5.2.2 ABA could be considered a biomarker of clementines and table grapes senescence 74
5.3 Impact to the industry 76
5.4 Conclusions 79
5.5 Recommendations for future work 80
6 References 82
7 Appendices 102

7.1 Appendix A: Statistical tables for the experiment on ‘Nadorcott’ clementines.. 102
7.2 Appendix B: Statistical tables for the experiment on ‘Krissy’ table grapes.. 111
7.3 Appendix C: Calibration curves.. 122
7.4 Appendix D: Meteorologic data for Lora del Rio, Seville, Spain on the months preceding the mandarins harvests 126

LIST OF FIGURES

Figure 1. Structure of a ripe clementine fruit adapted from Iglesias *et al*. (2007) and Sadka *et al*. (2019). 15

Figure 2. Metabolic changes taking place during the different citrus fruit growth stages (Iglesias *et al*., 2007)... 16

Figure 3. Grape berry development stages (from Rogiers *et al*., 2017)............ 20

Figure 4. The ABA metabolic pathway in plants (from Abrams and Loewen, 2019). 23

Figure 5. Diagram of the different canopy positions studied: upper outside, upper inside, lower outside, and lower inside. 33

Figure 6. Respiration rate (RR) expressed as CO_2 production (mL kg^{-1} h^{-1}) of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 36

Figure 7. CIELab colour parameters [Lightness (L*), Chroma index (C*), and Hue angle (Ho)] of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 37

Figure 8. Juice content, Total soluble solids (TSS) and titratable acidity (TA) expressed in percentage (%) of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 38

Figure 9. Individual sugars (sucrose, fructose and glucose) expressed as mg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 40

Figure 10. Organic acids (tartaric and malic acids) expressed as mg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 41

Figure 11. ABA and ABA catabolites expressed as µg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error. 42

Figure 12. Tasting session of 'Nadorcott' clementines carried out in March 2017. Consumers tasted one fruit per position and scored the individual eating quality attributes from 1 "Extremely dislike" to 9 "Extremely like". Bars represent standard error of the mean scores... 43

Figure 13. Transversal cut of a grape berry differentiating the proximal and distal sections where biochemical compounds were analysed. 55

Figure 14. Mould incidence (%) (left) and firmness (N) (right) of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 56

Figure 15. CIELab colour parameters [Lightness (L*), Chroma index (C*), and Hue angle (H°)] of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 58

Figure 16. Respiration rate (RR) expressed as CO_2 production (mL kg^{-1} h^{-1}) of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 59

Figure 17. Total soluble solids (TSS) and titratable acidity (TA) expressed in % of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 60

Figure 18. Individual sugars (sucrose, fructose and glucose) expressed as mg g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 62

Figure 19. Organic acids (tartaric and malic acids) expressed as mg g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows

when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 63

Figure 20. ABA and ABA catabolites expressed as ng g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error. 65

LIST OF TABLES

Table 1. Ranking of the citrus attributes preferred by consumers. 44

Table 2. Attributes taken into account by consumers when buying citrus fruits.44

LIST OF ABBREVIATIONS

1-MCP	1-Methylcyclopropane
ABA	Abscisic acid
ABA-GE	Abscisic acid glucose ester
ANOVA	Analysis of Variance
C*	Chroma index
DAD	Diode Array Detector
DPA	Dihydrophaseic acid
DW	Dry Weight
EBP	Ethylene Binding Protein
FAO	Food and Agriculture Organisation
FW	Fresh Weight
H°	Hue angle
HPLC	High Performance Liquid Chromatography
L*	Lightness
LC-MS	Liquid Chromatography Mass Spectrometry
LSD	Least Significant Differences
N	Newtons
NCED	9-cis-Epoxycarotenoid Dioxygenase
PA	Phaseic acid
RBD	Rind Breakdown Disorder
RH	Relative Humidity
RID	Refractive Index Detector
RR	Respiration Rate
SSC	Soluble Solids Content
TA	Titratable Acidity
TSS	Total Soluble Solids

1 Chapter One: General Introduction

1.1 Project background

The fresh produce supply chain constitutes the activities or processes from production to marketing of agri-fresh products (i.e., from farm to table), with the main purpose of satisfying consumers' demands (Ahumada and Villalobos, 2009; Shukla and Jharkaria, 2013). However, the natural perishability of the products makes the supply chain especially complex, limiting produce quality and safety and, as a result, shelf life. This must be taken into account when managing the supply chain in order to maintain the quality of the products and reduce their deterioration and waste (Ahumada and Villalobos, 2009; Terry *et al.*, 2011). Moreover, the production of agricultural produce is dependent on climate conditions, which affects produce demand and price variability (Ahumada and Villalobos, 2009; Shukla and Jharkaria, 2013). A recent study on the UK's fruit and vegetable supply chain (Scheelbeek *et al.*, 2020) showed that domestic production decreased from 42 % in 1987 to 22 % in 2013, making it necessary to import from other production countries to be able to cope with the internal demand. According to FAO (2022), the four main fruit crops imported in the UK in 2020 were bananas, apples, soft citrus (tangerines, mandarins, clementines, and satsumas) and table grapes, with the last two together having an import volume of circa 600,000 tonnes and an import value of more than $1b. However, Scheelbeek *et al.* (2020) identified that 32 % of UK's fruit and vegetable imports is supplied from climate-vulnerable countries, like Spain, Egypt, South Africa, Chile, Morocco, Israel, and Peru. These countries are characterised by having a peak daytime temperature higher than 25 °C, less than 200 mm of precipitation, and a likelihood to face high to extremely high water stress. Although climate change could improve the growing conditions in temperate climates like in the UK (Scheelbeek *et al.*, 2020), other agricultural and postharvest strategies should be considered to improve the produce resilience to this challenging future while supplying a product that has consistent quality and is safe and healthy for the consumer.

In order to do so, this chapter will review the physiology and biochemical composition of citrus fruit and table grapes, as well as the factors affecting their postharvest resilience and flavour-life.

1.1.1 Citrus fruit

1.1.1.1 Crop importance and production of citrus fruit

Citrus fruits originated in Southeaster Asia and have been cultivated for more than 4,000 years in almost every country within 40° north-south latitude (Davies and Albrigo, 1994). Besides being one of the main sources of vitamin C, citrus fruit contain an extensive variety of secondary compounds with nutritional, antioxidant and anti-inflammatory capacity. For instance, flavonoids have been shown to suppress cell inflammation and proliferation of cancer cells through modulation of cellular proteins in *in vitro* studies (Koolaji *et al.*, 2020). However, there is not much evidence that confirms this in *in vivo* cases, and more clinical studies with a broader population are needed in order to claim that citrus fruit prevent cardiovascular and degenerative diseases like cancer, cholesterol, and obesity.

There has been a continuous increase in marketing and consumption of citrus fruit, especially of easy-to-peel mandarins due to its sweet but tangy taste, year-round availability, and affordable price (Goldenberg *et al.*, 2015). Recent data show that the world production of tangerines, mandarins, clementines, and satsumas reached 35 million tonnes in 2019, with China and Spain being the main producers with a production share of 55.6 % and 5 %, respectively (FAO, 2022).

1.1.1.2 The growth cycle of citrus fruit and the factors affecting fruit quality

The physiology and biochemistry of the citrus fruit have been extensively reviewed in the last few years (Iglesias *et al.*, 2007; Katz *et al.*, 2007; Lado *et al.*, 2014; Lado *et al.*, 2018; Sadka *et al.*,2019). Some authors (Davies and Albrigo, 1994) have explained the citrus growth curve in four stages, but newer studies (Iglesias *et al.*, 2007; Sadka *et al.*, 2019) have considered it to be a sigmoid curve divided into three stages. These reviews have described citrus fruit set, growth,

and ripening, including the hormonal regulation of these processes as well as the abiotic factors influencing them. However, no known studies have investigated the biochemical and hormonal changes during citrus fruit senescence.

The fruit of the citrus is a special berry named a hesperidium, characterised by its unique structure (Figure 1). The outer part is called pericarp and is divided into three tissues: 1) the exocarp, the coloured peel referred to as flavedo, 2) the mesocarp, a white, spongy tissue, and 3) the endocarp, an internal cell layer that separates the pericarp from the pulp. In turn, the mesocarp and the endocarp form what is known as albedo, but the former disintegrates in mandarins during maturation (Sadka *et al.*, 2019). On the other hand, the pulp, which is the edible portion of the citrus fruit, is made of segments enclosed in a locular membrane or segment epidermis and filled with the juice vesicles (Iglesias *et al.*, 2007).

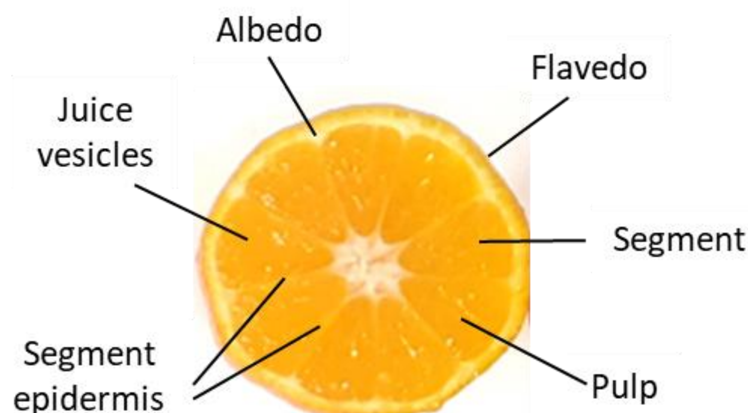

Figure 1. Structure of a ripe clementine fruit adapted from Iglesias *et al.* (2007) and Sadka *et al.* (2019).

In the Northern hemisphere, the initial stage of growth (stage I) (Figure 2) is a one to two months period of cell division, differentiation, and slow growth occurring between anthesis and the June drop. Following this, there is a four to six months period of rapid growth (stage II) and volume increase (*ca.* 1,000-fold) produced by cell enlargement and water accumulation (85-90 % of the fruit volume). At the same time, rind chlorophyll starts to decline, while sugars and carotenoids begin to accumulate, and colour starts to develop. In contrast, fruit acidity, which increases at the beginning of stage II, peaks at the middle of this period, and then decreases to initial concentration. This stage is highly dependent

on carbohydrate supply and environmental conditions (Iglesias, *et al.*, 2003; Iglesias, *et al.*, 2007; Rosa *et al.*, 2009). Consequently, in the case of unfavourable growth conditions, hormonal signals are generated to protect the plant by generating growth disruption, stomata closure, or even fruit abscission (Iglesias, *et al.*, 2007; Romero *et al.*, 2014; Sawicki *et al.*, 2015; Lado *et al.*, 2018). Finally, fruit ripening occurs during the last stage (stage III), which depends on nutrient and water availability, external environmental factors such as pollination, and the synthesis and expression of plant growth regulators (Iglesias *et al.*, 2007; McAtee *et al.*, 2013; Lado *et al.*, 2018). During this last stage, the fruit starts a non-climacteric ripening process, which is detailed in Section 1.1.3.

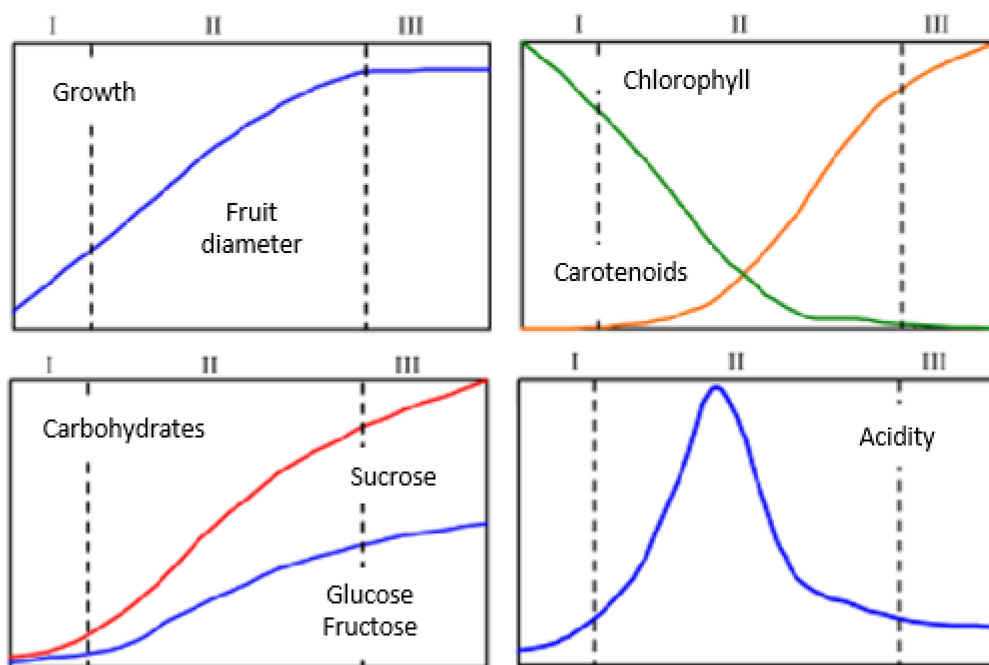

Figure 2. Metabolic changes taking place during the different citrus fruit growth stages (Iglesias *et al.*, 2007).

1.1.1.3 Factors influencing the postharvest resilience and quality of citrus fruit

The physiological and biochemical changes which occur during fruit growth and ripening, as well as biochemical substrates at the time of harvest, are determinant of the postharvest life and final quality perceived by consumers (Iglesias *et al.*, 2007; Khalid *et al.*, 2017). Generally, the taste of citrus fruit is determined by the levels of sugars and acids in the juice sacs (Goldenberg *et al.*, 2015). Still, other

attributes such as rind colour, appearance, freshness, and easiness to peel have been identified as drivers of consumer preference (Poole & Baron, 1996; Bi *et al.*, 2011; Baldwin *et al.*, 2014). Since consumer purchasing decisions are mostly based on external fruit quality and previous eating experience, it is important to understand the factors affecting them, which will be reviewed in this section.

In the last decades, citriculture has been affected by biotic and abiotic stresses that have compromised the development, yield, and quality of citrus fruits. However, several preharvest factors like rootstock, tree age, crop load, and fruit size have recently been shown to have a positive effect on the postharvest quality of citrus fruit. In this way, the introduction of new scion-rootstock combinations has been shown to improve plant growth, yield performance, and juice content of sweet orange trees (Carvalho *et al.*, 2019). In another study, Khalid *et al.* (2017) demonstrated that fruit from older trees had a higher respiration rate than younger trees, and this was due to a higher content of respiratory substrates (sugars and organic acids) in the former. At the same time, harvest time influenced the final composition of the fruits, since fruits harvested at an early stage of maturity had a higher content of organic acids and lower concentration of sugars and flavour compounds (Bermejo and Cano, 2012). It has been also demonstrated that a higher crop load produced more fruit splitting as rind growth was reduced (Cronje *et al.*, 2013), and this was later confirmed by Khalid *et al.* (2017), who reported that larger fruit had less juice content and a thicker rind than smaller fruit. The effect of fruit canopy position on different rind quality characteristics has had scientific interest in the last decade, with different authors reporting a direct effect on the mineral content of the citrus rind (Cronje *et al*, 2011a), the incidence of progressive rind defects (Cronje *et al.*, 2011b; Magwaza *et al.*, 2013), and the rind and pulp biochemical content and quality (Moon *et al.*, 2011; Thakre *et al.*, 2015; Magwaza *et al.*, 2019).

After harvest, citrus fruits are exposed to handling techniques that affect the quality of the product. Excessive washing when the fruits arrive at the packing house can produce water loss from the peel, increasing the resistance to gas exchange. In the same way, wounds created when fruit is transported through the packing line increase the fruit respiratory rate, and therefore the accumulation

of ethylene (Petraceck *et al.*, 1998). Although ethylene is mainly applied to citrus fruit to induce degreening, the hormone has proved to increase the incidence of the postharvest disorder known as rind breakdown (RBD) in 'Nules' clementines (Cronje *et al.*, 2011b), leading to the premature senescence of the flavedo, and therefore affecting the final fruit quality.

As seen, many studies have focused on determining the quality changes during fruit ripening and the factors affecting fruit quality, with a great interest in the citrus rind. However, despite the important role of hormones in regulating ripening processes, no known studies have investigated the effect of canopy position on the internal quality and hormonal changes during citrus fruit senescence.

1.1.2 Table grapes

1.1.2.1 Crop importance and production of table grapes

The first evidence of cultivation and domestication of the grapevine (*Vitis vinifera* L. ssp. *sativa*) seems to have occurred between the seventh and fourth millennia BC, in the northern regions of the near East, between the Black Sea and the Caspian Sea (Terral *et al.*, 2010; Myles *et al.*, 2011). From this geographical area, the domesticated cultivars were spread to the Jordan Valley (*c.* 4000 BC) and Egypt (*c.* 3000 BC). Mediterranean expansion of the wine culture is documented on the coasts of the Italian and Iberian peninsulas by c. 800 BC (Arroyo-Garcia *et al.*, 2006). Grapes are consumed as fresh fruit (table grapes) or processed in wine, grape juice, and raisins. In 2019, the production of grapes reached 77 million tonnes, with China, Italy, USA, Spain, and France being the top five producers in the world (FAO, 2021).

1.1.2.2 The growth cycle of table grapes

The fruit of the grapevine is a berry, and it is classified as a non-climacteric fruit. The basic parts of the berry are the pericarp, consisting of exocarp (skin) and mesocarp (pulp or flesh), and the seeds in seeded varieties. The outer layer of the berry is the skin, which consists of six to ten layers of walled cells. The external layer of the skin protects the berry with a wax-like coating called cuticle. The skin contains flavour and aroma compounds, tannins, minerals and pigments that give the green, red, and black colour to the grape skin. The pulp of the berry

is made up of cells with large vacuoles containing the juice, which consists of 70 to 80 % water and dissolved solids. The berry of seeded varieties presents two to four seeds located in the centre of the pulp (Dharmadhikari, 1994).

After the rapid growth of the vine in spring and the following setting of the flowers, the berries grow in three stages (Figure 3). Stage I usually occurs within 40 days from flowering. During this stage, the berries rapidly increase their size by cell division, acids start to accumulate, and seeds begin to develop (Coombe, 1987; Rogiers *et al.*, 2017). Organic acids, mainly tartaric and malic acid, are stored in the vacuoles of the berry cells during the first stage of berry development until the start of veraison, which is the onset of ripening, increasing the volume of the pericarp (Dharmadhikari, 1994; Soyer *et al.*, 2003). However, the tartaric to malic acid ratio is cultivar specific. For instance, in a characterisation study of organic acids in different commercial table grape varieties, 'Thompson Seedless' showed the lowest tartaric to malic acid ratio, compared with 'Red Globe' and 'Crimson Seedless' (Muñoz-Robredo *et al.*, 2011). After stage I, there is a short phase of slow growth (stage II) lasting seven to 40 days from anthesis during which the seeds mature. During this lag phase, photosynthesis and respiration rates are reduced, and organic acids are at their highest concentration. By this time, chlorophyll is the main pigment in the berry but at the end of the phase, it undergoes a slow reduction.

The final stage of berry development coincides with the beginning of the veraison and lasts 35 to 60 days, depending on cultivar, environmental conditions, and crop management practices (Coombe, 1987). During this stage, there is another fast increase in berry size due to cell expansion and the berries, which follow a non-climacteric ripening process (detailed in Section 1.1.3), start to ripen. This third stage is critical for the final quality of the berry, which changes colour, soften, and begins to accumulate sugars and lose acids (Coombe, 1987; Fischer, 2009). Sugars produced in the leaves are transported through the phloem to the berries and are accumulated during the ripening stage. Glucose, which is the most common carbohydrate in the grape berry, is produced in the chloroplasts of the plant leaves during the photosynthesis process. Coombe (1987) found that the concentration of glucose in unripe berries (6 % Brix) was double for that of

fructose, being the concentration of sucrose less than 2 mg g^{-1}. In ripe berries (17 % Brix), the amount of sucrose increased in the skin and near the style and the brush. Moreover, the concentrations of glucose and fructose were higher in the flesh than in the skin. When the berry was overripe (26 % Brix), the segments around the seeds, skin and flesh presented exceptionally high values of glucose and fructose, being the concentration of fructose higher than of glucose. The concentration of sucrose increased also in overripe berries. Despite the interesting findings of this study, the hormonal content in the different grape tissues was not investigated and no known studies have yet done so.

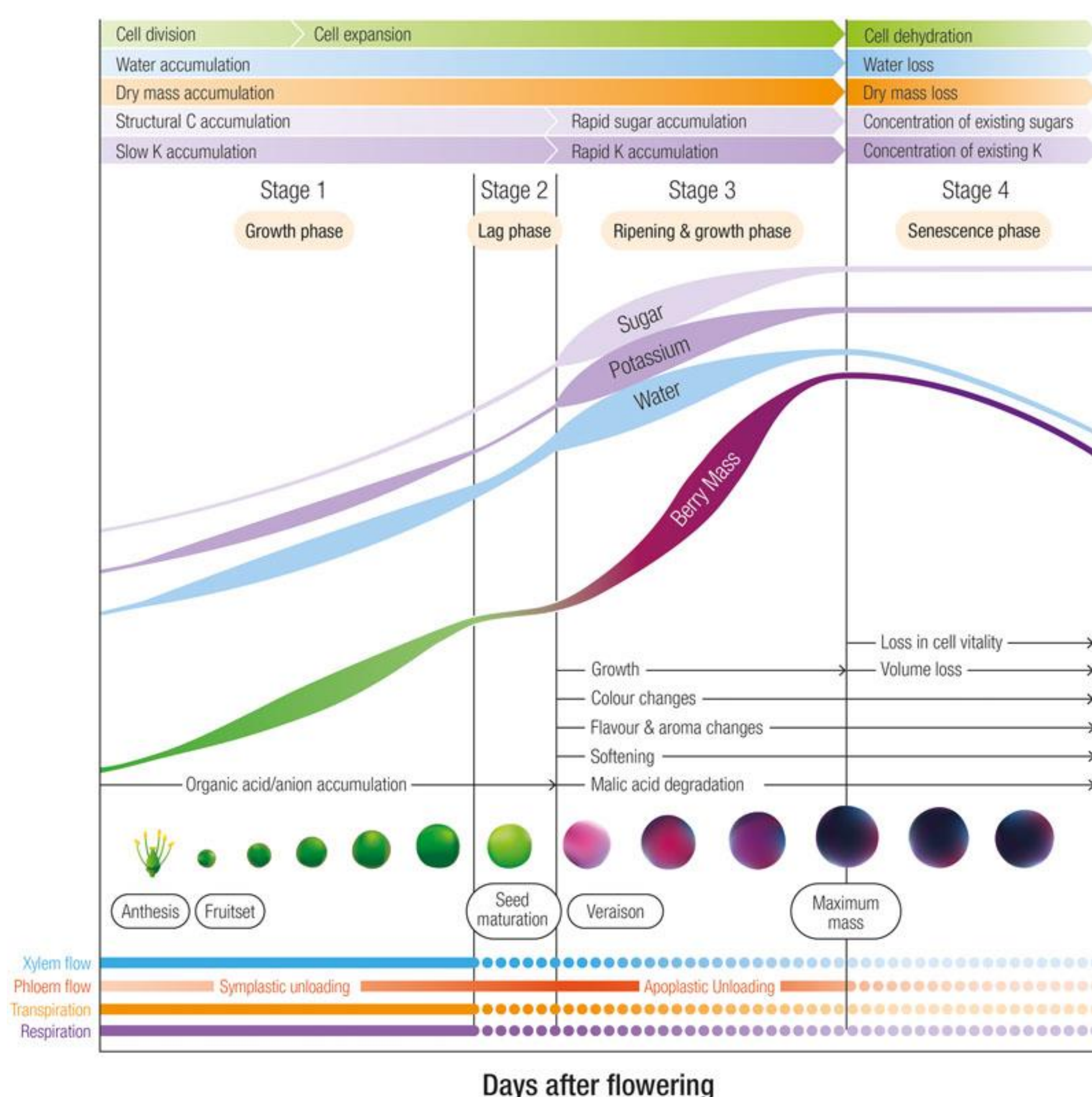

Figure 3. Grape berry development stages (from Rogiers *et al*., 2017).

1.1.2.3 Factors influencing quality and shelf-life of table grapes

The environmental and climatic conditions of the growing region, as well as the different agricultural and crop management practices, affect the chemical composition and quality traits of the grape berry (Granato *et al.*, 2016). At the same time, terroir, which is the soil and microclimate of a growing area that gives distinctive qualities to food produce, has been lately shown to influence the cell wall and carbohydrate composition of the grape skin (Apolinar-Valiente *et al.*, 2015). As a result, in a specific season, the environment, leaf area, and carbohydrate balance during the previous season determine the growth and ripening capacity of the plant (Fisher, 2009). Several authors have demonstrated that good sunlight exposure of leaves and berries enhances sugar and anthocyanin accumulation, while cold temperatures (<15 °C) prevent pollen tube growth, favour acids concentration and slow sugar accumulation (Dokoozlian and Wolpert, 2009; Fisher, 2009). Water availability is also important for the correct development of the berry; ripening and phenols accumulation will be delayed in case of heavy rains during berry growth, while water stress produced by droughts will produce a decrease in berry size and weight (Crupi *et al.*, 2012).

Table grapes show severe problems during postharvest handling, storage, and marketing. Rachis browning, grey mould infection, caused by the fungus *Botrytis cinerea*, and firmness loss are the main factors that reduce table grape postharvest quality. Mould infection can be caused by the pathogen remaining quiescent until the host conditions are favourable for infection (Petrasch *et al.*, 2019), or by wound infection, developing during the postharvest stage of the crop or during consumer life. In order to avoid or delay mould infection, the use of SO_2-generating pads is a common practice in the table grape industry (Youssef *et al.*, 2020). The pads contain sodium metabisulfite ($Na_2S_2O_5$), which reacts with moisture absorbed by the pads to release a small amount of SO_2 and inhibit germination of *Botrytis cinerea* spores (Ahmed *et al.*, 2018).

In addition, weight loss, change in colour, berry shatter, and accelerated softening are other problems affecting the final quality of the produce and therefore consumer acceptance (Crisosto *et al.*, 1994; Crisosto *et al.*, 2001; Lichter *et al.*, 2002; Valverde *et al.*, 2005). After harvest at optimum maturity, berries must be

rapidly cooled down to remove field heat and prolong the postharvest life of the fruits (Cameron, 2001). Cold storage is one of the main techniques used to maintain fruit quality and control decay of berry clusters (Balic *et al.*, 2012; Pinto *et al.*, 2015). It is thus important to set the appropriate relative humidity (RH) during cold storage since it will affect the water content of the berries and therefore their quality. For instance, levels of relative humidity near saturation (100 %) can enhance rot incidence and berry cracking (Pinto *et al.*, 2015). On the contrary, if the relative humidity is very low it will provoke intense water loss and rachis browning (Crisosto *et al.*, 2001). At the same time, water loss during cold storage will affect the respiratory rate and the accumulation of acids and soluble solids in the berry (Pinto *et al.*, 2015). Pinto *et al.* (2015) confirmed that rot severity has a direct relation with RH during postharvest cold storage, while rachis browning and weight loss are inversely related to RH. In the meantime, they found that the appropriate level of relative humidity to maintain the postharvest quality of table grapes range between 90 and 95 %. Nevertheless, storage of table grapes under low temperature is limited by high sensitivity to fungal attack (Sanchez-Ballesta *et al.*, 2006). Simultaneously, berry softening appeared after cold storage (Ejsmentewicz *et al.*, 2015) due to a decrease in the concentrations of cell wall pectin and hemicellulose polysaccharides.

1.1.3 Hormonal regulation of ripening in non-climacteric produce

Understanding fruit ripening is of high importance in postharvest management since fruit respiration rate affects the storage and shelf life of the final produce (Kader, 2013). In climacteric fruits, such as apples and bananas, the increase of ethylene concentration during fruit growth enhances ripening by a rise in respiration. On the contrary, the ripening of citrus fruit and table grapes follows a non-climacteric pattern and the endogenous ethylene, which has a low concentration before and during ripening, does not produce an increase in respiration (Coombe and Hale, 1973; Paul *et al.*, 2012). Although it has been reported that ethylene is somehow involved in the regulation of grape and citrus ripening (Chervin *et al.*, 2004; Li *et al.*, 2016a; Estables-Ortiz *et al.*, 2016), another plant hormone, abscisic acid (ABA), has shown to have a greater role than ethylene in regulating fruit ripening processes and response to postharvest stress

(McAtee *et al.*, 2013; Wang *et al.*, 2016; Jia *et al.*, 2017, Crupi *et al.*, 2019; Wang *et al.*, 2019). On the other hand, other authors have reported that the ripening of non-climacteric produce is mediated by a complex interplay between ethylene and ABA (Fortes *et al.*, 2015; Iqbal *et al.*, 2017; Tosetti *et al.*, 2020) and even between ABA, sucrose, and other plant hormones (Setha, 2012; Jia *et al.*, 2017; Olivares *et al.*, 2017; Coelho *et al.*, 2019).

In plants, ABA is produced from 9-*cis*-epoxycarotenoid metabolites, 9-*cis*-violaxanthin and 9'-*cis*-neoxanthin, after a series of oxidation and reduction reactions (Abrams and Loewen, 2019) (Figure 5). In addition to ABA synthesis, ABA can be hydroxylated to 8'-OH-ABA, isomerised to phaseic acid (PA), or esterified to ABA-glucose ester (ABA-GE), which is stored in the cell vacuoles or apoplast to respond to stress (Finkelstein, 2013; Jia *et al.*, 2017). According to Abrams and Loewen (2019), profiling the ABA metabolites during the different plant developmental stages provides more information than analysing ABA alone, since these metabolites have shown to have a role in seed germination, ripening, and responses to abiotic stress.

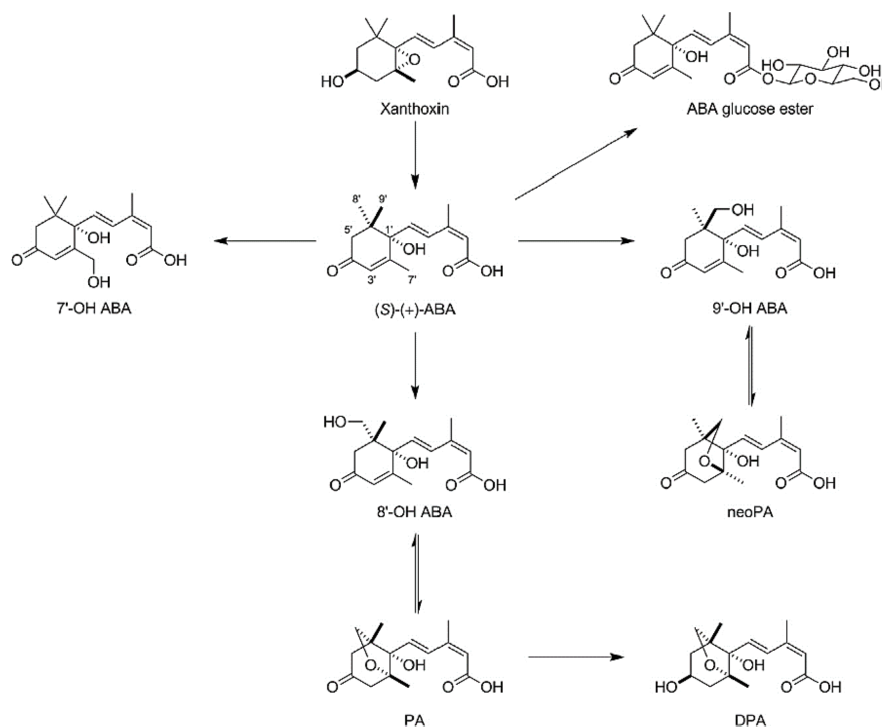

Figure 4. The ABA metabolic pathway in plants (from Abrams and Loewen, 2019).

In grape berry, the onset and rate of berry ripening is dependent on the level of endogenous ABA, as it gradually increases before veraison and accumulates rapidly once ripening starts. It has been proposed that berry ripening will not occur unless ABA accumulates to a certain level (Coombe and Hale, 1973), and three ABA peaks have been reported to initiate berry growth, ripening, and senescence (Sun *et al.*, 2010). ABA regulates berry ripening by influencing sugar accumulation, colour development and berry firmness (Zhang and Zhang, 2009; Pilati et al., 2017; Olivares *et al.*, 2017). During postharvest, ABA has been shown to be involved in water stress response and antioxidant defence (Wang *et al.*, 2019) and to have a strong interplay with calcium in modulating pectin development in grape berry cell wall (Martins *et al.*, 2018). In a recent study on 'Trincadeira' and 'Syrah' grapes, Coelho *et al.* (2019) suggested that ABA signalling varies with the organ and even the tissue. However, this is yet to be confirmed. Moreover, the role and profile of ABA and ABA catabolites during the senescence of table grapes is still not well understood.

In the same way, ABA has shown to promote citrus fruit ripening by regulating the expression of sucrose metabolism genes and signal transduction pathways. Similar to grape berry, the endogenous ABA content in the citrus pulp reached a peak before full ripening (Wang *et al.*, 2016). Moreover, ABA has shown to have a role in the stress response produced by postharvest fruit dehydration and mould infection (Romero *et al.*, 2013; Lafuente *et al.*, 2019). The role of ABA and catabolites in citrus rind susceptibility to peel damage has been lately studied. Magwaza *et al.* (2019) demonstrated that 'Nules' clementines located on the inside part of the tree canopy had lower ABA-GE and higher dihydrophaseic acid (DPA) content in the rind and showed a higher RBD incidence than fruit located on the outside of the canopy. However, neither these authors nor others have investigated the level of ABA and catabolites in the pulp of citrus fruit from different canopy positions, which would help understand the factors influencing citrus fruit internal quality after harvest and the role of ABA in regulating citrus fruit senescence.

1.2 Aim and Objectives

The aim of this thesis was to discover the influencing factors that mediate postharvest resilience and variability in imported table grapes and citrus and identify associated biomarkers of storage and flavour-life.

The specific objectives of this project were:

1. To conduct a literature review of previous research on postharvest resilience and biochemical composition of citrus and table grape.
2. To evaluate pre- and postharvest factors affecting the quality of clementines and table grapes.
3. To perform a detailed physiological and biochemical evaluation of specific consignments of clementines and table grapes.
4. To identify biomarkers of storage and flavour-life.

1.3 Declaration

All experimental work and analyses reported in this PhD thesis were conducted by the author.

2 Chapter Two: General Materials and Methods

2.1 Introduction

The experiments of this study were performed on clementines (*Citrus reticulata* Blanco) cv. 'Nadorcott' (Chapter 3) and table grapes (*Vitis vinifera* L.) cv 'Krissy' (Chapter 4) after harvest and during cold storage.

'Nadorcott' is a mid to late-maturing easy-to-peel clementine that grows from January to May in the Northern hemisphere. This variety naturally develops its bright orange colour on the tree, and has a deep citrus flavour and good sweet to acid balance, making it a success within the UK citrus consumers. Similarly, 'Krissy' is a mid-season, red seedless table grape variety that has an intense fruity flavour and good storage capacity. Therefore, understanding the factors influencing their postharvest quality and shelf-life is of high interest for the UK citrus and grape industries.

The details of the plant material and experimental design are specified in Chapters 3 and 4, while the general physiological and biochemical analyses are detailed below.

2.2 Physiological analysis

2.2.1 External colour

The external colour of the samples was determined using a hand-held tristimulus colourimeter with a D65 illuminant and 8 mm light aperture (model CR-400 Chroma Meter, Konica Minolta Inc., Cheshire, UK). The colourimeter was calibrated with a white plate, and it provided the lightness (L*), chroma index ($C^* = \sqrt{(a^*)^2 + (b^*)^2}$), and Hue angle ($H^0 = tan^{-1}(b^*/a^*)$) values of the samples, where a* and b* are the CIELAB colour space coordinates. These parameters are widely used as a measure of ripeness (Conesa *et al.*, 2016).

2.2.2 Respiration rate

Respiration Rate (RR) was measured as described in Collings *et al.* (2018). Samples were placed in 3 L airtight glass jars and dry clean air (300 mL min^{-1})

was passed through the jars into a Sable Respirometry System (model 1.3.8 Pro, Sable Systems International, Las Vegas, USA). Each sample jar (n = 3 reps per treatment) was measured for 2 min to achieve a stable reading. An empty jar (continuous air for 1 min) was used as a baseline to avoid cross-contamination between treatment measurements. The rate of CO_2 production was expressed in mL kg^{-1} h^{-1}.

2.2.3 Total Soluble Solids and Titratable Acidity

Total Soluble Solids (TSS) is a measure commonly used in the fruit industry to determine not only the maturity stage of the produce at a specific time, but also the best timing for harvesting the fruit. However, although TSS is made up mainly by the sugar content of the fruit, a small percentage of soluble proteins, amino acids, and other organic components complete this value (Kusumiyati *et al.*, 2020).

The TSS of the fruit juice was measured using a digital refractometer (model PR-32α, Atago Ltd., Tokyo, Japan) and expressed as %. The refractometer was calibrated using deionised water, and the measurements were done in triplicate.

TA was determined using an automatic titrator (model Rondolino, Mettler Toledo Ltd., Leicester, UK). The calibration of the equipment was done using a 3-point calibration curve with pH buffers ranging from pH 4 to pH 10. Then, 5 mL of juice were homogenised with 50 mL of deionised water, and the mL of NaOH consumed to reach an endpoint of pH 8.2 were recorded.

2.3 Biochemical analysis

2.3.1 Sample preparation

Before biochemical analysis, the samples were snap-frozen in liquid nitrogen and placed in a -50 °C CoolSafe freeze-dryer (Scanvac, Labogene, Denmark) for seven to ten days. After this, the freeze-dried samples were ground and kept at -40 °C until analysis (Garcia-Pastor *et al.*, 2021).

2.3.2 Chemicals

All HPLC and LC-MS grade solvents, solid KH_2PO_4 (99.9 %), D-sucrose (99.7 %), D-glucose (99 %), and D-fructose (99 %) were purchased from Fisher Scientific Ltd. (Leicester, UK). Citric (99.6 %), ascorbic (99 %), L-tartaric (99 %), and DL-malic acid (99 %) were purchased from Sigma-Aldrich Ltd. (Dorset, UK). Deuterated labelled and unlabelled ABA and ABA metabolites (PA, DPA, 7'-OH-ABA, and ABA-GE) were purchased from the National Research Council of Canada, Plant Biotechnology Institute (Saskatoon, Canada).

2.3.3 Individual sugars

Extraction and analysis of individual sugars (glucose, fructose, and sucrose) were done following Davis *et al*. (2007). Briefly, 150 mg of freeze-dried fruit samples were extracted with 3 mL of 62.5 % (v/v) aqueous HPLC-grade methanol in a water bath at 55 °C for 15 min, vortexing every 5 min for 30 s. The extract was then filtered through 0.2 µm PTFE filters and stored in 2 mL HPLC vials at -40 °C until analysis. Before analysis, the extracts were diluted 1:9 with HPLC grade water.

Detection was performed using a Refractive Index Detector (RID) coupled to an Agilent 1200 series HPLC system (Agilent Technologies, Germany). The column and system conditions for each analysis is described in the corresponding chapter. A calibration curve with a mixture of sucrose, fructose, and glucose was used for quantification, and the results were expressed as mg g^{-1} DW (Appendix C).

2.3.4 Organic acids

Analysis of non-volatile organic acids was done as described in Terry *et al*. (2007) with slight modifications. Briefly, freeze-dried samples were dissolved in 3 % aqueous metaphosphoric acid, kept at room temperature for 10 min, filtered through 0.2 µm cellulose filters into HPLC vials, and analysed immediately after extraction.

The acids content was determined using an Agilent 1200 series HPLC system with an Alltech Prevail Organic Acid 250 mm x 4.6 mm, 5 µm particle size column

fitted with an Alltech Prevail Organic Acid 7.5 mm x 4.6 mm, 5 µm particle size guard column (Alltech, CA, USA). The column oven temperature was set at 35 °C, and the compounds were detected by a diode array detector (DAD) set at 210 nm. The mobile phase used was 25 mM KH_2PO_4 HPLC-grade water at a flow rate of 1.5 mL min^{-1}, and the autosampler injection volume was 20 µL. A calibration curve with a mixture of citric, ascorbic, tartaric, and malic acids was used for quantification, and the results were expressed as mg g^{-1} DW.

2.3.5 Abscisic acid and ABA catabolites

ABA and ABA catabolites concentration was determined by following Serradilla *et al.* (2019) with modifications. Freeze-dried samples (5 mg) were mixed with 500 µL of methanol/formic acid/water (60:5:35, v/v) and 10 µL of the internal standards mix in a 1.5 mL Eppendorf tube, and shaken in a Star Beater (R&L Slaughter Ltd., Essex, UK) at 30 Hz for 2 min. The internal standard mix was made of the labelled forms of the compounds d4-ABA, d3-PA, d3-DPA, d5-ABA-GE, and d4-OH-ABA (100 ng mL^{-1}). The tubes were then placed in dry ice and kept in darkness for 20 min. Afterwards, the tubes were centrifuged at 4 °C and 14,000 rpm for 10 min, then transferred to 15 mL falcon tubes and freeze-dried overnight. The extracts were reconstituted with 500 µL of acetonitrile/formic acid/water (10:0.1:89.9, v/v), vortexed for 1 min, sonicated for 1 min, and centrifuged at 4 °C and 4,500 rpm for 1 min. The samples (20 µL) were then injected on a Phenomenex Luna C18 100 mm x 2 mm, 3 µm with guard column at 40 ºC, and analysed by an Agilent 1200 series HPLC system coupled to a Q-Trap 6500 mass spectrometer (AB Sciex, MA, USA). The concentration of ABA and ABA metabolites was quantified based on Morris *et al.* (2019) methodology. A 6-point calibration curve ranging from 0.1 to 100 ng mL^{-1} was used for quantification, and the results were expressed as ng g^{-1} DW (see Appendix C).

2.4 Statistical analysis

All statistical analyses were carried out using Statistica for Windows version 13 (Dell Inc., USA). Data were subjected to normality tests and outliers were removed if necessary. An analysis of variance (ANOVA) was performed to test the experimental hypotheses. When significant differences were found, the data

were subjected to a Fisher's post-hoc test. Least significant differences (LSD) were calculated for each analysis ($p < 0.05$). The differences between harvest time, storage time, canopy position, temperature, treatment, and berry section (for biochemical analysis) were studied. Figures were plotted in SigmaPlot 14 (Systat Software Inc., USA).

3 Chapter Three: Clementines

3.1 Introduction

Soft citrus or mandarins – tangerines, clementines, and satsumas – are liked by consumers due to their easiness to peel, juiciness, and sweet but tangy citrus flavour (Baldwin *et al.*, 2014). However, consumers are not able to evaluate these attributes at the moment of purchasing, and their decision is based on the external appearance and freshness of the fruit (Poole and Baron, 1996; Gao *et al.*, 2011; Baldwin *et al.*, 2014).

To be able to offer consistency in quality across the year, the citrus industry often source from multiple growing locations across different seasons and climatic regions. As a result, the correct management of both pre- and postharvest factors are determinant in the final quality of the fruit (Lado *et al.*, 2018; Mditshwa *et al.*, 2017). Citrus fruits are harvested depending on their horticultural maturity and following commercial standards. Therefore, fruit harvested at different times during the season will have varied quality (Thakre *et al.*, 2015; Rokaya *et al.*, 2016; Sun *et al.*, 2021).

The position of the fruit within the canopy has been widely reported to have an effect on citrus rind quality and susceptibility to postharvest disorders Generally, fruit harvested from the outer, sun-exposed parts of the canopy have better colour (deep orange), higher rind sugar and organic acid content, higher juice content, and less susceptibility to postharvest rind disorders (Khan *et al.*, 2009; Moon *et al.*, 2011; Cronje *et al.*, 2011b; Cronje and Barry, 2013; Magwaza *et al.*, 2013). However, opposing results have also been reported, with the fruit located in the inside of the canopy showing the highest rind carbohydrate levels (Magwaza *et al.*, 2013). These differences between canopy positions have been explained by the postharvest changes in hormonal content due to abiotic stresses (Rosa *et al.*, 2009; Romero *et al.*, 2013; Sawicki *et al.*, 2015; Magwaza *et al.*, 2019) as well as the different microclimatic conditions within the canopy, mainly light exposure, temperature, and osmotic pressures (Cronje *et al.*, 2011a; Moon *et al.*, 2011; Cronje and Barry, 2013).

Nevertheless, these studies have focused their attention on rind quality during postharvest storage but not on the biochemical properties of the citrus pulp and how they affect the eating quality and consumer experience after harvest. This gap in knowledge has been previously reported by many authors, who have identified the need of research relating produce attributes, shelf-life, and consumer preferences (Poole and Baron, 1996; Terry *et al*., 2011; Jianying *et al*., 2014; Goldenberg *et al*., 2015). Therefore, the present study was designed with the objectives of (i) evaluating the quality of clementines from different canopy positions at different horticultural maturity stages during postharvest cold storage, (ii) determining if these differences can be perceived by consumers during the eating experience, and (iii) identifying the attributes that drive purchasing decisions and if these coincide with the preferences of citrus consumers.

3.2 Materials and Methods

3.2.1 Plant material

The experiments were conducted on clementine fruits (*Citrus reticulata* Blanco) cv. 'Nadorcott' from a commercial field in Lora del Rio, Seville (South Spain). Three trees from three consecutive lines were selected for their good crop load and distribution of the fruit within the tree. Samples (n = 2,880) from four different canopy positions (Figure 5) – upper outside, upper inside, lower outside, and lower inside – were manually harvested at their optimal commercial maturity according to market standards at two different times during the 2017 season (March and April). The fruit was then sent to the Plant Science Laboratory (PSL) at Cranfield University, UK, by refrigerated lorry (5.5 °C and 85% relative humidity [RH]) within 5 days from harvest.

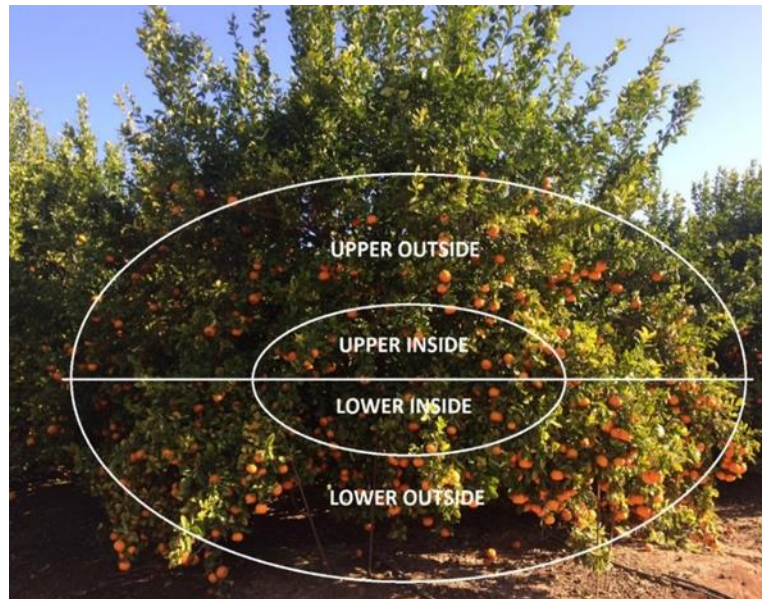

Figure 5. Diagram of the different canopy positions studied: upper outside, upper inside, lower outside, and lower inside.

3.2.2 Experimental design

At arrival at the PSL, samples showing skin damage or decay were removed, and sound fruit were placed in crates in cold storage (8.5 °C and 85 % RH) overnight to acclimatise.

Three replicates per canopy position and harvest time were analysed every two weeks, and each replicate consisted of six fruits.

3.2.3 Physiological analysis

3.2.3.1 Rind colour and respiration rate

Rind colour was determined as described in Chapter 2, section 2.2.1.

The respiration rate of six clementines (~500-600 g) per replicate was determined as in Chapter 2, section 2.2.2.

3.2.3.2 Total Soluble Solids and Titratable Acidity

The juice of six clementines per replicate was prepared using a commercial citrus juicer. TSS and TA were determined as described in Chapter 2, section 2.2.3.

The TA of the clementine juice (%) was calculated as mL NaOH * 0.1 N NaOH * milliequivalent factor * 100 * mL^{-1} juice. The milliequivalent factor for citric acid, which is the predominant acid in citrus juice, is 0.064.

3.2.4 Biochemical analysis

For biochemical analysis, portions of clementine segments were cut, snap-frozen and freeze-dried as described in Chapter 2, section 2.3.1.

3.2.4.1 Individual sugars

Extraction and analysis of individual sugars (glucose, fructose, and sucrose) were done as described in Chapter 2, section 2.3.3. Before analysis, the extracts were diluted 1:4 (v/v) with HPLC grade water. Detection was performed using an Agilent 1200 series HPLC system (Agilent Technologies, Germany) with a Phenomenex Rezex RCM monosaccharide Ca+ (8 %) 300 mm x 7.8 mm column, 8 µm particle size, fitted with a Phenomenex Carbo Ca+ 4 mm x 3 mm guard column (Phenomenex, CA). The column oven temperature was set at 80 °C and the refractive index detector at 50 °C. The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL min^{-1}, and the autosampler injection volume was 20 µL. During the analysis, the flow cell was purged and a calibration point was analysed as a quality check every ten samples. A 7-point calibration curve ranging from 1 to 4 mg mL^{-1} was used for quantification, and the results were expressed as mg g^{-1} of dry weight (DW).

3.2.4.2 Organic acids

Analysis of non-volatile organic acids was done as described in Chapter 2, section 2.3.4. In brief, 150 mg of freeze-dried clementines were dissolved into 6 mL of 3 % aqueous metaphosphoric acid, kept at room temperature for 10 min, and filter through 0.2 µm cellulose filters into HPLC vials. This work focused on citric and ascorbic acids because they are the main organic acids present in citrus fruit. During the analysis, a calibration point was analysed as a quality check every ten samples. An 8-point calibration curve ranging from 5 to 300 µg mL^{-1} was used for quantification, and the results were expressed as mg g^{-1} DW.

3.2.4.3 Abscisic acid and ABA catabolites

ABA and ABA catabolites concentration was determined as described in Chapter 2, section 2.3.5.

3.2.5 Consumer tasting evaluation

The consumer tasting session was performed by an external company, Wirral Sensory Services, which recommended a minimum of 50 panellists to obtain useful results. The tasters were regular consumers of citrus fruits with a mixture of gender and socio-economic demographics. The session took place at week 4 of storage for the fruit harvested in March. Consumers were presented with one fruit per canopy position in random order, and asked to score them for appearance, easiness to peel, aroma, flavour, juiciness, texture, and overall acceptance using a 9-point hedonic scale, where "1" meant "Extremely dislike" and "9" meant "Extremely like" (Kemp *et al.*, 2009).

3.2.6 Survey on consumer buying decisions

A survey was carried out with 50 regular consumers of citrus fruits to identify the most important citrus attributes for consumers and which ones were relevant for them at the moment of buying. The respondents ranked different citrus attributes (appearance, easiness to peel, aroma, flavour, sweetness, tanginess, juiciness, and texture) from 1 to 8, being 1 the most important attribute and 8 the least important.

3.2.7 Statistical analysis

All statistical analyses were carried out as described in Chapter 2, section 2.4.

3.3 Results

3.3.1 Respiration rate and rind colour

Fruit positioned on the upper part of the canopy presented a higher RR than lower fruit at both harvests (March and April) (Figure 6). For the clementines harvested in March, fruit located on the inside of the canopy (shadowed) had significantly higher (~48 % more) ($p = 0.000067$) RR at the end of storage than outside (sun-exposed) fruit. At the same time, a significant increase (31.5-90 %) ($p = 0.000000$)

in RR was observed from week 4 to week 6 of storage for both outside and inside fruit. Interestingly, this increase was observed in fruit harvested in April two weeks earlier, from weeks 2 to 4. However, as seen in March, all the positions reached similar RR values at the end of storage.

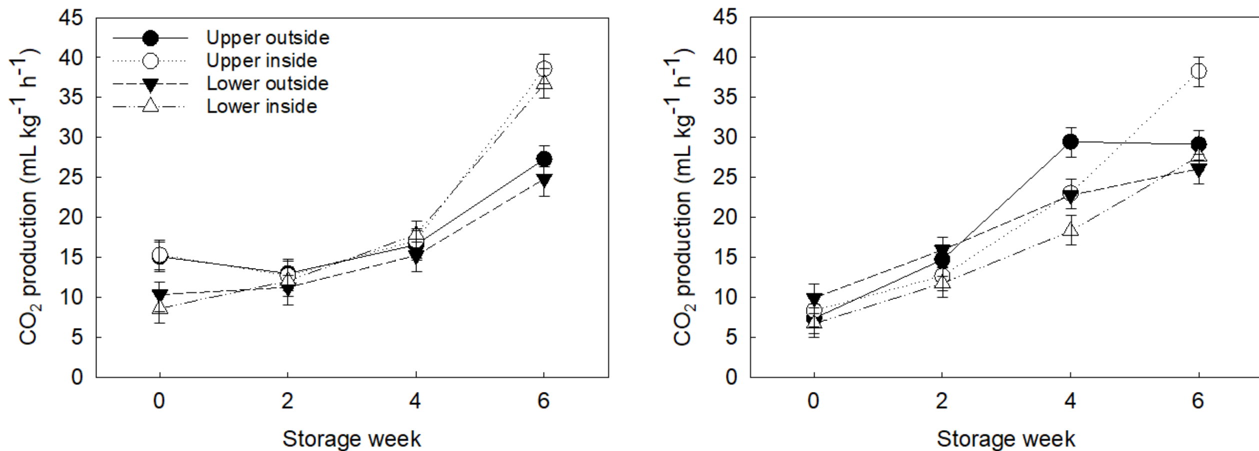

Figure 6. Respiration rate (RR) expressed as CO_2 production (mL kg^{-1} h^{-1}) of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

Regarding rind colour, fruit positioned on the lower outside part of the canopy and harvested in March presented lower lightness, chroma index, and hue angle values than the rest of the samples (Figure 7). While all the positions showed similar colour values during March, a higher variability within positions was seen for the fruit harvested in April, when inside fruit showed significantly higher lightness ($p = 0.000669$) and higher hue angle values than outside fruit during the first two weeks of storage.

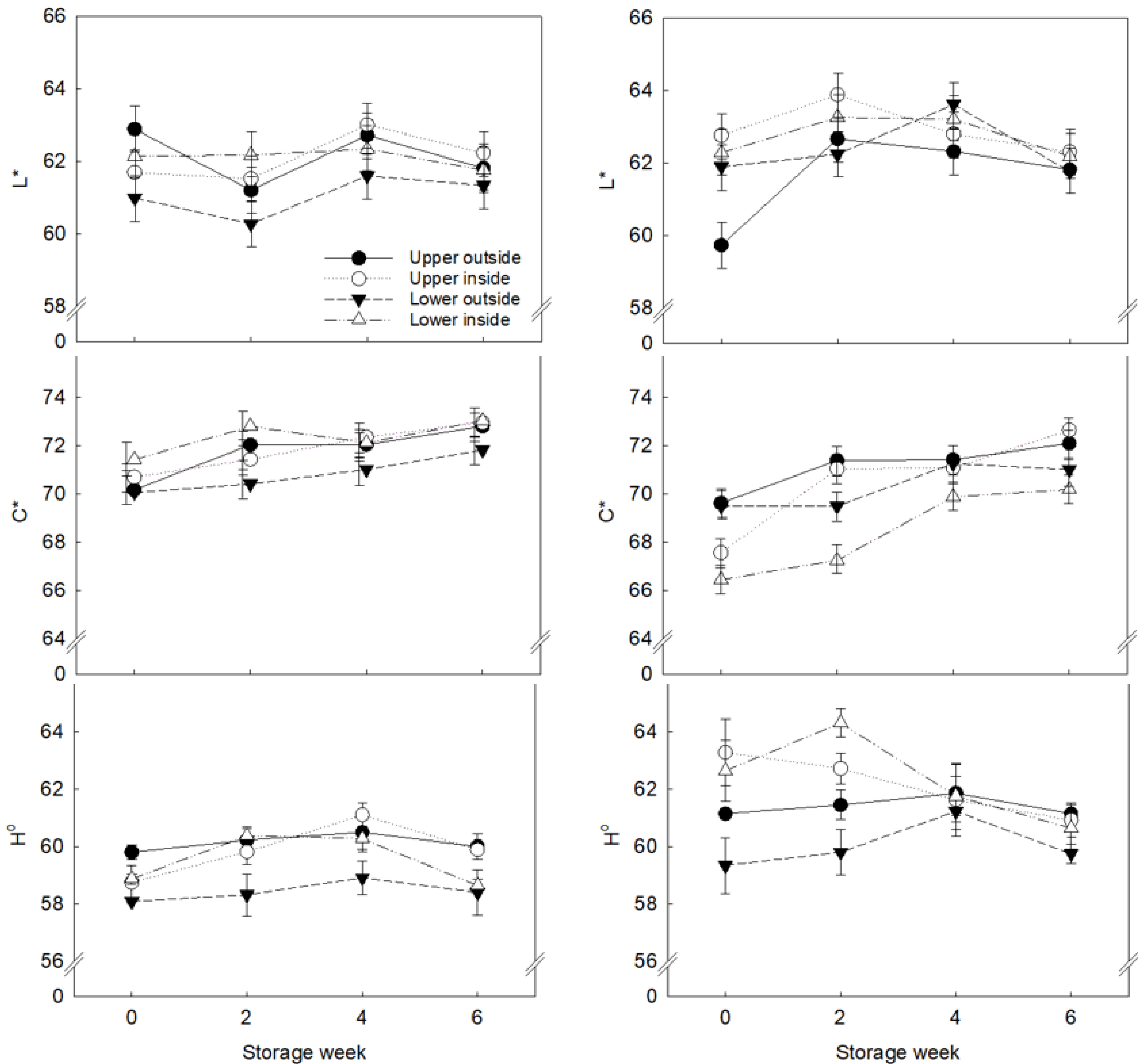

Figure 7. CIELab colour parameters [Lightness (L*), Chroma index (C*), and Hue angle (Ho)] of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

3.3.2 Juice content, Total Soluble Solids and Titratable Acidity

The interaction of canopy position, harvest month, and storage week significantly affected juice content ($p = 0.019689$), TSS ($p = 0.020273$), and TA ($p = 0.000261$) (Figure 8). Fruit harvested in March maintained similar TSS and TA values throughout cold storage, while the April harvest showed higher variability within

positions in TSS and TA values. Nevertheless, clear trends were observed during this month, when outside fruit had higher TSS and TA levels than inside fruit. Moreover, significant differences in TSS were observed between weeks 2 and 4 of storage for fruit harvested in April, with TSS increasing more than 1 % for all positions studied. By the end of storage, fruit located on the upper part of the canopy showed higher TSS and TA values than on the lower part, and the highest values were observed for the upper outside fruit. However, this position showed the lowest juice content at the end of storage independently of the harvest month.

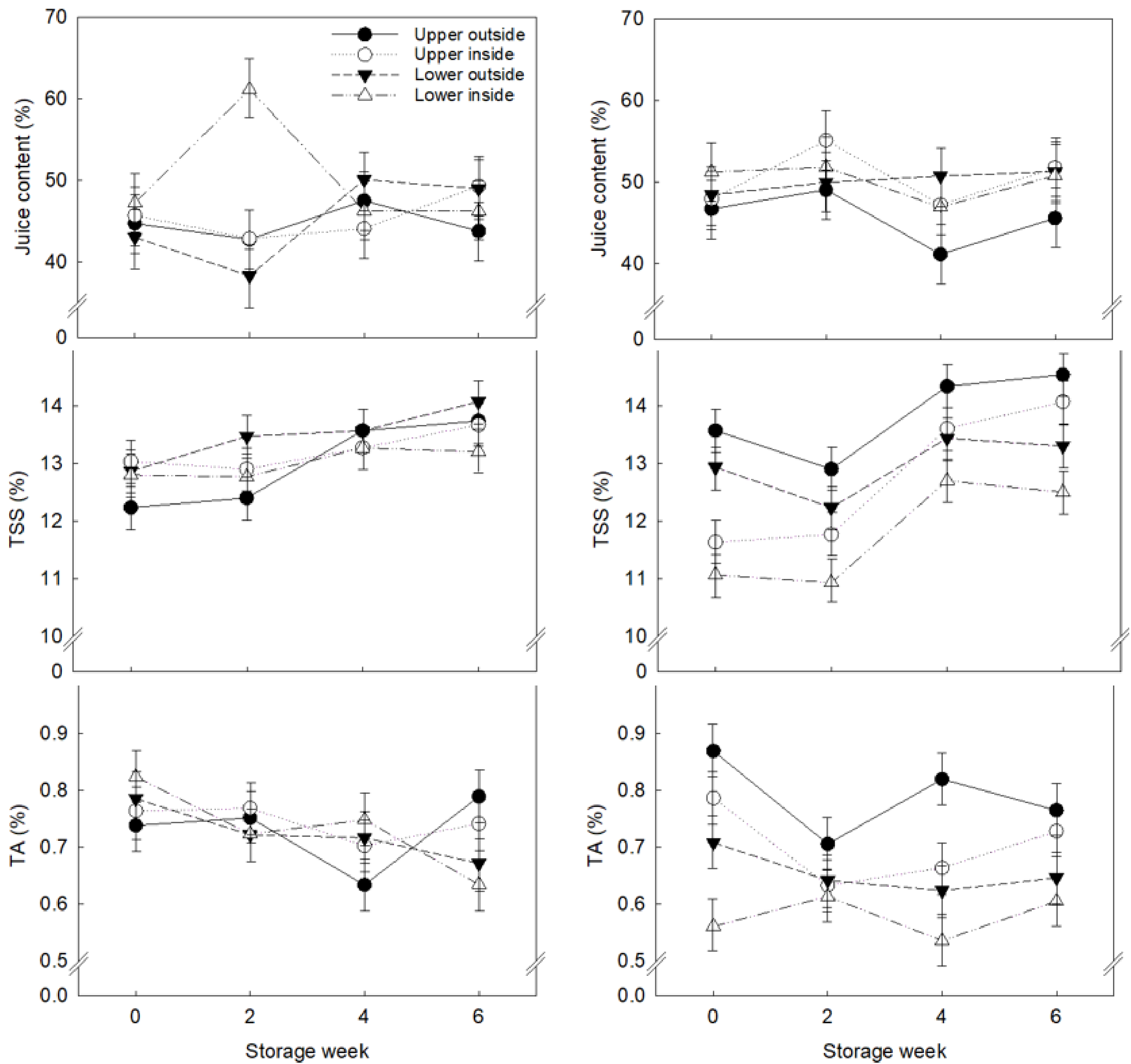

Figure 8. Juice content, Total soluble solids (TSS) and titratable acidity (TA) expressed in percentage (%) of 'Nadorcott' clementines harvested in March (left)

and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

3.3.3 Individual sugars

Sucrose was the primary sugar found in ‘Nadorcott’ clementines, followed by fructose and glucose (Figure 9). As observed for TSS, the sugar content of the fruit harvested in March was similar between the different canopy positions throughout storage, except for week 2, when upper outside and lower inside fruit had the highest and the lowest sucrose levels, respectively. However, lower inside fruit showed a significant increase in the sucrose content from week 2 to week 4 of storage. Besides that, the fructose ($p = 0.000000$) and glucose ($p = 0.000003$) levels significantly increased from week 0 until the end of storage. Significant differences in the fructose ($p = 0.000681$) and glucose ($p = 0.000001$) content were observed between canopy positions. On the other hand, the fructose and glucose content of fruit harvested in April was higher for upper outside fruit and lower for lower inside fruit independently of the storage week. However, there was a significant decrease in all sugars in the lower inside fruit at the end of storage.

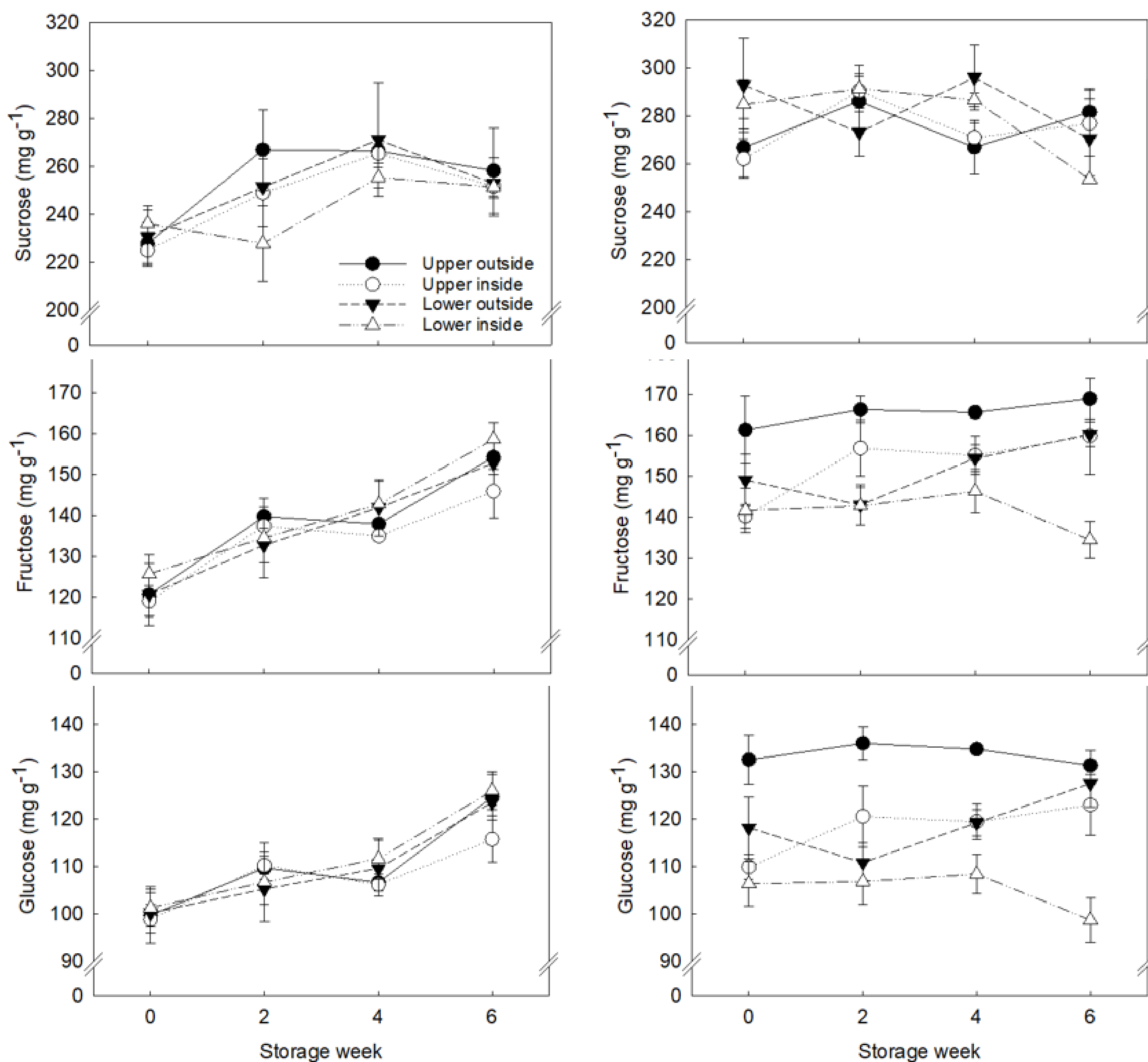

Figure 9. Individual sugars (sucrose, fructose and glucose) expressed as mg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

3.3.4 Organic acids

The primary organic acids found in clementines were citric and ascorbic acid. No apparent differences within canopy positions were observed in fruit harvested in March (Figure 10). On the contrary, fruit harvested in April and located on the upper part of the canopy showed significantly higher (17 %) citric acid content ($p = 0.000137$) throughout storage than on the lower part. Similarly, fruit located on

the inside part of the canopy had higher ascorbic acid levels than fruit on the outside.

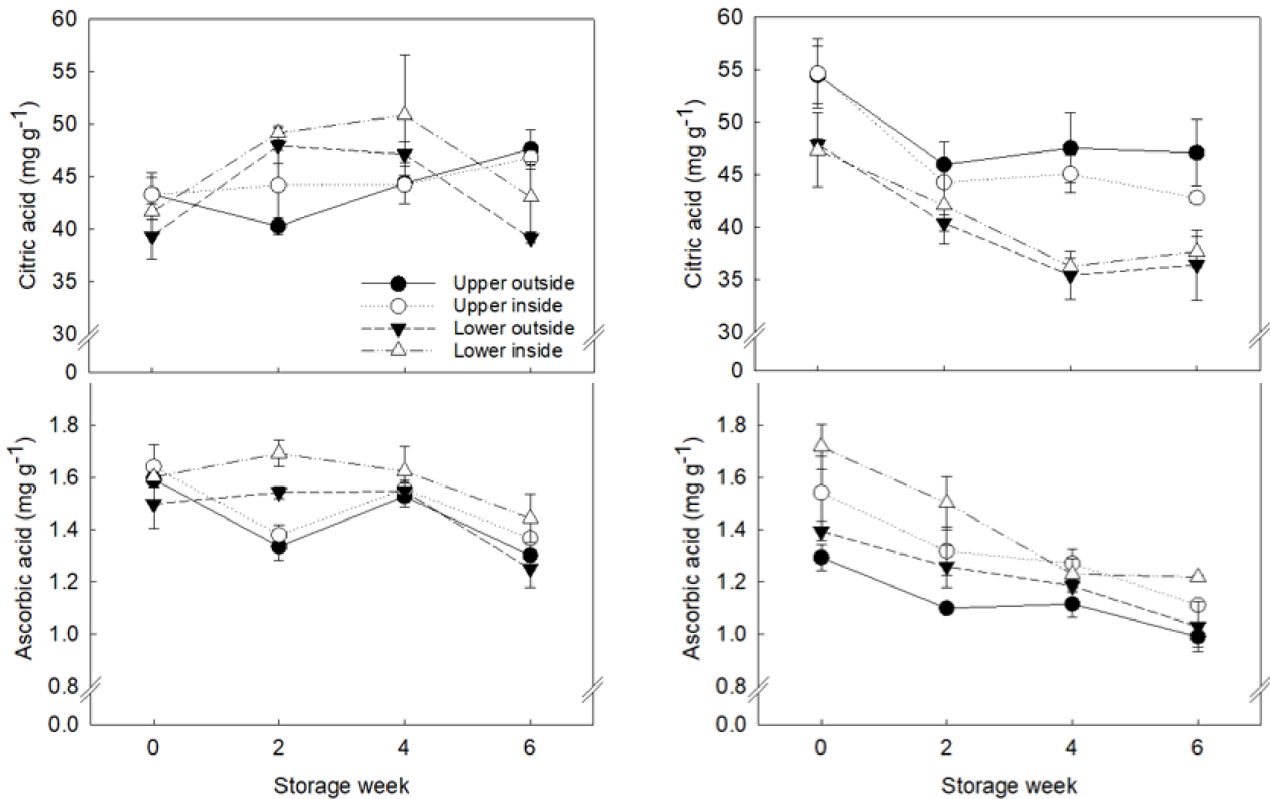

Figure 10. Organic acids (tartaric and malic acids) expressed as mg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

3.3.5 ABA and catabolites

ABA-GE was the main ABA catabolite found in clementines during storage, with ten times higher content than ABA ranging from 6 to 12 µg g^{-1} (Figure 11). Compared to April, there was greater variability in hormonal content between the different canopy positions for fruit harvested in March. At the same time, fruit located on the inside of the canopy showed significantly higher ($p = 0.024484$) ABA and catabolites content than fruit on the outside at the beginning of storage. In contrast, fruit harvested in April and located on the outside had a higher hormonal content than fruit on the inside part of the canopy. At the end of storage

and independent of the harvest month, upper outside fruit showed the highest level of ABA-GE, while lower inside fruit had the lowest values.

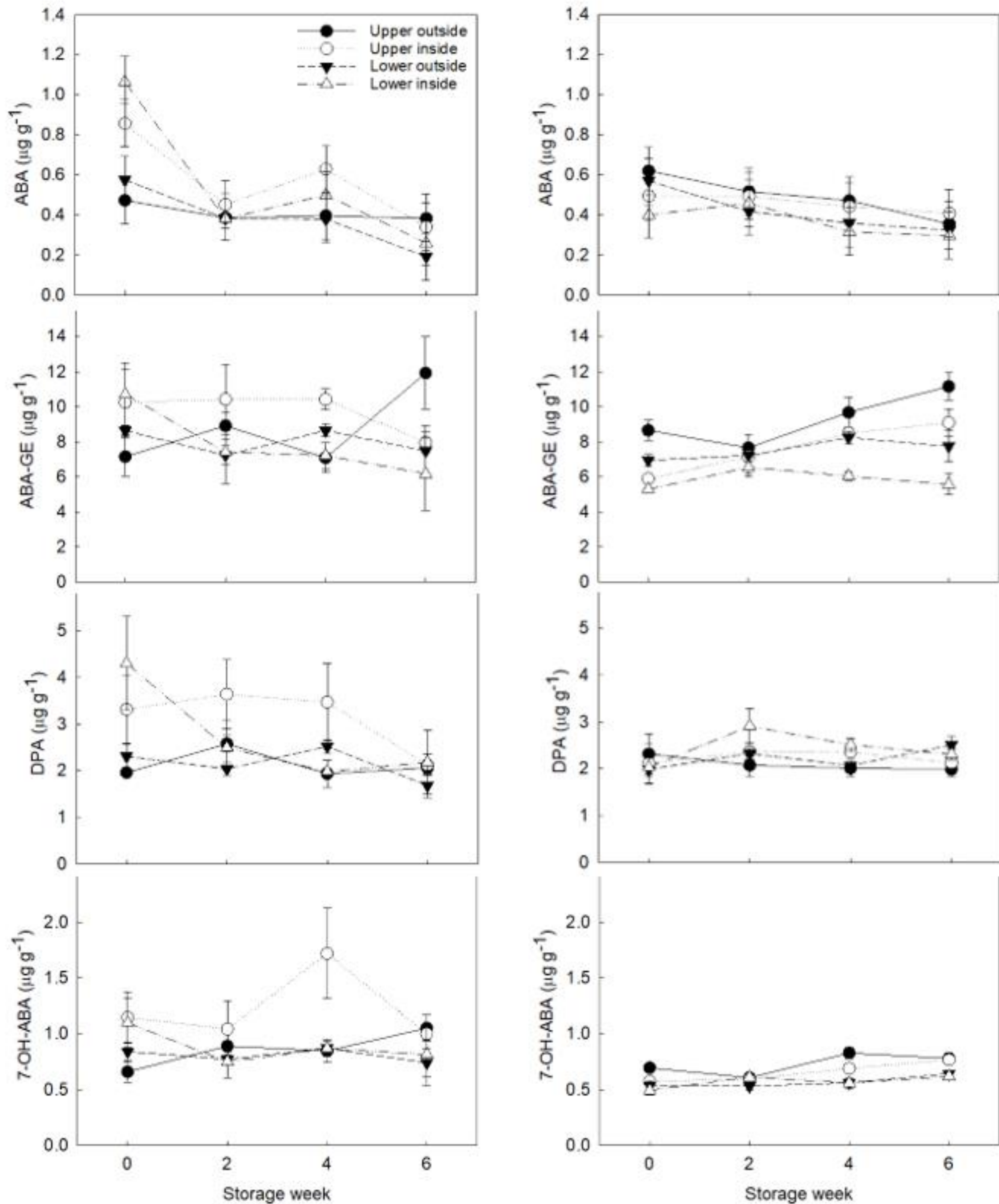

Figure 11. ABA and ABA catabolites expressed as µg g^{-1} DW of 'Nadorcott' clementines harvested in March (left) and April (right) 2017. The fruit was harvested

and stored for six weeks at 8.5 °C and 85 % RH. Data represents means (n = 18) ± standard error.

3.3.6 Consumer tasting evaluation

The consumer tasting session showed that canopy position significantly affected the overall acceptance of the fruit (Figure 12Figure 12). Clementines located on the lower part of the canopy were preferred over the upper fruit among the consumers. Accordingly, the upper fruit obtained the lowest liking scores for flavour, juiciness, texture, and overall acceptance. Specifically, the upper inside fruit scored significantly lower (6-11 %) for appearance than the rest of the samples, and obtained the lowest liking scores for peelability, flavour, and overall acceptance.

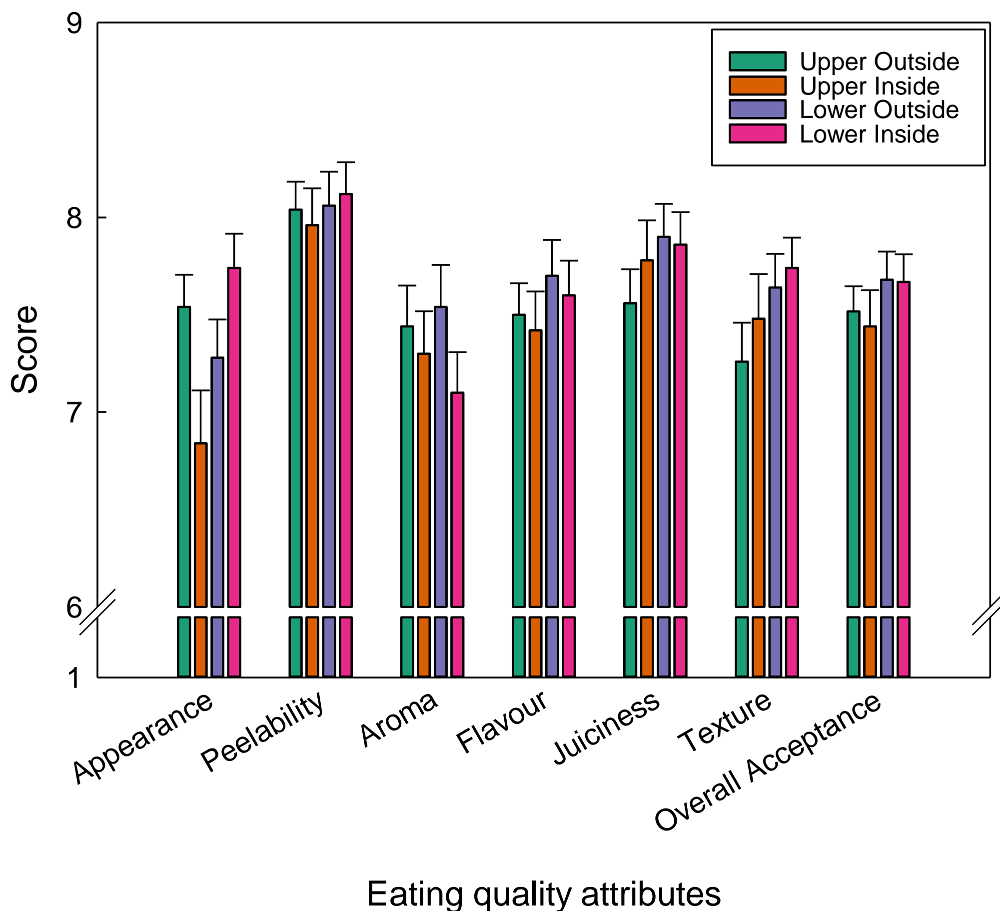

Figure 12. Tasting session of 'Nadorcott' clementines carried out in March 2017. Consumers tasted one fruit per position and scored the individual eating quality attributes

from 1 “Extremely dislike” to 9 “Extremely like”. Bars represent standard error of the mean scores.

3.3.7 Survey on consumer buying decisions

The results of the survey can be found in Table 1. The total attribute importance was calculated as:

$$Total\ attribute\ importance = (1st\ score * 8) + (2nd\ score * 7) + (3rd\ score * 6) + (4th\ score * 5) + (5th\ score * 4) + (6th\ score * 3) + (7th\ score * 2) + (8th\ score * 1)$$

As a result, the most important attribute for the panellists was flavour, followed by juiciness and sweetness. On the contrary, the least important attributes were aroma and texture. Interestingly, appearance obtained the 5th position in importance. However, when consumers were asked to identify the attributes they take into account when buying citrus fruits, skin colour, appearance, and size had the highest number of responses (Table 2).

Table 1. Ranking of the citrus attributes preferred by consumers.

Attribute	1st	2nd	3rd	4th	5th	6th	7th	8th	Total attribute importance
Appearance	5	2	11	6	1	7	11	7	204
Easiness to peel	8	6	5	8	7	7	6	3	240
Aroma	1	0	3	4	4	11	7	20	129
Flavour	18	12	3	8	3	2	3	1	311
Sweetness	10	10	7	3	5	7	5	3	261
Tanginess	0	6	8	7	12	3	5	9	201
Juiciness	8	9	12	8	5	3	3	2	276
Texture	0	5	1	6	13	10	10	5	178

Table 2. Attributes taken into account by consumers when buying citrus fruits.

Attribute	Number of responses
Colour	47
Size	42
Skin appearance	44
Aroma	19

Packaging	8
Other	1 (Squeeziness)

3.4 Discussion

3.4.1 The effect of harvest time and canopy position on the postharvest quality and consumer eating experience of 'Nadorcott' clementines

The quality profile of citrus fruit is highly dependent on the horticultural maturity at harvest (Lado *et al.*, 2018), and factors like fruit canopy position have been shown to have an effect on the postharvest quality of citrus. Previous research has demonstrated the effect of these factors on the pre- and postharvest quality of different citrus cultivars like 'Kinnow' mandarins (Thakre *et al.*, 2015), 'Nules' clementines (Cronje *et al.*, 2011a, 2011b; Magwaza *et al.*, 2013), and 'Iwasaki', 'Okitsu', and 'Goku Wase' satsumas (Sun *et al.*, 2021). However, to the best of our knowledge, this is the first time that the effect of fruit canopy position on consumer acceptance of 'Nadorcott' clementines has been studied.

'Nadorcott' is a seedless, mid to late-maturing clementine that is harvested from January to June in the Northern hemisphere. This variety does not need degreening (postharvest treatment with ethylene to develop peel colour), since carotenoids, which give the orange colour to the citrus peel and juice, are developed naturally as the fruit ripens. Peel colour is therefore a determinant factor of both fruit maturity for the citrus growers and eating quality for the final consumer. Nawaz *et al.* (2020) recently studied the effect of colour break on the internal quality of 'Kinnow' mandarins at early ripening stages, and demonstrated that green fruit had higher TA, ascorbic acid and lower TSS and sugars than yellow-coloured fruits. In this study, fruit harvested in March was brighter in colour, had significantly lower juice content, TSS:TA ratio, individual sugars, and higher ascorbic acid than fruit harvested in April. However, the results showed that the rind of fruit harvested in March was redder and there was less variability in colour hue within canopy positions. This confirms previous studies where 'Kinnow' mandarins harvested in India during the last week of December had higher ascorbic acid and total carotenoid content in the peel and juice than fruit

harvested two weeks later (Thakre *et al.*, 2015). Similarly, early maturing cultivars of satsumas (‘Iwasaki’, ‘Okitsu’, and ‘Goku Wase’) had higher absorption of carotenoids in the juice vesicles and advanced colour changes in the flavedo than late-maturing (Sun *et al.*, 2021).

Regarding canopy position, this study showed that outside (sun-exposed) fruit had higher TSS but lower juice content and colour lightness than inside (shadowed) fruit. This is in agreement with the results reported by Thakre *et al.* (2015), who found that fruit from the external canopy had higher TSS and total carotenoids than fruit from the internal canopy. From all the positions studied, fruit on the lower inside canopy showed the highest juice content and ascorbic acid, and the lowest TSS, TA, and fructose and glucose content, while the upper outside fruit had the opposite results. This means that, if harvested at the same time, upper outside fruit would be more ripe than lower inside fruit due to a more advanced degradation of ascorbic acid and sucrose, which may result in a shorter shelf-life.

However, no known research has investigated the effect of harvest time and canopy position on the CO_2 production of ‘Nadorcott’ clementines throughout the postharvest cold storage. Respiration rate and the potential postharvest life of fresh produce can depend on the content of respiratory substrates at the time of harvest (Khalid *et al.*, 2017), as once detached from the mother plant the photosynthetic substrates no longer accumulate. Citrus fruits are generally considered non-climacteric, since there is no peak in ethylene production and respiration at the beginning of fruit ripening. However, some authors have reported a rise in ethylene production after harvest. For instance, Khalid *et al.*, (2017) observed two ethylene peaks during the shelf-life of ‘Kinnow’ mandarins stored for seven days at ambient temperature, which were attributed to varietal effect, variation in fruit maturity, or even tree age. The same authors reported that CO_2 production decreased throughout ambient storage, which contrasts with our results. Herein, although harvest time did not affect fruit RR during the first two weeks of storage, there was a significant increase in RR from week 4 to 6 in March. Interestingly, this change was observed two weeks earlier in fruit harvested in April, from week 2 to 4, and could be explained by differences in the

climactic conditions before the two harvests. Meteorologic data for Lora del Rio, Seville (Appendix D), showed that the month preceding the second harvest (11th March – 7th April) had higher maximum temperature, solar radiation, and reference evapotranspiration values compared to the month before the first harvest (10th February – 10th March).

Cronje *et al.* (2011b) found that sun light availability decreases within the first 10 cm into the canopy resulting in outside fruit having greater exposure to higher temperatures, and lower RH compared to the inside position. However, the authors stated that this would result in the outer fruit having a higher transpiration rate than inside fruit due to higher atmospheric evaporative demand, which is contrary to our observations at the end of storage. Here, fruit located on the upper part of the canopy had significantly higher CO_2 production on average than fruit located on the lower part. Moreover, inside (shadowed) fruit showed the highest RR at the end of storage. It could be thought that these fruits should be less stressed than those on the outside part of the canopy as they are not exposed to direct sun light. Furthermore, the upper inside fruit showed the highest RR at the end of storage, probably caused by a higher osmotic pressure on the top of the canopy compared to the lower part and a poor ventilation inside the canopy.

The results of the tasting session carried out at the fourth week of the March harvest showed that consumers preferred the fruit located on the lower part of the canopy, which had higher juice content and TSS and lower TA than the upper fruit. This is in agreement with the survey results, which showed that consumers value flavour, juiciness, and sweetness more than other citrus attributes. When asked to rank the attributes they take into account at the moment of purchase, consumers positioned skin colour and appearance as the most important factors, demonstrating the mismatch between the attributes that citrus consumers consider for buying decisions and those for eating quality. This finding confirms the results reported by Poole & Baron (1996), where the most important characteristics for citrus consumers were juiciness, skin quality, sweetness, and texture, of which only skin quality can be perceived at the moment of purchase.

Overall, ‘Nadorcott’ clementines harvested in March had less physiological and biochemical variability between the different canopy positions throughout storage than fruit harvested in April. This suggests that, if there is no differentiation in canopy positions at the moment of harvest, fruit picked in March would have more consistent quality than in April. In contrast, when harvesting at the end of the season, the variability in fruit quality for the different canopy positions will be higher, making it challenging for the citrus industry to supply a product with consistency of quality and flavour throughout the season. To mitigate these variations between the different canopy positions, the implementation of shading nets could be an effective solution. As reported by Lee *et al.* (2015), the use of white shade nets in ‘Ponkan’ mandarin farms produced a significant reduction in fruit surface temperature and sunburn, higher juice percentage, and lower weight loss and decay than in non-shaded fruit while postharvest peel colour, TSS and TA were unaffected. Therefore, the use of shading nets could decrease the variability in temperature, sun light exposure, and transpiration of the different canopy positions and, as a result, improve the consistency in fruit quality at harvest.

3.4.2 The role of ABA and ABA catabolites on clementine senescence

ABA is a phytohormone that is synthesised in higher plants from C40 epoxycarotenoids, natural pigments that give the red-orange colour to plants, through several oxidation steps regulated by the enzyme 9-cis-Epoxycarotenoid Dioxygenase (NCED) (Endo *et al.*, 2014). Alongside ethylene, ABA regulates physiological processes such as fruit maturation, ripening, and stress adaptation in both climacteric and non-climacteric produce. However, previous studies have demonstrated that ABA has a greater role in regulating ripening and abscission than ethylene in non-climacteric fruit (Wang *et al.*, 2016; Jia *et al.*, 2016; Magwaza *et al.*, 2019; Garcia-Pastor *et al.*, 2021). At the same time, several authors have confirmed the implication of ABA in citrus rind quality (Lafuente and Sala, 2002; Magwaza *et al.*, 2019), response to water stress and dehydration (Romero *et al.*, 2013), and mould infection (Lafuente *et al.*, 2021). However, very few studies have focused their attention on the changes in endogenous ABA and

ABA catabolites during the postharvest life of citrus fruit and their role in citrus pulp quality. In addition, and to the best of our knowledge, there are no published studies relating the endogenous ABA and catabolites content in the citrus pulp with fruit canopy position and consumer acceptance.

Wang *et al.* (2016) reported that applying exogenous ABA to 'Ponkan' mandarins significantly affected the ripening of citrus fruit by accelerating fruit colouring and decreasing the organic acid content. Moreover, ABA regulated the expression of most sugar-related genes, confirming the coordinated role of ABA and sugars in regulating citrus fruit ripening. The results presented herein supported these findings, since both the organic acid and the ABA content in the pulp declined throughout storage and season, while sugars demonstrated the opposite behaviour. Interestingly, the levels of the catabolite ABA-GE were ten times higher than those of ABA and increased over time. This coincides with the results reported by Magwaza *et al.* (2019), who observed that the ABA-GE content in citrus rind increased throughout postharvest storage. Moreover, the ABA-GE content in the pulp reported here was four times higher than those in the citrus rind observed by Magwaza *et al.* (2019). These observations could be explained by the fact that the maturation processes of the citrus rind and pulp are considered autonomous and not coordinated (Lado, *et al.*, 2018; Sadka *et al.*, 2019), and higher ABA catabolites in the pulp than in the rind could indicate that the pulp is overmature and that senescence processes in the pulp could have started much earlier than in the rind.

The citrus rind has previously been regarded as a modified leaf (Cronje *et al.*, 2011a). As such, photoassimilates, mainly sucrose, are synthesised in the chloroplasts during the early stages of development when the fruit is still green. Then, chloroplasts in the citrus fruit epicarp are converted to chromoplasts in order to develop external colour through the synthesis of carotenoids, which are the precursors of ABA. This chloroplast to chromoplast conversion is stimulated by sucrose accumulation (Iglesias *et al.*, 2007), which is in turn exported from the leaves to the vacuoles of the juice sacs in the citrus pulp through the transport phloem (Lado *et al.*, 2018). However, the juice sacs are disconnected from the fruit vascular system ending in the albedo (Sadka *et al.*, 2019), therefore a

translocation of sucrose and other solutes back to the citrus rind would be unlikely. At the same time, it has been reported that ABA can induce its own biosynthesis in the juice sacs (Lado *et al.*, 2018). These facts would explain the higher nutrient and hormonal content in the citrus pulp compared to the rind.

Previously, a higher level of ABA catabolites has been related to a higher moisture loss and RBD development in the citrus rind (Magwaza *et al.*, 2019), which could explain the low score in external appearance of the upper inside fruit. When analysing the hormonal content in the pulp of fruit from different canopy positions, the upper inside samples showed significantly higher levels of ABA catabolites than the rest, and this position, interestingly, scored significantly lower than the others for external appearance during the consumers' tasting session. Apart from this, no significant differences were observed for any of the quality attributes evaluated by the consumers between the different canopy positions.

3.5 Conclusion

This study has demonstrated that canopy position has an effect on the postharvest quality of 'Nadorcott' clementines, specifically on external appearance, which is one of the main citrus attributes that consumers take into account at the moment of purchasing. Fruit located on the upper inside part of the canopy obtained the lowest consumer score for external appearance and had the highest levels of ABA catabolites.

ABA-GE has shown to have a greater role in the citrus senescence processes and the external quality of citrus fruit than ABA, demonstrating that ABA catabolites could be used as biomarkers of postharvest resilience and consumer acceptance. Moreover, it has been confirmed that the internal and external ripening processes in citrus fruit are independent and not coordinated. Therefore, it is recommended that further studies focus their efforts on elucidating the changes in ABA-GE during the ripening and senescence of citrus fruit to establish the hormonal content at different stages of citrus shelf-life and relate it to consumer acceptance.

At the same time, canopy position should be considered a determining factor of quality at harvest and be included in the agricultural practices for two reasons. Firstly, to improve the consistency of citrus flavour and quality throughout the season; and secondly, to meet retail specifications and consumer needs. For this, the implementation of shading nets in the field needs further testing.

4 Chapter Four: Table grapes

4.1 Introduction

Table grapes follow a non-climacteric ripening pattern, and thus do not show a burst in respiration and ethylene production at the onset of ripening (Coombe and Hale, 1973). However, it has been reported that ethylene is somehow involved in regulating grape ripening processes, such as the decrease in acids and the accumulation of sugars and anthocyanins during berry development (Chervin *et al*., 2004). Nonetheless, many studies have highlighted a more influential role of ABA on these ripening processes (Coombe and Hale, 1973; Sun *et al*., 2010; McAtee *et al*., 2013; Fortes *et al*., 2015; Pilati *et al*., 2017; Coelho *et al*., 2019; Gao-Takai *et al*., 2019).

There are three ABA peaks, which occur during grape berry growth, at ripening, and at postharvest senescence, the latter being an irreversible process if a certain ABA threshold is achieved. ABA can be esterified to ABA-GE and stored in the vacuoles to respond to stresses, hydroxylated to 7' -, 8' -, or 9'-OH-ABA, or isomerised to PA or DPA (Jia *et al*., 2017). Although some research has been done on ABA and ABA metabolites during ripening and senescence in fruits like cherries (Serradilla *et al*., 2019) and strawberries (Tosetti *et al*., 2020), little is known about the role of ABA and the different ABA catabolites during grape berry postharvest senescence.

The ethylene antagonist 1-methylcyclopropene (1-MCP) has been widely used in postharvest studies to better understand the ripening process of fresh produce (Watkins, 2006). However, there is a lack of information on the effect of 1-MCP on grapes postharvest quality and senescence. Moreover, the results obtained from the few published studies are not conclusive. Chervin *et al*. (2004) applied 1-MCP at the time of the ethylene peak during on-vine berry maturation. They found that the treatment delayed the increase in berry diameter, slowed down the decline in acidity during the post-veraison period, and inhibited the accumulation of anthocyanins in the berry skin. In another study, Bellincontro *et al*. (2006) investigated the effect of 1-MCP on stored grapes. Their results showed that 1-MCP lowered ethylene production, whilst no change in grape berry respiration

rate was observed. Moreover, 1-MCP-treated berries showed a higher incidence of grey mould and a significant loss of terpenols and esters. More recently, Silva *et al*. (2013) applied different doses of 1-MCP (0, 500, 1,000 and 2,000 nL L^{-1} for 12 hours) to stored berries and found that the treatment maintained the ascorbic acid and total anthocyanins contents without affecting berry firmness and decreased berry drop as the doses increased.

Therefore, despite the efforts made to understand the mechanisms behind the ripening and senescence of non-climacteric fruit, the role of the different ripening hormones remains unclear. The objective of this study was three-fold: (i) to understand the role of ABA and ABA catabolites on grape berry senescence; (ii) to study the spatial distribution of these hormones within the grape berry during senescence and its effect on berry quality, and (iii) to evaluate the effect of 1-MCP and storage temperature on the postharvest quality of 'Krissy' table grapes.

4.2 Materials and Methods

4.2.1 Plant material

The experiment was conducted on grapes (*Vitis vinifera* L.) from a commercial vineyard of drip irrigated 3-year-old 'Krissy' vines grafted onto Paulsen 1103 (3.5 × 3 m spacing) located in Murcia (SE Spain).

The grape clusters (n = 200) were monitored during the 2018 growing season and harvested at their optimal commercial maturity according to market standards. The samples were then transported to the Plant Science Laboratory at Cranfield University (UK) by refrigerated lorry (0.5 °C and 85 % RH) within five days of harvest.

4.2.2 Experimental design

Grape clusters showing decay or mechanical damage were discarded, and sound fruit was placed in cold storage (0.5 °C and 85 % RH) overnight to acclimatise. Then, two treatment groups were considered: i) control (untreated); and ii) 1-MCP, where one hundred grape clusters were placed inside 100 L hermetically sealed boxes and treated with 1-MCP (1 µL L^{-1}) for 12 hours at 15 °C, as described in Foukaraki *et al*. (2016) with slight modifications.

Following the 1-MCP application, two storage scenarios were considered: i) scenario 1: 15 days at 0.5 °C and 85 % RH as per normal storage conditions, followed by five days at 5.5 °C and 85 % RH to simulate shelf-life; and ii) scenario 2: 20 days at 5.5 °C and 85 % RH to simulate a higher storage temperature followed by shelf-life.

Three replicates per treatment group and storage scenario were analysed every five days. Each replicate consisted of three grape clusters from which thirty random berries were analysed (n = 90).

4.2.3 Physiological analysis

4.2.3.1 Disease incidence, berry firmness, and berry skin colour

Disease incidence was recorded as the percentage of mouldy berries per replicate, according to Youssef *et al.* (2019).

The firmness of individual berries was determined using a uniaxial testing machine calibrated with a 5 N cell (Instron, model 5542, MA, USA). Maximum compressive load in Newtons (N) was measured by compressing the berry 1 mm deep with a 4 mm diameter probe at a speed of 100 mm min^{-1} (Alamar *et al.*, 2017).

Grape skin colour was determined as described in Chapter 2, section 2.2.1.

4.2.3.2 Respiration Rate

The respiration rate of three grape clusters (*ca*. 1.5 kg) per replicate was determined as in Chapter 2, section 2.2.2.

4.2.3.3 Total Soluble Solids and Titratable Acidity

The juice of 50 berries per replicate was prepared using a commercial blender. TSS and TA were determined as described in Chapter 2, section 2.2.3.

The TA of the grape juice (%) was calculated as mL NaOH * 0.1 N NaOH * milliequivalent factor * 100 * mL^{-1} juice. The milliequivalent factor for tartaric acid, which is the predominant acid in grape juice, is 0.075.

4.2.4 Biochemical analysis

For biochemical analysis, berries were cut transversely at *ca.* one third from the top of the berry (Figure 13), and then snap-frozen and freeze-dried as described in Chapter 2, section 2.3.1.

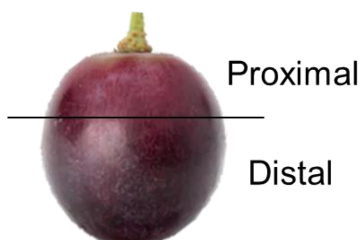

Figure 13. Transversal cut of a grape berry differentiating the proximal and distal sections where biochemical compounds were analysed.

4.2.4.1 Individual sugars

Extraction and analysis of individual sugars (glucose, fructose, and sucrose) were done as described in Chapter 2, section 2.3.3. Before analysis, the extracts were diluted 1:9 (v/v) with HPLC grade water. Detection was performed using an Agilent 1200 series HPLC system (Agilent Technologies, Germany) with a Phenomenex Rezex RCM monosaccharide Ca+ (8 %) 300 mm x 7.8 mm column, 8 µm particle size, fitted with a Phenomenex Carbo Ca+ 4 mm x 3 mm guard column (Phenomenex, CA). The column oven temperature was set at 80 °C and the refractive index detector at 50 °C. The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL min^{-1}, and the autosampler injection volume was 20 µL. A 6-point calibration curve ranging from 0.05 to 2.5 mg mL^{-1} was used for quantification, and the results were expressed as mg g^{-1} DW.

4.2.4.2 Organic acids

Analysis of non-volatile organic acids was done as described in Chapter 2, section 2.3.4. In brief, 50 mg of freeze-dried grapes were dissolved into 3 mL of HPLC-grade water, kept at room temperature for 10 min, and filter through 0.2 µm cellulose filters into HPLC vials. This work focused on tartaric and malic acids because they are the main organic acids present in grape berries. A 5-point

calibration curve ranging from 0.05 to 1 mg mL^{-1} was used for quantification, and the results were expressed as mg g^{-1} DW.

4.2.4.3 Abscisic acid (ABA) and ABA catabolites

ABA and ABA catabolites concentration was determined as described in Chapter 2, section 2.3.5.

4.2.5 Statistical analysis

All statistical analyses were carried out as described in Chapter 2, section 2.4.

4.3 Results

4.3.1 Mould incidence, berry firmness, and berry skin colour

Mould incidence in grapes stored at 5.5 °C was five times higher than the incidence found on grapes stored at 0.5 °C, which did not show decay until the end of storage. 1-MCP did not have a significant effect on disease incidence (p = 0.871739) in any of the scenarios. With respect to firmness, it was observed that storage time was the key factor involved. Berry firmness declined during storage, with a significant decrease (p = 0.000001) from day 10 to day 15, and then remained unchanged until the end of shelf-life. 1-MCP did not maintain firmness during the simulated supply chains ().

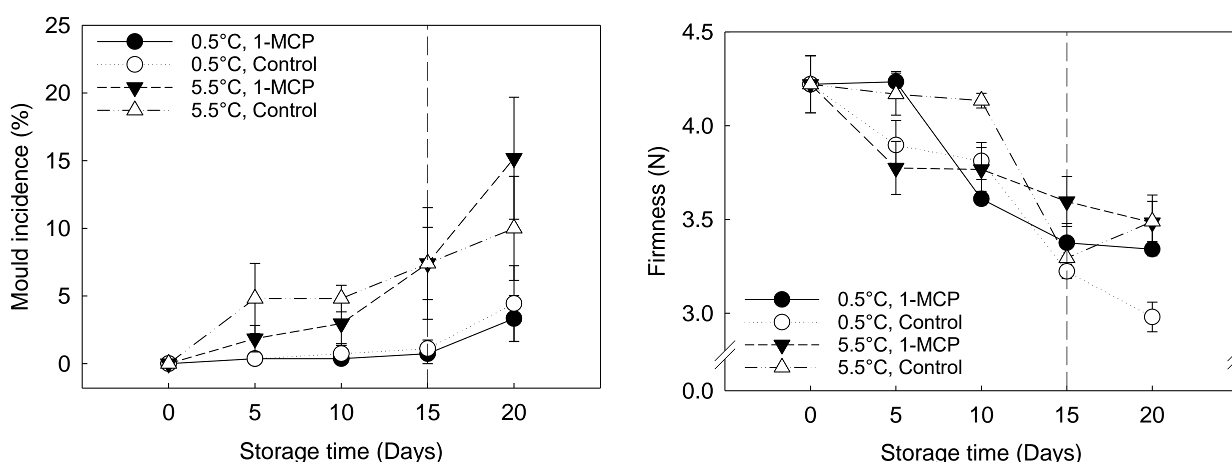

Figure 14. Mould incidence (%) (left) and firmness (N) (right) of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios:

i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

The treatment with 1-MCP did not affect any of the berry skin colour parameters studied (L*, C* and H°). Storage temperature only affected lightness, with grapes stored at 5.5 °C showing significantly higher values at day 5 and day 20 than at 0.5 °C. Chroma index progressively decreased over storage (Figure 15), and hue angle significantly increased ($p = 0.000000$) at the end of storage, with berries acquiring a dull, dark red skin colour.

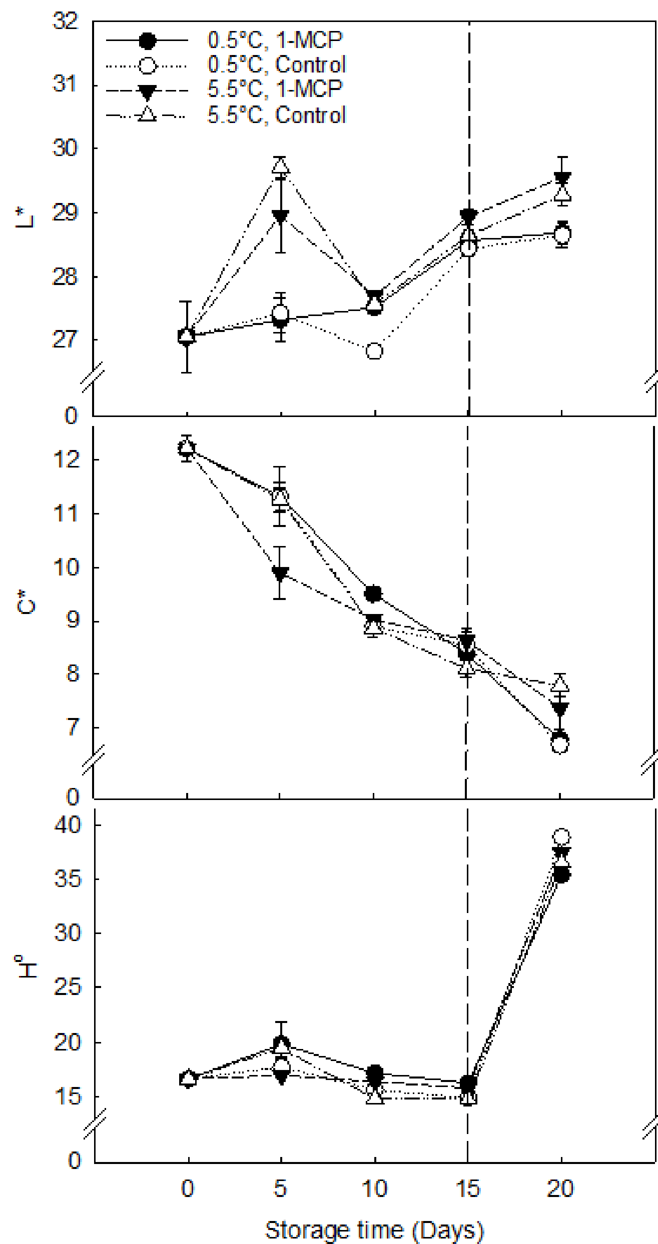

Figure 15. CIELab colour parameters [Lightness (L*), Chroma index (C*), and Hue angle (H°)] of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 μL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.3.2 Respiration rate

The treatment with 1-MCP significantly decreased ($p = 0.012801$) the RR of the grape berries stored at 5.5 °C compared to control, from a mean of 2.6 to 2.3 mL CO_2 kg^{-1} h^{-1} for control and treated grapes, respectively. However, 1-MCP did not affect grapes stored at 0.5 °C. The CO_2 production of grape berries significantly decreased ($p = 0.000000$) at the beginning of the storage, from 1.5 to 0.8 mL CO_2 kg^{-1} h^{-1} at day 0 and day 10, respectively. Then, RR significantly increased to 3.0 mL CO_2 kg^{-1} h^{-1} at day 15. Storage temperature was the main effect for RR: grapes stored at 5.5 °C showed significantly higher ($p = 0.000000$) (2.5 mL CO_2 kg^{-1} h^{-1}) RR than grapes stored at 0.5 °C (1.4 mL CO_2 kg^{-1} h^{-1}) (Figure 16).

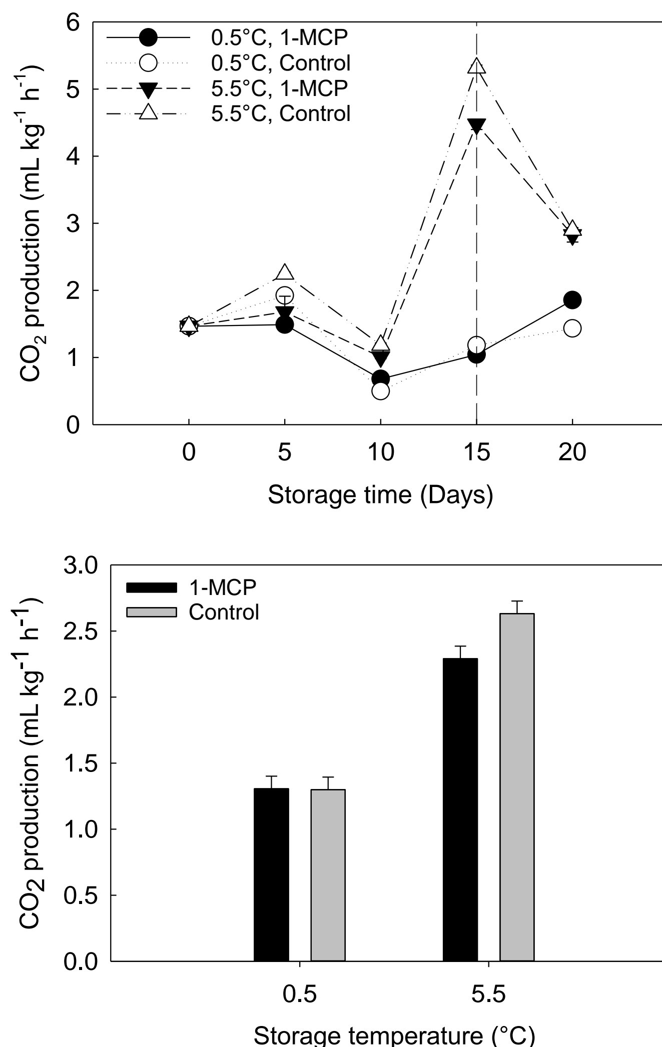

Figure 16. Respiration rate (RR) expressed as CO_2 production (mL kg^{-1} h^{-1}) of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C

[1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.3.3 Total Soluble Solids and Titratable Acidity

TSS and TA levels of the grape berries were not affected by 1-MCP, and the changes in these parameters were only dependent on storage temperature. As such, treated grapes stored at 0.5 °C maintained similar TSS and TA levels to those at the beginning of the storage by the end of shelf-life whilst TSS decreased, and TA increased for grapes stored at 5.5 °C (Figure 17).

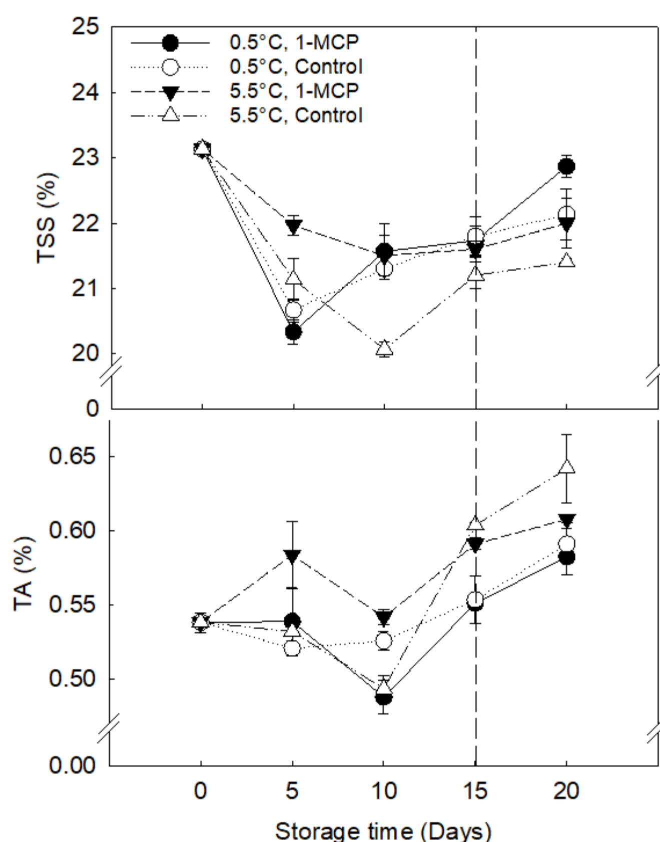

Figure 17. Total soluble solids (TSS) and titratable acidity (TA) expressed in % of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.3.4 Individual sugars

The main sugars found in the grape berry were fructose and glucose, which shown similar trends throughout storage, while the concentration of sucrose was ten times lower (Figure 18). Storage time and temperature were the key factors affecting sugar content, whilst 1-MCP only had a significant effect at the end of shelf-life. At this time, the distal section of the treated berries stored at 0.5 °C showed a significantly lower content of fructose and glucose than the rest of the samples. Sucrose content significantly increased ($p = 0.000000$) from day 5 (20 mg g^{-1} DW) to day 10 (26 mg g^{-1} DW) of storage and then significantly decreased to values recorded at day 5 for both storage temperatures and sections.

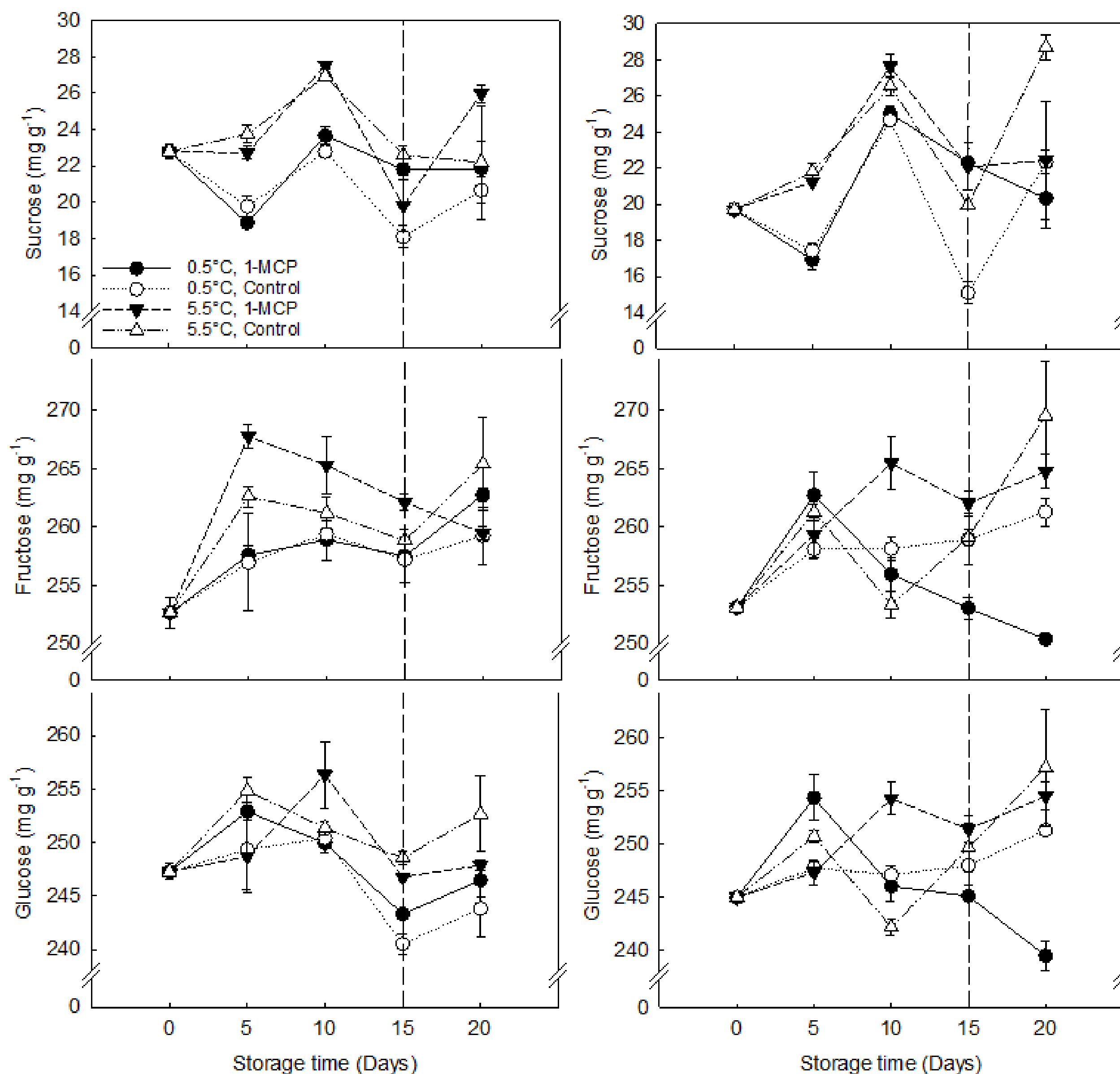

Figure 18. Individual sugars (sucrose, fructose and glucose) expressed as mg g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.3.5 Organic acids

The concentration of both tartaric and malic acid experienced a significant decrease (p = 0.000000) from day 0 to day 10, independent of the berry section

sampled, and then remained constant until the end of shelf-life. Significant differences between temperatures and treatments were only observed at day 5, with treated grapes stored at 0.5 °C showing a higher level of malic acid than the rest of the samples. The effect of 1-MCP on the tartaric (p = 0.990023) and malic acid content (p = 0.467297) was not significant at 5.5 °C; however, it did affect the organic acids content at the beginning of storage when the berries where kept at 0.5 °C. Thus, malic acid levels on day 5 were significantly higher (p = 0.034116) for treated grapes stored at 0.5 °C than control, and this difference was more pronounced in the distal section of the berries. In contrast, the proximal section of control samples stored at 0.5 °C had higher tartaric acid content than treated grapes on the same day. Moreover, a higher tartaric acid concentration was found in the proximal section at the beginning of storage, while malic acid was more concentrated in the distal section (Figure 19).

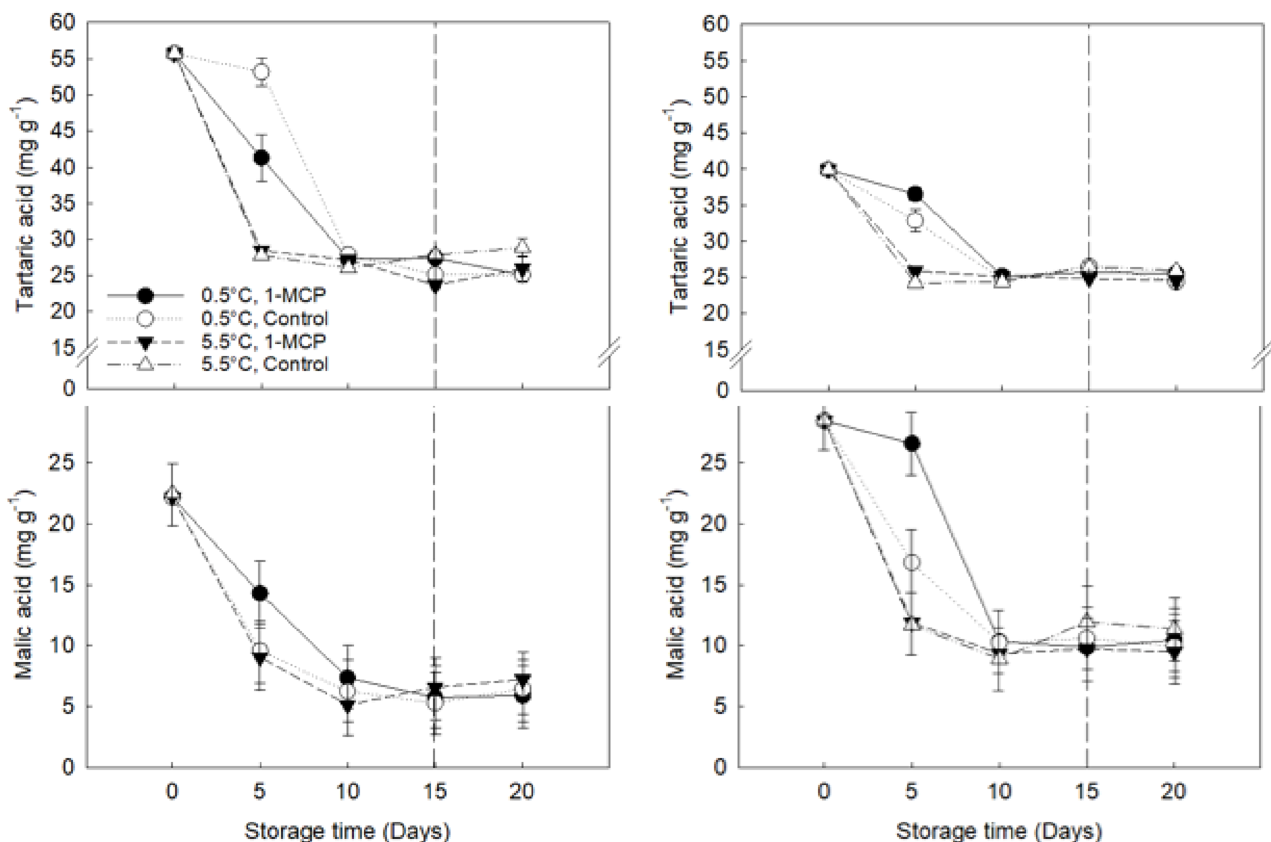

Figure 19. Organic acids (tartaric and malic acids) expressed as mg g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air

[control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH, followed by 5 days at 5.5 °C and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.3.6 ABA and catabolites

ABA and ABA catabolites significantly increased ($p = 0.000001$) throughout storage and decreased by the end of shelf-life. Grapes stored at 5.5 °C had 20 % lower ABA and 15 % higher ABA-GE content than grapes stored at 0.5 °C. The distal section of the berries showed a significantly higher (3-fold) ($p = 0.000000$) ABA content than the proximal section. However, the ABA content in the distal section of 1-MCP-treated grapes was approximately half (238 ng g^{-1} DW) of that in the control (435 ng g^{-1} DW) at the end of storage (Figure 20).

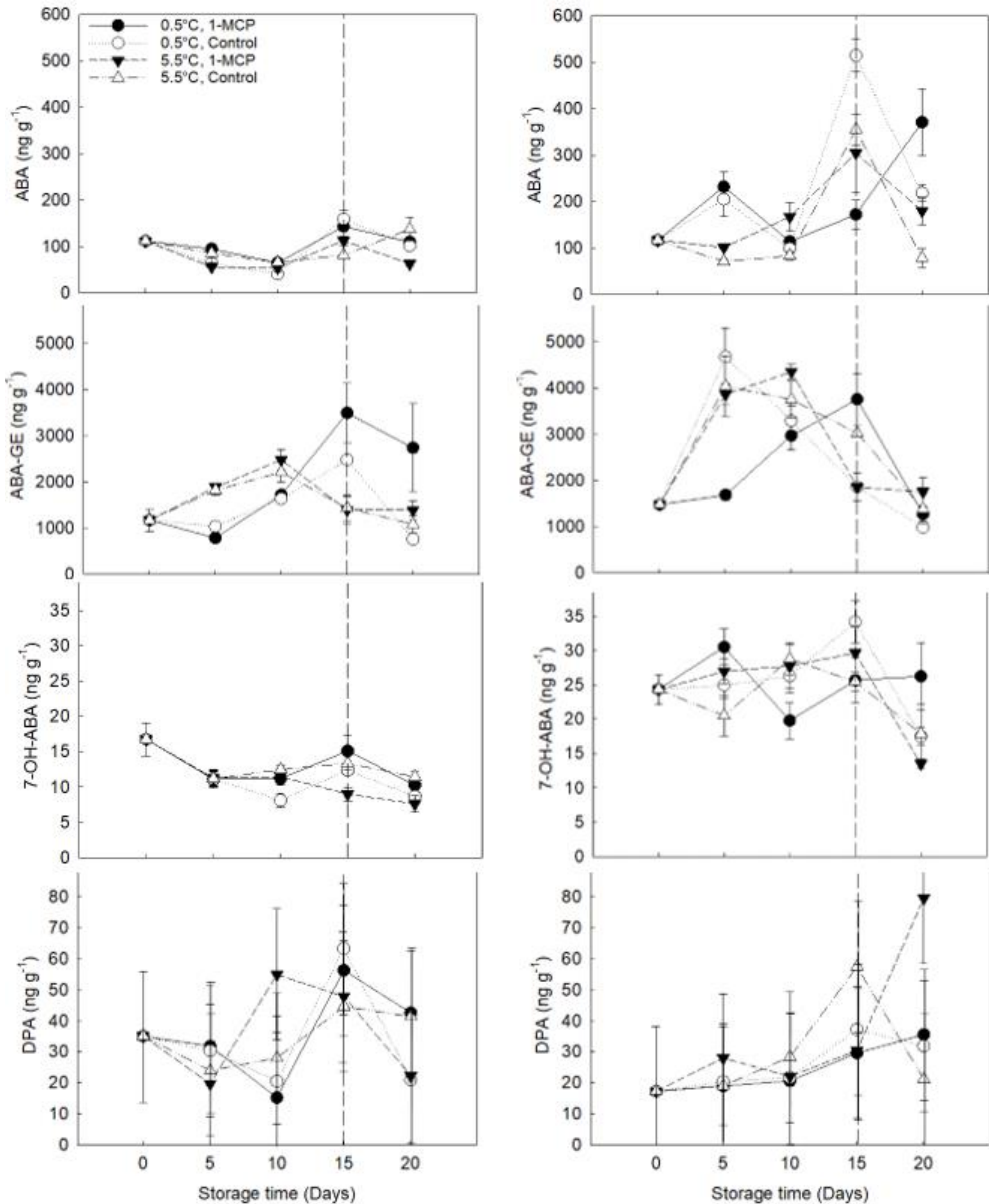

Figure 20. ABA and ABA catabolites expressed as ng g^{-1} DW in the proximal (left) and distal (right) sections of ´Krissy´ grapes. Table grapes were treated with 1-MCP (1 µL L^{-1} for 12 hours at 15 °C [1-MCP]) or without 1-MCP (air [control]) prior to storage and subjected to two postharvest storage scenarios: i) 15 days at 0.5 °C and 85 % RH,

followed by 5 days at 5.5 °C, and ii) 20 days at 5.5 °C and 85 % RH. The vertical stripped line shows when all samples were stored at 5.5 °C. Data represents means (n = 90) ± standard error.

4.4 Discussion

4.4.1 The effect of 1-MCP and storage temperature on the postharvest quality of 'Krissy' table grapes

The ethylene antagonist 1-MCP is a compound that inhibits ethylene perception by binding to and hence inactivating the ethylene binding protein (EBP) (Mullins *et al.*, 2000). For instance, 1-MCP delays the increases in ethylene production associated with ripening, softening, and colour changes in apples (Watkins and Nock, 2005), banana (Pathak *et al.*, 2003; Pelayo *et al.*, 2003), and plums (Valero et al., 2004; Martinez-Romero *et al.*, 2003). However, contradictory results have also been reported for other climacteric and non-climacteric fruit crops. Rasori *et al.* (2002) found that 1-MCP-treated peaches had higher ethylene production than untreated fruits; and that the increase in soluble solids content (SSC) was either not affected or delayed (Liu *et al.*, 2005). On grapes, a single dose of 1-MCP (1 mg L^{-1}) applied for 15 h at 20 °C in 300 L boxes during postharvest storage reduced ethylene production yet only for two days after treatment application. In addition, there was an increase in grey mould incidence after six days of storage at 20 °C (Bellincontro *et al.*, 2006).

In the study herein, treated grapes stored at 5.5 °C were highly affected by mould incidence at the end of shelf-life. This agrees with the above-mentioned study (Bellincontro *et al.*, 2006) and with previously published research on non-climacteric fruit like grapefruit (Mullins *et al.*, 2000) and strawberry (Bower *et al.*, 2003). It has been demonstrated previously that local resistance to *Botrytis cinerea* (grey mould) in Arabidopsis requires the upregulation of defence genes that use ethylene as a secondary messenger (Ferrari *et al.*, 2003). Therefore, blocking ethylene production with 1-MCP could be stopping the expression of these defence genes and, as a result, decreasing the crop resistance to a fungal attack.

Regarding RR, table grapes stored at 5.5 °C experienced a significant increase in CO_2 production from day 10 to day 15, which was lower in 1-MCP treated grapes than in control. It has been previously proposed that the increase in RR at the onset of ripening occurs due to a change in the berries' metabolism and that sugars and not malate are the primary substrate used for respiration (Famiani *et al.*, 2014). This hypothesis is confirmed by the results seen here, which showed that a lower RR due to the 1-MCP treatment delayed both the hydrolysis of sucrose into fructose and glucose and the decline in the organic acid content compared to untreated grapes. In contrast to these results, Bellincontro *et al.* (2006) and Li *et al.* (2016b) found that 1-MCP applied after harvest did not significantly affect the RR of 'Aleatico' wine grapes and 'Thompson Seedless' white table grapes, respectively. However, it is necessary to highlight that Li *et al.* (2016b) only measured the RR of five berries clipped off at the end of the pedicel after three and six days from applying the 1-MCP treatment, while in this study the RR of three whole bunches per condition was measured every five days for 20 days.

Therefore, while 1-MCP delays the increase in RR and the degradation of organic acids and sugars in table grapes stored at 5.5 °C, the treatment is not able to counteract the effect of a higher storage temperature, which results in a higher mould incidence and, hence, the unsuitability of the grapes to be commercialised and accepted by the end consumer.

4.4.2 The role of ABA and ABA catabolites on grape berry senescence

ABA is known to regulate plant growth and developmental processes, such as cell division, seed dormancy, maturation, ripening, and abscission, as well as stress responses to drought, cold, or pathogen attack (Taylor and Whitelaw, 2001; Zhang and Zhang, 2009; Finkelstein, 2013; Jia *et al.*, 2017; Coelho *et al.*, 2019). While a few studies have investigated the profile and role of ABA and ABA catabolites during the preharvest stages of grape berry development and ripening, less attention has been given to the hormonal profile of berries during postharvest and senescence. According to Coombe and Hale (1973), the onset and rate of berry ripening are a function of ABA accumulation. The authors

suggested that berry ripening starts once ABA accumulates to a certain level (*viz.* 62 ng g^{-1} fresh weight [FW]), below which the berry will not ripen. In the literature, there is a considerable variation in the value of this peak and the moment in berry development when it appears. For instance, Coombe and Hale (1973) observed that 'Doradillo' grape veraison started with an ABA concentration of 62 ng g^{-1} FW, which then increased to 212 ng g^{-1} FW 13 days after veraison. In contrast, Sun *et al.* (2010) reported an ABA peak of 500 ng g^{-1} FW two days after 'Muscat Hamburg' grape veraison and a second ABA peak at similar levels six days after harvest after which the berries started to senesce. In a study by Owen *et al.* (2009), the ABA concentration in the pulp of immature 'Merlot' grape berries was 4,000 ng g^{-1} DW, while mature berries harvested at commercial maturity had an ABA content of 400 ng g^{-1} DW. Moreover, the authors found that ABA was catabolised to form ABA-GE by conjugation instead of producing DPA by oxidation at the onset of veraison. However, the mentioned study did not quantify ABA or its catabolites during the postharvest stage of berry senescence. In this work, ABA accumulation up to 100 ng g^{-1} DW was observed during the first ten days of storage, followed by an ABA peak of 500 ng g^{-1} FW at day 15. This ABA rise coincided with a decrease in the organic acid content and firmness of the berries and an increase in berry respiration rate, sugar hydrolysis, and disease incidence, which are factors determining quality acceptance for the final consumer (Wolf, 2002; Jianying *et al.*, 2014). These results suggest that the grape berries started to senescence after reaching an ABA concentration higher than 100 ng g^{-1} DW and that ABA played a role in regulating grape berry metabolism during storage and shelf-life. The variability between the results of this study and previous research could be explained by the differences in cultivars, maturity stages, and storage conditions studied (Coelho *et al.*, 2019).

In addition, it was observed here that ABA-GE concentration was ten times higher than ABA, whilst DPA concentration was ten times lower than ABA. High levels of DPA have been observed during the early developmental stages (Owen *et al.*, 2009). Therefore, a low level of this hormone could indicate an advanced level of ripening or senescence. ABA is stored in the form of ABA-GE in the vacuoles and the apoplast during the early berry developmental stages, being released to the

endoplasmic reticulum under water stress or dehydration (Finkelstein, 2013; Jia *et al.*, 2017). Thus, it could be hypothesised that the proximal section would have a higher hormonal content due to dehydration in the abscission zone, as ripening related changes in the cell wall undergo from the formation of a dehiscence zone to the softening of the flesh tissue (McAtee *et al.*, 2013). Contrastingly, here it was found that the concentration of ABA and its catabolites, mainly ABA-GE, was significantly higher (3-fold) in the distal section when compared to the proximal section, confirming the hypothesis of a different hormonal distribution within the grape berry during senescence. Two facts could explain this: firstly, the distal section of the berries had a darker colour than the proximal, and secondly, it was observed that this section also presented a higher mould incidence during the last stages of cold storage and shelf-life.

The crosstalk between ABA and sucrose in regulating fruit ripening processes has been recently reviewed (Duran-Soria *et al.*, 2020; Alferez *et al.*, 2021). Jia *et al.* (2017) found that treatments with exogenous ABA and sucrose regulate berry ripening of 'Fujiminori', a black table grape, and that sucrose can induce the ABA synthesis gene expression. According to Luo *et al.* (2020), ABA and sucrose play a synergistic role in regulating the ripening of strawberry, a non-climacteric produce. Conversely, our results showed a negative correlation between sucrose and ABA during cold storage and shelf-life, suggesting that the breakdown of sucrose could act as signalling for ABA to start accumulating and regulate senescence processes.

In addition to regulating sugar levels, ABA is involved in the biosynthesis of secondary metabolites like anthocyanins, which are pigments that give red, purple, and blue colours to plants (Owen *et al.*, 2009; Zhang *et al.*, 2014). For this reason, ABA is applied to coloured grape varieties during berry maturation to improve skin colour development by enhancing the accumulation of anthocyanin biosynthetic enzyme genes (Jeong *et al.*, 2004; Yamamoto *et al.*, 2015; Domingues *et al.*, 2017). In this study, an increase in the fructose and glucose content coincided with darker berry colour and a higher ABA content by the end of storage and shelf-life, confirming the role of sugars in the stimulation of pigmentation (Solfanelli *et al.*, 2006). Darker berry colour is related to higher

consumer acceptability, and therefore, the understanding of ABA metabolism and its interaction with pigment development is key for the grape industry.

As seen before, ABA is involved in the biosynthesis of colour pigments and the regulation of stress responses due to pathogen attack. Therefore, it can be confirmed the crucial role of ABA and its catabolites in regulating senescence during the postharvest storage of table grapes.

4.5 Conclusions

The changes in ABA, respiration rate, and sucrose observed at the end of cold storage confirmed the crucial role of ABA in regulating processes associated with the senescence of the non-climacteric grape berry. However, whether respiration or the changes in endogenous ABA concentration are the triggers of the different senescence mechanisms undergone in the grape berry needs further investigation. It is also recommended that these studies focus their attention on the distal section of the berries, as it has shown a higher hormonal content than the proximal part. The benefit of ethylene antagonists like 1-MCP on non-climacteric produces during postharvest storage is not entirely evident yet, and further research is suggested.

5 Chapter Five: General Discussion and Conclusions

5.1 Introduction

The aim of this research project was to identify biomarkers of postharvest resilience and flavour life of imported clementines and table grapes. For that, the effect of different pre- and postharvest factors on the postharvest quality of these products were investigated. The quality profile of these non-climacteric produces has been widely studied and reviewed; therefore, the mere determination of their quality profile was not an objective of this work. Instead, the effect of a preharvest factor, i.e., fruit canopy position, on the postharvest quality and eating experience of 'Nadorcott' clementines, and the effect of a storage treatment with 1-MCP on the postharvest quality of 'Krissy' table grapes were examined.

Both citrus fruit and table grapes are classified as non-climacteric, not producing a peak in respiration rate at the onset of ripening. Moreover, it has been demonstrated that ethylene is not the main hormone mediating the ripening processes in this kind of produce, and that ABA has a more predominant role in their ripening and senescence processes. However, the changes in ABA and ABA catabolites during clementine and grape senescence as well as their effect on postharvest quality and resilience have not yet been defined. Given the key role of ABA in understanding the postharvest quality and senescence, both experiments focused on the following: 1) the determination of pre- or postharvest factors influencing the produce postharvest quality – both physiological and biochemical, and 2) the role of ABA and ABA catabolites on fruit senescence. Moreover, the effect of fruit canopy position on the consumer eating experience of clementines was investigated.

5.2 Main findings and contribution to knowledge

5.2.1 Senescence processes in clementines and table grapes start after a significant increase in respiration rate

Respiration rate is a major determinant of fruit quality, as decreasing the metabolic activity of the produce can extend its shelf-life (Falagán and Terry, 2018). In the study herein, the RR, sugars, and organic acids content of

clementines harvested in March did not change dramatically during the first four weeks of storage and there were not significant differences between canopy positions. However, at the end of storage, fruit positioned on the inside (shadowed) part of the canopy had significantly higher RR than outside (sun-exposed) fruit, probably caused by a higher RH inside the canopy before harvest compared to the external branches. An increase in RR is produced when the fruit is under stress for different reasons, like fungal development, high osmotic pressure or dehydration, or adverse climatic conditions, among others (Romero *et al.*, 2013; Lafuente *et al.*, 2019). This increase in fruit RR by the end of storage was accompanied by a significant increase in fructose and glucose content produced by sucrose hydrolysis, and a decrease in ascorbic acid (vitamin C). In contrast, the RR of fruit harvested in April increased throughout storage, with a significant increase from week 2 to 4 of storage, two weeks earlier than in March, when the average temperature, solar radiation, and evapotranspiration was higher. Differences in quality between the canopy positions studied were more evident for fruit harvested in April throughout postharvest storage.

In order to reduce these differences between canopy positions and offer a more consistent fruit quality throughout the season, two alternatives are suggested. Firstly, harvesting fruit located on the shadowed parts of the canopy one to two weeks earlier than sun-exposed fruit will offer a more consistent quality and shelf life over the supply season than harvesting both at the same time. Skin colour and external appearance have been established by consumers as the most important factors at the time of purchase. In fact, upper inside fruit obtained the lowest score for external appearance in a consumer tasting session carried out with fruit harvested in March. At the same time, inside fruit scored lower for flavour and aroma than outside fruit. This is the first time that consumers evaluate the quality of clementines from different canopy positions. Given the interesting results of the tasting session, it is recommended that further attention is given to the effect of canopy position in the consumer eating experience, in order to offer a more consistent quality at the end of the supply chain. The second strategy would be the use of shading nets, as they have been proved (Lee *et al.*, 2015) to significantly reduce fruit temperature and sunburn in ‘Ponkan’ mandarins without

affecting skin colour or pulp TSS and TA, as well as decrease postharvest decay. Further studies evaluating whether the differences in postharvest quality in fruit from different canopy positions can be reduced using shade nets are suggested.

In the case of table grapes, all samples showed a significant increase in RR after 15 days of cold storage, although it was higher for grapes stored at 5.5 °C and non-treated grapes. This significant increase in RR at the end of storage, resulted in an increase in mould incidence and berry firmness loss by the end of shelf-life. This has been as well observed in other produces. For instance, Serradilla *et al.* (2019) reported significant changes in firmness, weight loss, colour, and ABA content of ‘Burlat’ sweet cherries after 15 days of cold storage, while Falagán *et al.* (2020) observed that control samples of ‘Duke’ blueberries suffered a significant increase in decay incidence after 15 days of cold storage, supporting the results showed herein.

Different postharvest technologies are used in the fresh produce industry to decrease the RR of the produce and extend its shelf-life. The ethylene antagonist 1-MCP binds to and inactivates the ethylene binding proteins (EBP), resulting in the inhibition of this ripening hormone. The use of 1-MCP on fruits and vegetables was reviewed by Watkins *et al.*, (2006), and more recently by Li *et al.* (2016) on non-climacteric fruit crops. However, opposing results have been found and the effects of this ethylene inhibitor during postharvest storage are still not clear, depending on the produce and treatment conditions. For instance, Bellincontro *et al.* (2006) applied a single dose of 1 mg L^{-1} for 15 h to ‘Aleatico’ wine grapes stored at 20 °C, while Silva *et al.* (2013) tested different doses ranging from 0 to 2,000 ng L^{-1} for 12 h on 'Thompson Seedless' white table grapes stored at 25 °C. Both studies found that the RR of the grapes was not affected, which contrasts with the results of this study. Herein, the use of 1-MCP delayed the increase in RR of grapes stored at 0.5 °C and 5.5 °C until the end of storage (15 days). However, grapes stored at 0.5 °C for 15 days and then moved to 5.5 °C for five days had higher RR than the control. In addition, although the use of 1-MCP delayed the increase in berry RR, storing the samples at a higher temperature caused an increase in mould incidence, confirming previous research

(Bellincontro *et al.*, 2006) and resulting in the immediate unsuitability of the product for consumption.

As previously discussed, ethylene is required to upregulate defence-related genes against fungal attack in Arabidopsis (Ferrari *et al.*, 2003). Therefore, blocking ethylene production with 1-MCP could lower the grape resistance against mould infection, resulting in a higher mould incidence at the end of shelf life, when the conditions for mould growth are more favourable due to berry firmness loss and degradation of organics acids and sucrose. Other ethylene removal methods for the storage and transportation of fruit and vegetables have been reviewed recently (Ying *et al.*, 2021). Thus, while 1-MCP inhibits ethylene, other technologies like ozone, low-temperature catalytic oxidation, and plasma catalysis act by eliminating ethylene. For instance, Feliziani *et al.*, (2014) demonstrated that storing 'Crimson Seedless' table grapes in chambers containing 0.1 µL L^{-1} ozone or higher at 2° C for three weeks reduced grey mould incidence by 65 % after 5 to 8 weeks of storage. Therefore, since the use of 1-MCP has not been proved to have a positive effect on extending the postharvest life of table grapes, it is recommended to test other ethylene removal techniques like the mentioned above.

5.2.2 ABA could be considered a biomarker of clementines and table grapes senescence

Citrus fruits and table grapes are considered non-climacteric, not showing an increase in respiration rate and ethylene production during ripening (Li *et al.*, 2016). However, several authors have reported that the ripening of these produces is somehow regulated by ethylene (Chervin *et al.*, 2004; Li *et al.*, 2016a; Estables-Ortiz *et al.*, 2016), ABA (McAtee *et al.*, 2013; Wang *et al.*, 2016; Jia *et al.*, 2017, Crupi *et al.*, 2019; Wang *et al.*, 2019), a complex interplay between these two hormones (Fortes *et al.*, 2015; Iqbal *et al.*, 2017; Tosetti *et al.*, 2020), or even between ABA, sucrose, and other plant hormones (Setha, 2012; Jia *et al.*, 2017; Olivares *et al.*, 2017; Coelho *et al.*, 2019).

In the study herein, the esterified form of ABA, ABA-GE, was found to be the most abundant hormone in the citrus pulp and grape berry, with a content ten times

higher than ABA. However, ABA-GE does not have biological activity, and acts as a reservoir of ABA, releasing the hormone as a stress response (Nazareno and Hernandez, 2017; Duran-Soria *et al*., 2020; Alferez *et al*., 2021). In the same way, the ABA catabolites evaluated here (7-OH-ABA and DPA) are considered inactive molecules (Nambara and Marion-Poll, 2005).

The results presented here showed that ABA content in the citrus pulp declined throughout postharvest storage. Moreover, significant differences were found at the beginning of storage for fruit harvested in March, when fruit located on the inside of the canopy (shadowed) had significantly higher ABA content than outside (sun-exposed) fruit. These results coincide with Magwaza *et al.* (2019), who found that ABA content in the citrus rind of 'Nules' clementines declined throughout storage and that bagged fruit had higher ABA content than non-bagged fruit at the beginning of postharvest storage. This could be explained by the different microclimatic conditions within the canopy before harvest. Inside fruit would have been exposed to higher RH levels than sun-exposed fruit (Cronje *et al*., 2011b), increasing their stress levels and, as a result, ABA content. However, no significant differences in ABA between the different canopy positions were observed throughout storage, probably due to an acclimation of the samples to the storage conditions. Interestingly, ABA significantly decreased at the end of storage compared to two weeks earlier, and this coincided with an increase in fruit RR, ascorbic acid degradation, and fructose and glucose accumulation independent of the canopy position.

Contrary to clementines, the ABA content in the grape berry increased throughout storage, with the distal section having three times higher hormonal content than the proximal section. This could mean that berry senescence processes start earlier in the distal section than in the proximal, softening berry skin in this area and making it more susceptible to fungal attack. In addition, ABA significantly increased by the end of storage and then declined by the end of shelf-life, when mould incidence and berry RR significantly increased. This ABA peak coincided with a significant reduction in sucrose, confirming the hypothesis that ABA and sucrose jointly regulate grape berry senescence as demonstrated before in 'Crimson Seedless' table grape (Olivares *et al*., 2017) and strawberry (Luo *et al*.,

2020). Moreover, this result confirms previous studies in which an ABA peak six days after harvest preceded the start of senescence in 'Muscat Hamburg' grapes (Sun *et al.*, 2010). To the best of our knowledge, this is the first study that quantifies ABA and ABA catabolites in the different sections of the grape berry. However, a limitation of this study is that, although a spatial distinction was done, there was no differentiation between berry skin and pulp. It has been proposed that ABA signalling varies with the tissue (Coelho *et al.*, 2019), and that ripening processes in the berry cell wall result in the formation of a dehiscence zone in the skin and softening of the pulp (McAtee *et al.*, 2013). Therefore, further studies evaluating hormonal content not only in the proximal and distal sections of the berry but also in the skin and pulp of these sections are recommended.

5.3 Impact to the industry

The fresh produce supply chain is more complex than other supply chains due to some specific factors such as the natural perishability of the crop and the climatic conditions during its development and ripening. It is estimated that 32 % of UK's fruit and vegetable imports is supplied from climate-vulnerable countries, like Spain, Egypt, South Africa, Chile, Morocco, Israel, and Peru (Scheelbeek *et al.*, 2020). At the same time, the economic situation in growing regions and global events, like the exit of the United Kingdom from the European Union (Brexit) and the recent pandemic produced by the SARS-CoV-2 virus, disrupt the supply chain and availability of fresh produce, resulting in demand and price variability (Ahumada & Villalobos, 2009; Shukla & Jharkaria, 2013).

Another global challenge is the reduction of food waste, since it has been calculated that 17 % of the global food produced is wasted (UNEP, 2021). The United Nations Environment Programme's Food Waste Index Report 2021 estimated that 61 % of this figure is produced in consumers' households, while 26 % and 13 % comes from food service and retail, respectively. Waste is defined by the EU Council Directive Waste 75/442/EEC [91/156/EEC] (EU, 1991) as 'any substance or object the holder discards, intends to discard, or is required to discard'. In order to tackle this problem, the UN Sustainable Development Goal (SDG) target 12.3 aims 'to halve per capita global food waste at the retail and

consumer level by 2030 and reduce food losses along production and supply chains, including postharvest losses' (UNEP, 2022). Typically, waste during the supply chain of fruit and vegetables occurs during food grading, transportation, storage, and marketing (Murthy *et al.*, 2009; Terry *et al*, 2011; Mena *et al.*, 2014). The main reason of waste is postharvest decay due to poor adherence to the required low temperature during all stages of the supply chain, but more commonly at the retail level. In addition, short or lack of shelf-life was determined as the main reason for waste by some retailers in the UK (Mena *et al.*, 2014).

According to Lancaster & Massingham (1993), consumer purchase decision follows a stimulus-reaction model mostly influenced by last experiences. Consumers expect a standard quality and safety of the food they purchase but, at the same time, these expectations are continually increasing (Terry *et al.*, 2011; Mena *et al.*, 2014) due to changes in eating habits and food safety awareness of the consumers (Shukla & Jharkaria, 2013). To meet these standards, it is necessary that producers and retailers perform an exhaustive quality control during the whole supply chain. Quality control enables the retailers to determine product specifications, which guarantees that the product satisfies consumer demands and, therefore, the success of the retailer and the supply chain (Terry *et al.*, 2011). Food quality assessment is the most time-consuming activity of the supply chain due to the different nature of the products that a supplier manages (Terry *et al.*, 2011). For example, Manikas & Terry (2009) determined that quality control in a distribution centre was significantly faster in citrus than in grapes because of the limited perishability of the latter. However, quality control is necessary and required by retailers, who expect the produce to meet agreed quality specifications. The determination of food specifications and attributes may lead to a well-informed consumer and, therefore, changes in the purchasing patterns (Poole & Baron, 1996). For instance, in the case of citrus fruits, some quality attributes such as juiciness, sweetness, and texture cannot be determined before consumption, and the purchase decision depends on the external appearance of the fruit (Poole & Baron, 1996). In the same way, grape producers should inform the consumer of grapes attributes and benefits to increase consumption and sales (Jianying *et al.*, 2014).

In order to face these adverse factors, supply a safe and nutritious produce, and satisfy retailer specifications and consumer expectations, the fresh produce industry requires more efficient management strategies and technologies that extend shelf-life without affecting the quality and nutrition of the produce.

Previous research has determined the biochemical profiles of citrus fruit (Masuda *et al.*, 2003; Abeysinghe *et al.*, 2007; Bermejo and Cano, 2012) and table grapes (Soyer *et al.*, 2003; Muñoz-Robredo *et al.*, 2011; Zhang, et al., 2015). Furthermore, other authors have studied the attributes determining consumer acceptability of grapes (Jianying *et al.*, 2014) and citrus fruit (Poole and Baron, 1996; Goldenberg *et al.*, 2015). However, little is known about the pre- and postharvest factors that influence the postharvest quality and senescence processes of clementines and table grapes. Moreover, many authors have stated the lack of research relating consumer preferences and product attributes (Poole & Baron, 1996; Terry *et al.*, 2011; Jianying *et al.*, 2014; Goldenberg *et al.*, 2015).

The study herein has determined how canopy position affects the postharvest quality, shelf-life, and senescence of 'Nadorcott' clementines, as well as how canopy position affects consumer preference. At the same time, strategies to extend the shelf-life of clementines and supply a more consistent quality have been identified. Similarly, it has been confirmed that the maintenance of a proper cold chain after harvest is crucial for extending the shelf-life of table grapes and reduce waste produced by mould infection. The use of 1-MCP on table grapes has been demonstrated to negatively affect the quality of table grapes at the end of postharvest life. Therefore, its use is not recommended here.

ABA has been confirmed to be a biomarker of postharvest senescence and shelf-life in both clementines and table grapes. It would be interesting for the industry to work alongside research organisations in developing non-destructive techniques that allow a rapid analysis of hormonal content. This would allow to determine the senescence stage of the produce at a specific moment during transportation or storage and make more accurate decisions to supply the best quality possible.

Following these recommendations would have a direct impact from the beginning to the end of the supply chain of citrus fruits and table grapes. Improved farming and harvesting programs as well as developing postharvest technologies that reduce fruit RR and delays senescence will offer a more consistent fruit quality, extending the postharvest storage and shelf-life of the produce. In turn, more consistent fruit quality not only will increase consumer satisfaction and reduce household waste and consumer complaints, but also increase purchase and intake of a more nutritious and healthier product, having a positive impact on consumers' health.

5.4 Conclusions

In summary, the overall conclusions of this study in relation to the objectives listed in Chapter 1, Section 1.2 are:

- Senescence processes in clementines and table grapes start after a significant increase in RR.
- ABA could be considered a biomarker of clementines and table grapes senescence since an ABA peak during postharvest storage preceded an increase in RR, mould incidence, organic acids, and sucrose hydrolysis, and a decrease in berry firmness.
- The crosstalk between ABA and sucrose in regulating the postharvest senescence of non-climacteric produce has been confirmed.
- ABA-GE was the predominant ABA catabolite at the end of the shelf-life of clementines and tables grapes, with a content ten times higher than that of ABA.
- 1-MCP delays the increase in grape berry RR and the hydrolysis of tartaric acid and sucrose during postharvest storage at 0.5 °C, but has a negative effect on mould incidence when storing grapes at 5.5 °C.
- Canopy position has a significant effect in the postharvest quality of 'Nadorcott' clementines, with shadowed fruit having a shorter shelf-life if harvested at the same time than sun-exposed fruit.

- Fruit from different canopy positions harvested in March had less differences in quality compared to fruit harvested in April, when quality was more inconsistent during postharvest storage.
- Fruit located on the upper inside canopy obtained the lowest consumer score for external appearance.

5.5 Recommendations for future work

Although clear findings have been identified, the results of this study were limited by time, resources, climatic conditions, and other factors. Therefore, further work is recommended to:

- Expand the knowledge about the role of ABA in regulating senescence processes in citrus fruit and table grape by carrying out molecular studies on the crosstalk between ABA and ethylene, and ABA and sucrose.
- Identify strategies to delay the increase in ABA during the postharvest storage of citrus fruits and table grapes. Preharvest practices focused on reducing abiotic stresses, like the use of shade nets, and postharvest treatments aiming to reduce fruit RR and sucrose hydrolysis could result in an extended fruit shelf-life and more consistent quality.
- Determine if grape berry respiration or the endogenous concentration of ABA in the grape pulp are the triggers of grape berry senescence. Further studies monitoring grape RR and hormonal content during both pre- and postharvest stages are needed in order to understand the lifecycle of the non-climacteric grape.
- Evaluate ethylene-removal technologies like ozone, low-temperature catalytic oxidation, and plasma catalysis to extend the shelf-life of table grapes.
- Evaluate hormonal content not only in the proximal and distal sections of the grape berry but also in the skin and pulp to understand berry senescence processes and develop mechanisms to delay mould growth.
- Evaluate the effect of shading nets on the quality of citrus fruit harvested from different canopy positions, in order to offer a more consistent quality at the end of the supply chain.

- Study the effect of canopy position in the consumer eating experience, in order to meet retailer specifications and consumer expectations.

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7 Appendices

7.1 Appendix A: Statistical tables for the experiment on 'Nadorcott' clementines

Table A.1. Univariate test of significance for juice content (%) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Juice % (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 4.3968				
	SS	Degr. of Freedom	MS	F	p
Intercept	218859.7	1	218859.7	11321.37	0.000000
Position	310.0	3	103.3	5.35	0.002385
Month	186.4	1	186.4	9.64	0.002830
Week	85.7	3	28.6	1.48	0.229119
Position*Month	127.5	3	42.5	2.20	0.096786
Position*Week	529.2	9	58.8	3.04	0.004330
Month*Week	101.8	3	33.9	1.76	0.164498
Position*Month*Week	420.9	9	46.8	2.42	0.019689
Error	1237.2	64	19.3		

Table A.2. Univariate test of significance for TSS (%) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for TSS (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.4554				
	SS	Degr. of Freedom	MS	F	p
Intercept	16247.41	1	16247.41	78340.09	0.000000
Position	13.82	3	4.61	22.22	0.000000
Month	2.63	1	2.63	12.70	0.000698
Week	28.50	3	9.50	45.81	0.000000
Position*Month	13.28	3	4.43	21.34	0.000000
Position*Week	1.95	9	0.22	1.04	0.417003
Month*Week	3.71	3	1.24	5.97	0.001184
Position*Month*Week	4.49	9	0.50	2.41	0.020273
Error	13.27	64	0.21		

Table A.3. Univariate test of significance for TA (%) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for TA (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.0554				
	SS	Degr. of Freedom	MS	F	p
Intercept	47.95284	1	47.95284	15651.95	0.000000
Position	0.14189	3	0.04730	15.44	0.000000
Month	0.06148	1	0.06148	20.07	0.000032
Week	0.07638	3	0.02546	8.31	0.000095
Position*Month	0.14135	3	0.04712	15.38	0.000000
Position*Week	0.02456	9	0.00273	0.89	0.538546
Month*Week	0.01610	3	0.00537	1.75	0.165305
Position*Month*Week	0.11615	9	0.01291	4.21	0.000261
Error	0.19608	64	0.00306		

Table A.4. Univariate test of significance for TSS:TA ratio of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for TSS:TA ratio (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 1.3285				
	SS	Degr. of Freedom	MS	F	p
Intercept	33407.91	1	33407.91	18929.08	0.000000
Position	43.56	3	14.52	8.23	0.000104
Month	23.20	1	23.20	13.15	0.000572
Week	165.23	3	55.08	31.21	0.000000
Position*Month	29.06	3	9.69	5.49	0.002032
Position*Week	14.57	9	1.62	0.92	0.516296
Month*Week	3.21	3	1.07	0.61	0.613639
Position*Month*Week	88.89	9	9.88	5.60	0.000011
Error	112.95	64	1.76		

Table A.5. Univariate test of significance for respiration rate (mL kg-1 h-1) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for RR (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 2.2282				
	SS	Degr. of Freedom	MS	F	p
Intercept	33134.89	1	33134.89	6673.722	0.000000
Position	206.67	3	68.89	13.875	0.000000
Month	8.81	1	8.81	1.774	0.187672
Week	6230.63	3	2076.88	418.305	0.000000
Position*Month	128.87	3	42.96	8.652	0.000067
Position*Week	522.79	9	58.09	11.700	0.000000
Month*Week	398.03	3	132.68	26.723	0.000000
Position*Month*Week	188.34	9	20.93	4.215	0.000260
Error	317.76	64	4.96		

Table A.6. Univariate test of significance for L* of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for L* (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.7543				
	SS	Degr. of Freedom	MS	F	p
Intercept	370748.8	1	370748.8	651545.7	0.000000
Position	11.2	3	3.7	6.6	0.000613
Month	7.8	1	7.8	13.7	0.000456
Week	11.7	3	3.9	6.9	0.000437
Position*Month	11.1	3	3.7	6.5	0.000669
Position*Week	4.1	9	0.5	0.8	0.622733
Month*Week	12.8	3	4.3	7.5	0.000226
Position*Month*Week	13.7	9	1.5	2.7	0.010481
Error	36.4	64	0.6		

Table A.7. Univariate test of significance for C* of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for C* (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.7189				
	SS	Degr. of Freedom	MS	F	p
Intercept	482905.2	1	482905.2	934369.5	0.000000
Position	18.4	3	6.1	11.9	0.000003
Month	60.2	1	60.2	116.4	0.000000
Week	90.8	3	30.3	58.5	0.000000
Position*Month	45.3	3	15.1	29.2	0.000000
Position*Week	10.1	9	1.1	2.2	0.036257
Month*Week	6.9	3	2.3	4.4	0.006777
Position*Month*Week	13.7	9	1.5	3.0	0.005345
Error	33.1	64	0.5		

Table A.8. Univariate test of significance for H angle of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for H angle (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 1.0983				
	SS	Degr. of Freedom	MS	F	p
Intercept	351228.5	1	351228.5	291177.9	0.000000
Position	51.2	3	17.1	14.2	0.000000
Month	93.5	1	93.5	77.5	0.000000
Week	17.0	3	5.7	4.7	0.005085
Position*Month	8.3	3	2.8	2.3	0.085970
Position*Week	16.3	9	1.8	1.5	0.165795
Month*Week	8.2	3	2.7	2.3	0.088024
Position*Month*Week	14.8	9	1.6	1.4	0.223655
Error	77.2	64	1.2		

Table A.9. Univariate test of significance for fructose (mg g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Fructose (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 9.0450				
	SS	Degr. of Freedom	MS	F	p
Intercept	2025857	1	2025857	24762.22	0.000000
Position	1588	3	529	6.47	0.000681
Month	5699	1	5699	69.66	0.000000
Week	4766	3	1589	19.42	0.000000
Position*Month	2194	3	731	8.94	0.000050
Position*Week	677	9	75	0.92	0.514179
Month*Week	1677	3	559	6.83	0.000458
Position*Month*Week	652	9	72	0.89	0.542935
Error	5236	64	82		

Table A.10. Univariate test of significance for glucose (mg g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Glucose (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 7.6619				
	SS	Degr. of Freedom	MS	F	p
Intercept	1256434	1	1256434	21402.48	0.000000
Position	2318	3	773	13.16	0.000001
Month	2048	1	2048	34.88	0.000000
Week	2052	3	684	11.65	0.000003
Position*Month	2685	3	895	15.24	0.000000
Position*Week	512	9	57	0.97	0.473898
Month*Week	1199	3	400	6.81	0.000471
Position*Month*Week	538	9	60	1.02	0.435854
Error	3757	64	59		

Table A.11. Univariate test of significance for sucrose (mg g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Sucrose (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 19.6876				
	SS	Degr. of Freedom	MS	F	p
Intercept	6677902	1	6677902	17228.71	0.000000
Position	671	3	224	0.58	0.632437
Month	20414	1	20414	52.67	0.000000
Week	4746	3	1582	4.08	0.010261
Position*Month	820	3	273	0.71	0.552449
Position*Week	3925	9	436	1.13	0.358425
Month*Week	4166	3	1389	3.58	0.018462
Position*Month*Week	3168	9	352	0.91	0.523764
Error	24807	64	388		

Table A.12. Univariate test of significance for citric acid (mg g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Citric acid (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 4.1073				
	SS	Degr. of Freedom	MS	F	p
Intercept	188638.1	1	188638.1	11181.90	0.000000
Position	320.8	3	106.9	6.34	0.000786
Month	4.5	1	4.5	0.27	0.606880
Week	191.9	3	64.0	3.79	0.014425
Position*Month	403.0	3	134.3	7.96	0.000137
Position*Week	250.8	9	27.9	1.65	0.119584
Month*Week	778.9	3	259.6	15.39	0.000000
Position*Month*Week	184.5	9	20.5	1.22	0.301624
Error	1079.7	64	16.9		

Table A.13. Univariate test of significance for ascorbic acid (mg g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for Ascorbic acid (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.1125				
	SS	Degr. of Freedom	MS	F	p
Intercept	183.2239	1	183.2239	14466.96	0.000000
Position	0.6510	3	0.2170	17.13	0.000000
Month	1.2357	1	1.2357	97.57	0.000000
Week	1.2463	3	0.4154	32.80	0.000000
Position*Month	0.0807	3	0.0269	2.13	0.105724
Position*Week	0.1986	9	0.0221	1.74	0.097572
Month*Week	0.2252	3	0.0751	5.93	0.001241
Position*Month*Week	0.1124	9	0.0125	0.99	0.460496
Error	0.8106	64	0.0127		

Table A.14. Univariate test of significance for DPA (ng g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for DPA (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.6786				
	SS	Degr. of Freedom	MS	F	p
Intercept	549.1814	1	549.1814	1192.610	0.000000
Position	5.9578	3	1.9859	4.313	0.007829
Month	1.9944	1	1.9944	4.331	0.041428
Week	2.9258	3	0.9753	2.118	0.106639
Position*Month	3.4450	3	1.1483	2.494	0.067874
Position*Week	3.9263	9	0.4363	0.947	0.491305
Month*Week	3.5343	3	1.1781	2.558	0.062800
Position*Month*Week	8.1833	9	0.9093	1.975	0.056948
Error	29.4712	64	0.4605		

Table A.15. Univariate test of significance for 7-OH-ABA (ng g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for 7-OH-ABA (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.2213				
	SS	Degr. of Freedom	MS	F	p
Intercept	59.32040	1	59.32040	1210.977	0.000000
Position	0.90268	3	0.30089	6.142	0.000976
Month	2.36698	1	2.36698	48.320	0.000000
Week	0.28008	3	0.09336	1.906	0.137527
Position*Month	0.62416	3	0.20805	4.247	0.008450
Position*Week	0.53357	9	0.05929	1.210	0.304545
Month*Week	0.16482	3	0.05494	1.122	0.347011
Position*Month*Week	0.70979	9	0.07887	1.610	0.131354
Error	3.13508	64	0.04899		

Table A.16. Univariate test of significance for ABA (ng g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for ABA (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.1427				
	SS	Degr. of Freedom	MS	F	p
Intercept	19.91202	1	19.91202	977.9697	0.000000
Position	0.15851	3	0.05284	2.5951	0.060094
Month	0.04649	1	0.04649	2.2834	0.135686
Week	1.20524	3	0.40175	19.7315	0.000000
Position*Month	0.27477	3	0.09159	4.4983	0.006309
Position*Week	0.15335	9	0.01704	0.8368	0.585122
Month*Week	0.32993	3	0.10998	5.4015	0.002240
Position*Month*Week	0.42673	9	0.04741	2.3287	0.024484
Error	1.30308	64	0.02036		

Table A.17. Univariate test of significance for ABA-GE (ng g-1 DW) of 'Nadorcott' clementines

Effect	Univariate Tests of Significance for ABA-GE (All data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 1.8000				
	SS	Degr. of Freedom	MS	F	p
Intercept	6273.904	1	6273.904	1936.296	0.000000
Position	68.689	3	22.896	7.066	0.000355
Month	24.851	1	24.851	7.670	0.007339
Week	4.909	3	1.636	0.505	0.680204
Position*Month	29.180	3	9.727	3.002	0.036890
Position*Week	71.236	9	7.915	2.443	0.018586
Month*Week	23.695	3	7.898	2.438	0.072610
Position*Month*Week	43.250	9	4.806	1.483	0.173424
Error	207.370	64	3.240		

7.2 Appendix B: Statistical tables for the experiment on ‘Krissy’ table grapes

Table B.1. Univariate test of significance for mould incidence (%) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Mould incidence(Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 3.1780				
	SS	Degr. of Freedom	MS	F	p
Intercept	528.0667	1	528.0667	52.28383	0.000000
Sampling Date	382.1000	4	95.5250	9.45792	0.000018
Temperature	224.2667	1	224.2667	22.20462	0.000029
Treatment	0.2667	1	0.2667	0.02640	0.871739
Sampling Date*Temperature	110.2333	4	27.5583	2.72855	0.042428
Sampling Date*Treatment	18.2333	4	4.5583	0.45132	0.770804
Temperature*Treatment	0.6000	1	0.6000	0.05941	0.808683
Sampling Date*Temperature*Treatment	30.2333	4	7.5583	0.74835	0.564956
Error	404.0000	40	10.1000		

Table B.2. Univariate test of significance for berry firmness (N) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Berry firmness (N) (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.3772				
	SS	Degr. of Freedom	MS	F	p
Intercept	847.3371	1	847.3371	5956.572	0.000000
Sampling Date	7.2591	4	1.8148	12.757	0.000001
Temperature	0.2664	1	0.2664	1.873	0.178806
Treatment	0.0012	1	0.0012	0.008	0.927954
Sampling Date*Temperature	0.4164	4	0.1041	0.732	0.575638
Sampling Date*Treatment	0.4492	4	0.1123	0.789	0.538961
Temperature*Treatment	0.2191	1	0.2191	1.540	0.221829
Sampling Date*Temperature*Treatment	0.3784	4	0.0946	0.665	0.620013
Error	5.6901	40	0.1423		

Table B.3. Univariate test of significance for L* of 'Krissy' table grapes

Effect	Univariate Tests of Significance for L* (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 1.1128				
	SS	Degr. of Freedom	MS	F	p
Intercept	47335.17	1	47335.17	38227.09	0.000000
Sampling Date	32.65	4	8.16	6.59	0.000358
Temperature	6.68	1	6.68	5.40	0.025365
Treatment	0.13	1	0.13	0.11	0.746874
Sampling Date*Temperature	6.89	4	1.72	1.39	0.254711
Sampling Date*Treatment	1.28	4	0.32	0.26	0.902358
Temperature*Treatment	0.05	1	0.05	0.04	0.845219
Sampling Date*Temperature*Treatment	0.69	4	0.17	0.14	0.966315
Error	49.53	40	1.24		

Table B.4. Univariate test of significance for C* of 'Krissy' table grapes

Effect	Univariate Tests of Significance for C* (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.9137				
	SS	Degr. of Freedom	MS	F	p
Intercept	5485.722	1	5485.722	6570.760	0.000000
Sampling Date	196.131	4	49.033	58.731	0.000000
Temperature	0.033	1	0.033	0.039	0.843838
Treatment	0.041	1	0.041	0.049	0.825257
Sampling Date*Temperature	3.965	4	0.991	1.187	0.331118
Sampling Date*Treatment	1.983	4	0.496	0.594	0.669068
Temperature*Treatment	0.409	1	0.409	0.490	0.488040
Sampling Date*Temperature*Treatment	1.716	4	0.429	0.514	0.725792
Error	33.395	40	0.835		

Table B.5. Univariate test of significance for H angle of 'Krissy' table grapes

Effect	Univariate Tests of Significance for H angle (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 2.2285				
	SS	Degr. of Freedom	MS	F	p
Intercept	25821.99	1	25821.99	5199.711	0.000000
Sampling Date	4128.00	4	1032.00	207.812	0.000000
Temperature	1.42	1	1.42	0.285	0.596236
Treatment	0.58	1	0.58	0.117	0.734293
Sampling Date*Temperature	1.84	4	0.46	0.093	0.984230
Sampling Date*Treatment	18.51	4	4.63	0.932	0.455201
Temperature*Treatment	0.23	1	0.23	0.046	0.830830
Sampling Date*Temperature*Treatment	25.90	4	6.47	1.304	0.285048
Error	198.64	40	4.97		

Table B.6. Univariate test of significance for respiration rate (mL kg-1 h-1) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for RR (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.2579				
	SS	Degr. of Freedom	MS	F	p
Intercept	212.5816	1	212.5816	3196.289	0.000000
Sampling Date	31.9796	4	7.9949	120.208	0.000000
Temperature	20.1231	1	20.1231	302.563	0.000000
Treatment	0.4198	1	0.4198	6.313	0.016123
Sampling Date*Temperature	28.4468	4	7.1117	106.929	0.000000
Sampling Date*Treatment	1.1091	4	0.2773	4.169	0.006473
Temperature*Treatment	0.4517	1	0.4517	6.792	0.012801
Sampling Date*Temperature*Treatment	0.2494	4	0.0623	0.937	0.452204
Error	2.6604	40	0.0665		

Table B.7. Univariate test of significance for TSS (%) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for TSS (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.8010				
	SS	Degr. of Freedom	MS	F	p
Intercept	28553.65	1	28553.65	44499.20	0.000000
Sampling Date	37.00	4	9.25	14.42	0.000000
Temperature	0.16	1	0.16	0.25	0.620087
Treatment	1.70	1	1.70	2.65	0.111428
Sampling Date*Temperature	5.73	4	1.43	2.23	0.082826
Sampling Date*Treatment	1.26	4	0.31	0.49	0.742640
Temperature*Treatment	0.70	1	0.70	1.10	0.301126
Sampling Date*Temperature*Treatment	1.80	4	0.45	0.70	0.595245
Error	25.67	40	0.64		

Table B.8. Univariate test of significance for TA (%) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for TA (Grapes Physiology_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 0.0398				
	SS	Degr. of Freedom	MS	F	p
Intercept	18.48267	1	18.48267	11673.55	0.000000
Sampling Date	0.06300	4	0.01575	9.95	0.000011
Temperature	0.00903	1	0.00903	5.70	0.021747
Treatment	0.00008	1	0.00008	0.05	0.825128
Sampling Date*Temperature	0.00427	4	0.00107	0.67	0.613820
Sampling Date*Treatment	0.00524	4	0.00131	0.83	0.515850
Temperature*Treatment	0.00107	1	0.00107	0.68	0.415958
Sampling Date*Temperature*Treatment	0.00596	4	0.00149	0.94	0.450433
Error	0.06333	40	0.00158		

Table B.9. Univariate test of significance for fructose (mg g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Fructose (Grapes Biochemistry_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 5.3375				
	SS	Degr. of Freedom	MS	F	p
Intercept	7472332	1	7472332	262285.2	0.000000
{1}Date	1107	4	277	9.7	0.000002
{2}Temperature	337	1	337	11.8	0.000964
{3}Treatment	1	1	1	0.0	0.830071
{4}Section	20	1	20	0.7	0.405165
Date*Temperature	115	4	29	1.0	0.410544
Date*Treatment	189	4	47	1.7	0.167794
Temperature*Treatment	45	1	45	1.6	0.213861
Date*Section	40	4	10	0.3	0.844564
Temperature*Section	1	1	1	0.0	0.879740
Treatment*Section	19	1	19	0.7	0.413441
Date*Temperature*Treatment	137	4	34	1.2	0.316962
Date*Temperature*Section	222	4	55	1.9	0.111666
Date*Treatment*Section	72	4	18	0.6	0.639225
Temperature*Treatment*Section	28	1	28	1.0	0.324608
Date*Temperature*Treatment*Section	145	4	36	1.3	0.288791
Error	2108	74	28		

Table B.10. Univariate test of significance for glucose (mg g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Glucose (Grapes Biochemistry_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 5.2984				
	SS	Degr. of Freedom	MS	F	p
Intercept	6892545	1	6892545	245522.8	0.000000
{1}Date	371	4	93	3.3	0.015093
{2}Temperature	239	1	239	8.5	0.004674
{3}Treatment	0	1	0	0.0	0.973725

{4}Section	3	1	3	0.1	0.734836
Date*Temperature	262	4	65	2.3	0.063724
Date*Treatment	174	4	43	1.5	0.197340
Temperature*Treatment	0	1	0	0.0	0.971267
Date*Section	279	4	70	2.5	0.051074
Temperature*Section	0	1	0	0.0	0.908071
Treatment*Section	0	1	0	0.0	0.915141
Date*Temperature*Treatment	276	4	69	2.5	0.053016
Date*Temperature*Section	54	4	13	0.5	0.750489
Date*Treatment*Section	77	4	19	0.7	0.606895
Temperature*Treatment*Section	78	1	78	2.8	0.100670
Date*Temperature*Treatment*Section	66	4	17	0.6	0.669724
Error	2077	74	28		

Table B.11. Univariate test of significance for sucrose (mg g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Sucrose (Grapes Biochemistry_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 2.6205				
	SS	Degr. of Freedom	MS	F	p
Intercept	55774.77	1	55774.77	8121.904	0.000000
{1}Date	490.30	4	122.58	17.849	0.000000
{2}Temperature	181.09	1	181.09	26.370	0.000002
{3}Treatment	3.42	1	3.42	0.498	0.482397
{4}Section	20.17	1	20.17	2.937	0.090631
Date*Temperature	64.04	4	16.01	2.331	0.063383
Date*Treatment	44.82	4	11.20	1.632	0.174991
Temperature*Treatment	11.99	1	11.99	1.746	0.190401
Date*Section	65.28	4	16.32	2.377	0.059299
Temperature*Section	0.38	1	0.38	0.056	0.813771
Treatment*Section	1.11	1	1.11	0.162	0.688505

Date*Temperature*Treatment	38.78	4	9.70	1.412	0.238143
Date*Temperature*Section	8.72	4	2.18	0.317	0.865540
Date*Treatment*Section	79.46	4	19.87	2.893	0.027593
Temperature*Treatment*Section	1.37	1	1.37	0.199	0.656533
Date*Temperature*Treatment*Section	14.33	4	3.58	0.522	0.720137
Error	521.91	76	6.87		

Table B.12. Univariate test of significance for tartaric acid (mg g-1 DW) of 'Krissy' table grapes

	Univariate Tests of Significance for Tartaric acid (Grapes Biochemistry_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 3.0834				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	105806.4	1	105806.4	11129.16	0.000000
{1}Date	6484.2	4	1621.1	170.51	0.000000
{2}Temperature	208.5	1	208.5	21.93	0.000014
{3}Treatment	9.0	1	9.0	0.94	0.334758
{4}Section	766.0	1	766.0	80.57	0.000000
Date*Temperature	975.3	4	243.8	25.65	0.000000
Date*Treatment	14.0	4	3.5	0.37	0.830741
Temperature*Treatment	0.0	1	0.0	0.00	0.990023
Date*Section	775.4	4	193.8	20.39	0.000000
Temperature*Section	18.0	1	18.0	1.89	0.173442
Treatment*Section	24.6	1	24.6	2.58	0.112639
Date*Temperature*Treatment	72.5	4	18.1	1.91	0.119509
Date*Temperature*Section	114.9	4	28.7	3.02	0.023567
Date*Treatment*Section	75.6	4	18.9	1.99	0.106274
Temperature*Treatment*Section	6.4	1	6.4	0.68	0.413759
Date*Temperature*Treatment*Section	83.9	4	21.0	2.21	0.077308

Error	646.5	68	9.5		

Table B.13. Univariate test of significance for malic acid (mg g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for Malic acid (Grapes Biochemistry_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 2.5498				
	SS	Degr. of Freedom	MS	F	p
Intercept	17658.61	1	17658.61	2716.056	0.000000
{1}Date	4018.08	4	1004.52	154.505	0.000000
{2}Temperature	92.70	1	92.70	14.258	0.000337
{3}Treatment	0.01	1	0.01	0.002	0.964383
{4}Section	483.32	1	483.32	74.338	0.000000
Date*Temperature	376.35	4	94.09	14.471	0.000000
Date*Treatment	16.44	4	4.11	0.632	0.641232
Temperature*Treatment	3.47	1	3.47	0.534	0.467297
Date*Section	29.26	4	7.31	1.125	0.352063
Temperature*Section	0.69	1	0.69	0.106	0.745700
Treatment*Section	10.51	1	10.51	1.617	0.207823
Date*Temperature*Treatment	5.37	4	1.34	0.206	0.934015
Date*Temperature*Section	5.01	4	1.25	0.193	0.941480
Date*Treatment*Section	78.93	4	19.73	3.035	0.023078
Temperature*Treatment*Section	23.21	1	23.21	3.571	0.063075
Date*Temperature*Treatment*Section	71.99	4	18.00	2.768	0.034116
Error	442.11	68	6.50		

Table B.14. Univariate test of significance for DPA (ng g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for DPA (Grapes PGRs_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 17.2394				
	SS	Degr. of Freedom	MS	F	p
Intercept	105128.3	1	105128.3	353.7345	0.000000
{1}Date	7586.5	4	1896.6	6.3817	0.000198
{2}Temperature	333.8	1	333.8	1.1232	0.292930
{3}Treatment	131.9	1	131.9	0.4437	0.507557
{4}Section	1143.3	1	1143.3	3.8469	0.053876
Date*Temperature	1039.8	4	259.9	0.8746	0.483731
Date*Treatment	1628.6	4	407.1	1.3700	0.253369
Temperature*Treatment	84.6	1	84.6	0.2845	0.595449
Date*Section	2266.0	4	566.5	1.9062	0.119199
Temperature*Section	301.2	1	301.2	1.0134	0.317601
Treatment*Section	6.1	1	6.1	0.0206	0.886328
Date*Temperature*Treatment	245.3	4	61.3	0.2063	0.934084
Date*Temperature*Section	1479.0	4	369.8	1.2441	0.300472
Date*Treatment*Section	1555.4	4	388.9	1.3084	0.275513
Temperature*Treatment*Section	128.5	1	128.5	0.4325	0.512935
Date*Temperature*Treatment*Section	3161.6	4	790.4	2.6595	0.039856
Error	20506.5	69	297.2		

Table B.15. Univariate test of significance for 7-OH-ABA (ng g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for 7-OH-ABA (Grapes PGRs_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 7.5077				
	SS	Degr. of Freedom	MS	F	p
Intercept	38645.82	1	38645.82	685.6254	0.000000
{1}Date	571.70	4	142.92	2.5357	0.046875
{2}Temperature	15.77	1	15.77	0.2798	0.598400
{3}Treatment	0.59	1	0.59	0.0104	0.919033

{4}Section	4471.60	1	4471.60	79.3318	0.000000
Date*Temperature	177.22	4	44.30	0.7860	0.537797
Date*Treatment	79.45	4	19.86	0.3524	0.841617
Temperature*Treatment	7.82	1	7.82	0.1388	0.710508
Date*Section	335.98	4	83.99	1.4902	0.213557
Temperature*Section	14.87	1	14.87	0.2639	0.608958
Treatment*Section	2.69	1	2.69	0.0478	0.827548
Date*Temperature*Treatment	112.31	4	28.08	0.4981	0.737138
Date*Temperature*Section	72.60	4	18.15	0.3220	0.862396
Date*Treatment*Section	101.76	4	25.44	0.4513	0.771121
Temperature*Treatment*Section	35.35	1	35.35	0.6272	0.430843
Date*Temperature*Treatment*Section	154.43	4	38.61	0.6849	0.604563
Error	4283.80	76	56.37		

Table B.16. Univariate test of significance for ABA (ng g-1 DW) of 'Krissy' table grapes

Effect	Univariate Tests of Significance for ABA (Grapes PGRs_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 84.7581				
	SS	Degr. of Freedom	MS	F	p
Intercept	2252356	1	2252356	313.5270	0.000000
{1}Date	305449	4	76362	10.6296	0.000001
{2}Temperature	35889	1	35889	4.9958	0.028347
{3}Treatment	29	1	29	0.0040	0.949716
{4}Section	246049	1	246049	34.2499	0.000000
Date*Temperature	41228	4	10307	1.4347	0.230703
Date*Treatment	70412	4	17603	2.4503	0.053181
Temperature*Treatment	2343	1	2343	0.3261	0.569655
Date*Section	141750	4	35438	4.9329	0.001371
Temperature*Section	15352	1	15352	2.1370	0.147908
Treatment*Section	213	1	213	0.0296	0.863865

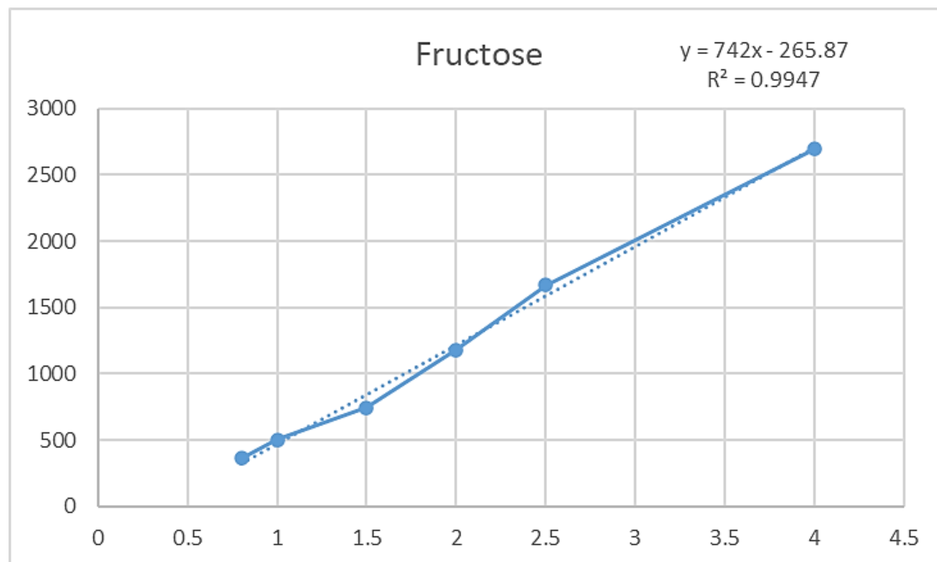
Date*Temperature*Treatment	46759	4	11690	1.6272	0.176080
Date*Temperature*Section	44060	4	11015	1.5333	0.201034
Date*Treatment*Section	97628	4	24407	3.3975	0.013041
Temperature*Treatment*Section	14150	1	14150	1.9697	0.164553
Date*Temperature*Treatment*Section	13395	4	3349	0.4661	0.760367
Error	545979	76	7184		

Table B.17. Univariate test of significance for ABA-GE (ng g-1 DW) of 'Krissy' table grapes

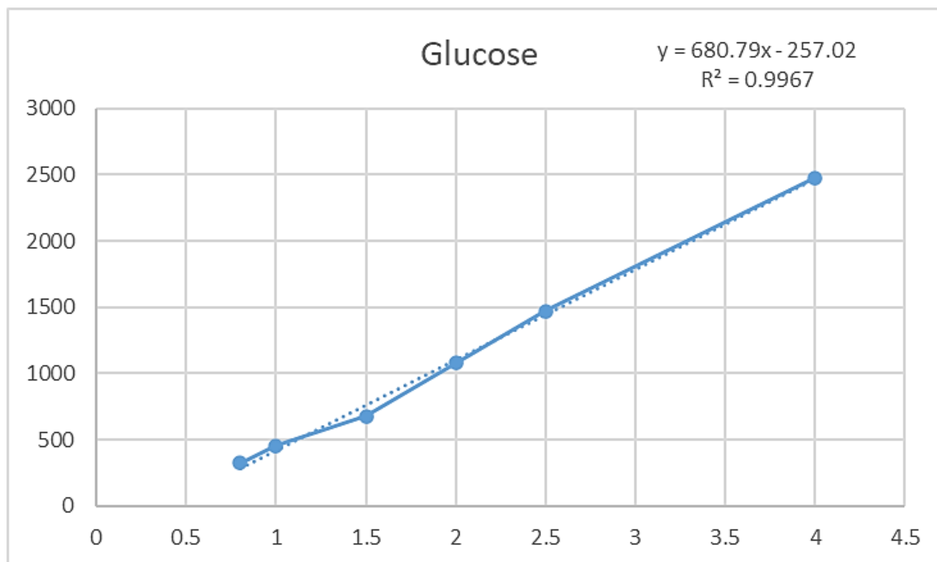
	Univariate Tests of Significance for ABA-GE (Grapes PGRs_Data) Sigma-restricted parameterization Effective hypothesis decomposition; Std. Error of Estimate: 1073.4832				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	491809908	1	491809908	426.7826	0.000000
{1}Date	40285166	4	10071291	8.7397	0.000008
{2}Temperature	465164	1	465164	0.4037	0.527139
{3}Treatment	258577	1	258577	0.2244	0.637093
{4}Section	21490905	1	21490905	18.6494	0.000047
Date*Temperature	13181398	4	3295349	2.8596	0.029086
Date*Treatment	7941816	4	1985454	1.7229	0.153800
Temperature*Treatment	154674	1	154674	0.1342	0.715125
Date*Section	21264685	4	5316171	4.6133	0.002198
Temperature*Section	1427805	1	1427805	1.2390	0.269215
Treatment*Section	1721955	1	1721955	1.4943	0.225382
Date*Temperature*Treatment	11148858	4	2787215	2.4187	0.055852
Date*Temperature*Section	2101247	4	525312	0.4559	0.767828
Date*Treatment*Section	2490878	4	622719	0.5404	0.706518
Temperature*Treatment*Section	636093	1	636093	0.5520	0.459827
Date*Temperature*Treatment*Section	4464890	4	1116223	0.9686	0.429839
Error	86427466	75	1152366		

7.3 Appendix C: Calibration curves

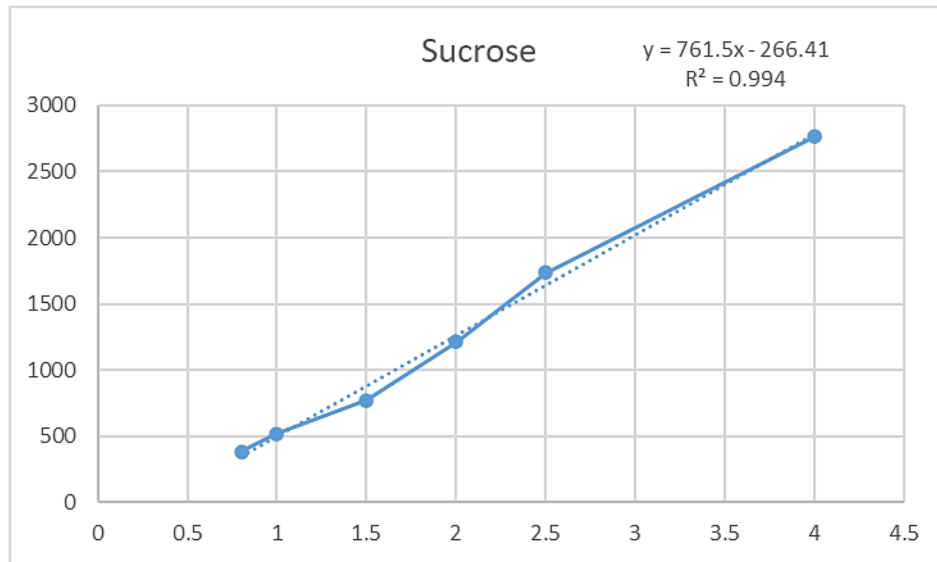
Calibration curve for fructose:



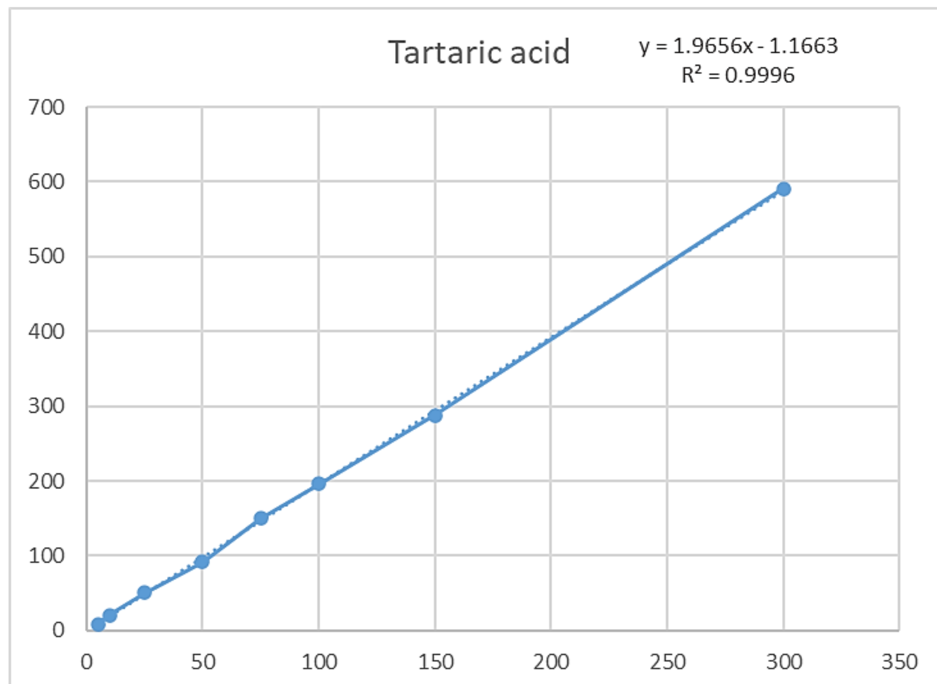
Calibration curve for glucose:



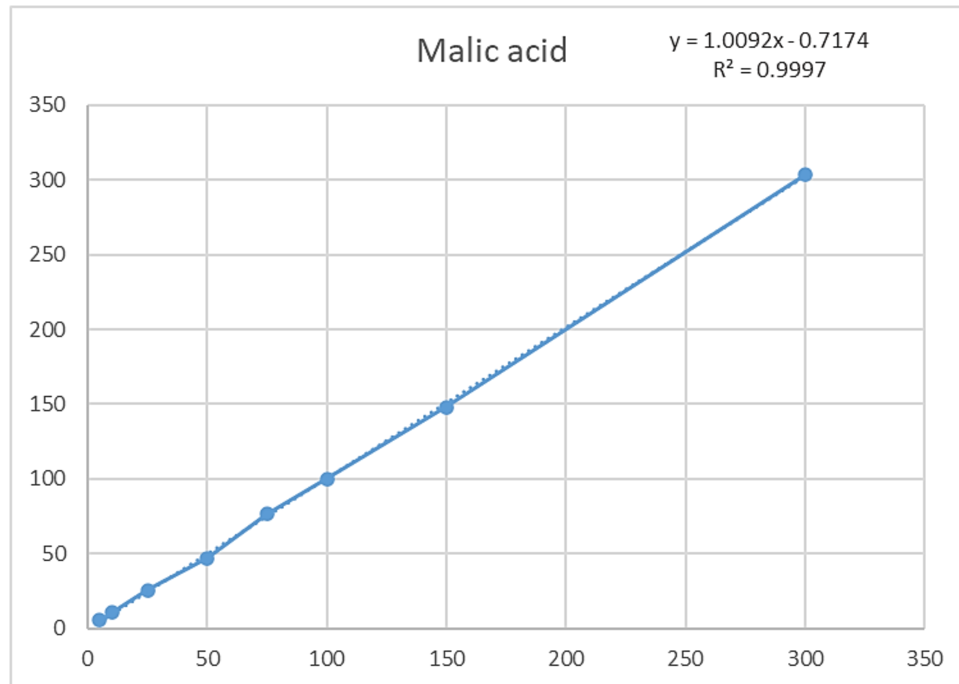
Calibration curve for sucrose:



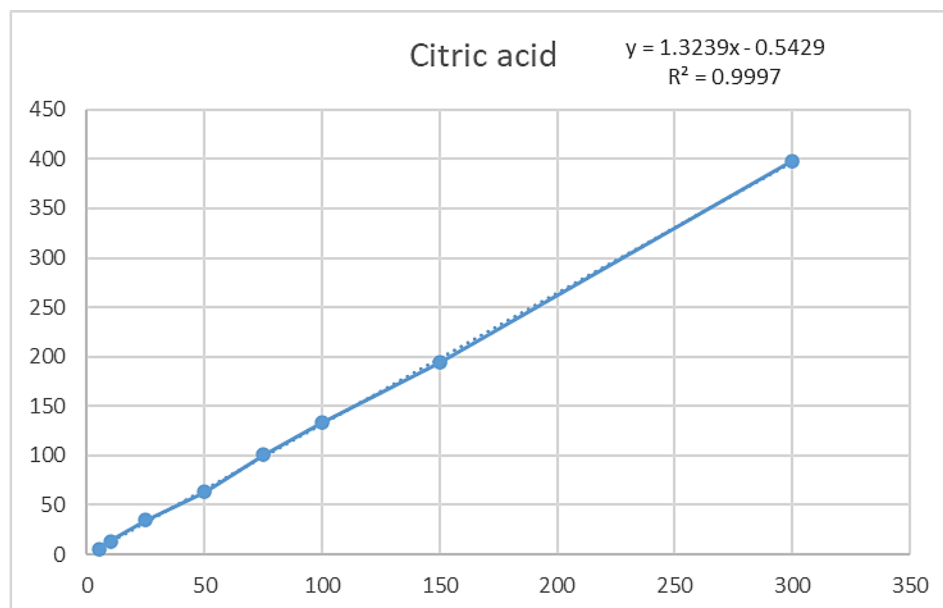
Calibration curve for tartaric acid:



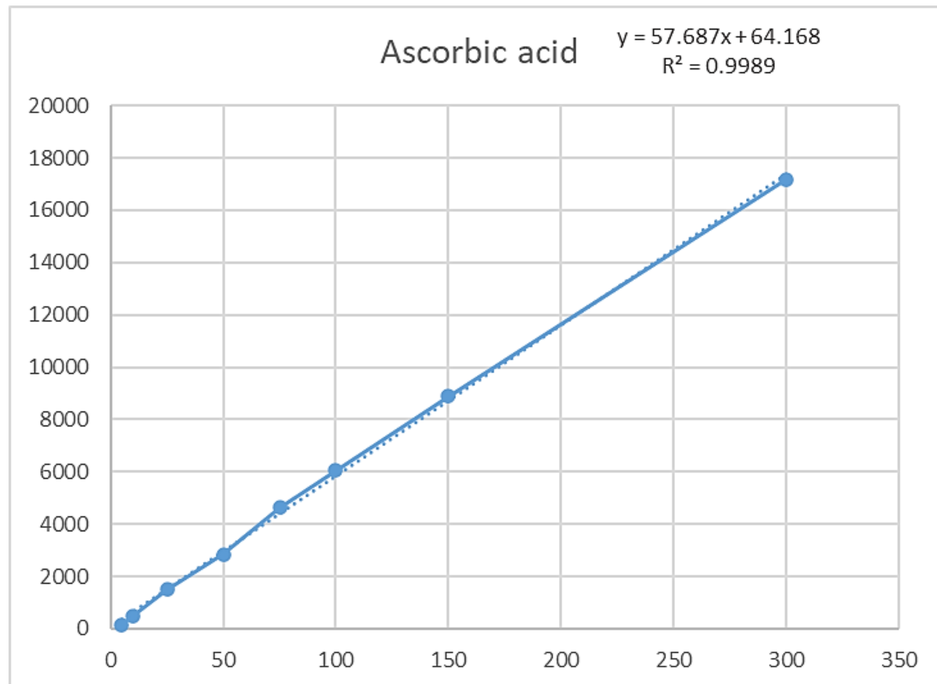
Calibration curve for malic acid:



Calibration curve for citric acid:



Calibration curve for ascorbic acid:



7.4 Appendix D: Meteorologic data for Lora del Rio, Seville, Spain on the months preceding the mandarins harvests

Date	Julian date	Rain (mm)	T max (°C)	T min (°C)	T med (°C)	RH max (%)	RH min (%)	RH med (%)
10 Feb - 10 Mar	41 - 69	3.5	18.97	7.53	13.25	97.69	52.52	75.1
11 Mar - 7 Apr	70 - 97	1.14	22.44	7.41	14.92	93.57	32.21	62.89

Date	Julian date	Avg wind speed (m/s)	Wind direction (0-360)	Solar radiation (MJ m-2 day)	Reference Evapotranspiration (mm/day)
10 Feb - 10 Mar	41 - 69	1.37	138	9.69	1.86
11 Mar - 7 Apr	70 - 97	1.47	147	16.34	3.23