Programa de Doctorado en Ciencia,

Tecnología y Gestión Alimentaria (RD 99/2011)

Effect of rootstock on the fruit quality of mandarins ‘Clemenules’ and ‘Tango’, and blood oranges ‘Tarocco Rosso’ and ‘Moro’

PhD Thesis

Presented by:
Julia Morales Alfaro

Thesis supervisors:
Ph.D. Alejandra Salvador Pérez
Ph.D. Almudena Bermejo del Castillo

Academic tutor:
Ph.D. Isabel Hernando Hernando

Valencia, November 2020

Lo mejor para emprender una búsqueda,

la compañía de un dragón

Michael Ende

Agradecimientos

Escribir estas líneas es darme cuenta de que una etapa importante está finalizando. Un largo camino que ha sido mi realidad estos últimos años. Hay trayectos difíciles de recorrer sin compañía y apoyo y, éste, no ha sido una excepción.

Parto del contexto en qué esta tesis no es fruto de ninguna beca doctoral si no del interés en realizarla gracias al disfrute de la beca concedida por el Fondo Social Europeo para la formación de tecnólogos de alimentos en el Instituto Valenciano de Investigaciones Agrarias. Es por ello que esta tesis ha sido llevada a cabo gracias a que un gran número de personas han estado a mi lado, y con su esfuerzo, le han dado forma y fondo a cada una de las partes.

Uno de mis principales pilares ha sido Alejandra Salvador: desde el primer momento que le mostré mi interés por realizar la tesis confió en mí y en el proyecto, orientando mis pasos hasta el último día.

También desde el inicio de este camino, Almudena Bermejo desempeñó un papel fundamental. A ella le debo todo lo que sé acerca de los biocompuestos y agradezco enormemente el vínculo creado tras estos años entre nosotras. Con ella compartí mis primeros años en el Departamento de Citricultura del IVIA, junto con Lidia y Amparo. Pasé inmejorables momentos con ellas tres y con el resto del equipo: Ángeles, Belén, Mary Rus, Carmen, Teresa, Charo, Ramón y Javi. Igualmente, me gustaría hacer especial mención a María Ángeles Forner, quien nos propuso la temática de esta Tesis, lo que nos permitió enfocar la calidad físico-química y nutricional de los cítricos y la repercusión que tenía el patrón sobre ésta.

Dentro del Departamento de Postcosecha, al cual considero como mi propia familia, además de Alejandra he tenido siempre otro apoyo básico en Pilar Navarro. Pilar siempre sabe escuchar y siempre nos arroja luz cuando las dificultades se presentan. Además de ellas, me han ayudado a crecer tanto dentro como fuera del laboratorio el resto de compañeros: Cristina, Rebeca, Juani, Carmen, Begoña, Ayoub, Mari Cruz, Lluís y Berna.

También quiero expresar mi más sentido agradecimiento a Ana Quiñones la cual, y de manera altruista, ha desempeñado un papel fundamental para que esta Tesis este hoy llegando a buen puerto. Junto a ella tuve la oportunidad de trabajar estrechamente con el Centro para el Desarrollo de Agricultura Sostenible, donde conocí a un equipo humano increíble: Isabel, Merche, Pepe, Rodolfo, Carmen, Irene, Piedad, María Ángeles y José Miguel.

No puedo terminar sin agradecer la colaboración de Anecoop S. Coop. y Frutaria Agricultura S.L. las cuales nos han cedido la mayoría del material vegetal necesario para llevar a cabo los ensayos reflejados en esta Tesis.

Finalmente, para llevar a cabo una Tesis es casi tan importante el ámbito académico como el personal. Por suerte he contado con el apoyo de mi familia y amigos y más especialmente con mi compañero de vida. Es por ello que esta tesis se la agradezco a Dani y a los que están a punto de llegar a desordenar nuestra vida un poco más, Julián y Teresa.

Valenica, 25 de octubre de 2020

ABSTRACT

Citriculture constantly faces changing environmental scenarios that cause different biotic and abiotic stress that can make production difficult or might affect fruit quality. The rootstock onto which a specific variety is grafted is an important tool to help to improve its agronomic adaptability to each crop area.

The present Thesis was carried out to study the effect of rootstock on physico-chemical and nutritional fruit quality in some varieties of much commercial interest today: ‘Clemenules’ and ‘Tango’ mandarins, and ‘Tarocco Rosso’ and ‘Moro’ blood oranges.

In ‘Clemenules’, the most representative mandarin variety in the Mediterranean Region, the fruit of the trees grafted into eight rootstocks at three harvest times was evaluated by performing studies during two seasons. Of the evaluated rootstocks, Forner-Alcaide 13 and C-35 Citrange stood out for their earlier color change, which is very interesting for this variety, in which early harvesting is a relevant aspect from the commercial point of view. Forner-Alcaide V17 also stood out for maintaining optimum acidity levels until the season ended, and also presented the highest contents in vitamin C, flavonoids, glucose and fructose. Carrizo Citrange brought about high concentrations of sucrose and vitamin C in fruit.

‘Tango’ is a mandarin variety that has been recently introduced into the Mediterranean Region. Its harvest time is very interesting because it starts when that of clementines ends. The present Thesis studies changes in the physico-chemical, nutritional and sensorial quality of the ‘Tango’ mandarin grafted onto two rootstocks (Carrizo Citrange and Forner-Alcaide 5) during the harvest period in the two main production areas in Andalusia (S Spain). The results revealed that fruit quality during harvest was influenced by the location of orchards, which was particularly related to soil texture composition. However, in both areas, Forner-Alcaide 5 was the rootstock that induced higher acidity content, and more total soluble solids, sucrose, vitamin C and citric acid in fruit. The physico-chemical determinations, along with the sensorial evaluation, allowed the optimum harvest time to be established depending on the different studied conditions.

This Thesis also includes a study about this variety's postharvest behavior as no data are available for our crop conditions. The 'Tango' mandarin presented outer chilling injury symptoms after being stored for 20 days at 1°C and 5°C. A microstructural study was done to characterize the alteration caused by low temperatures. The Forner-Alcaide 5 rootstock fruit showed a lower chilling injury incidence. Storage at 9°C did not compromise this variety's external or internal quality.

Among oranges, demand for blood oranges to be eaten fresh has grown in recent years, basically due to their high content in anthocyanins and their positive effect for human health. To assess the effect that rootstock had on blood oranges, two reference varieties were taken, 'Moro' and 'Tarocco Rosso', grafted onto eight rootstocks. Internal quality was strongly influenced by harvest time, which was more evident for 'Moro'. In both varieties, rootstock affected changes in the quality parameter studied during maturity. In 'Moro', juice color faded as anthocyanins degraded, and rootstocks C-35 Citrange, Macrophylla and Volkameriana showed the most marked reduction. Such anthocyanin degradation was related to the change in temperature that took place during the harvest period. In 'Tarocco Rosso', anthocyanins did not undergo degradation, which suggests that this variety is less sensitive to changes in ambient temperature. In this variety, rootstocks Forner-Alcaide 5 and Forner-Alcaide 13 gave fruit with a higher content of anthocyanins and sugars.

This Thesis also includes a study of the suitability of refrigerated conservation of two blood orange varieties: 'Tarocco Rosso' and 'Sanguinelli'. Although storage at any tested temperature (1°C, 5°C and 9°C) did not affect internal quality for 45 days, fruit displayed chilling injury symptoms at 1°C, with a higher incidence for 'Sanguinelli' than for 'Tarocco Rosso'. Both varieties can be stored between 5°C and 9°C for 30 days for 'Sanguinelli' and for up to 45 days with 'Tarocco Rosso'.

RESUMEN

La citricultura constantemente se enfrenta a escenarios ambientales cambiantes que provocan diferentes estreses bióticos y abióticos que pueden dificultar la producción o afectar a la calidad de los frutos. El patrón sobre el cual se injerta una variedad específica es una importante herramienta para mejorar su adaptabilidad agronómica en cada área de cultivo.

En la presente Tesis se ha llevado a cabo el estudio del efecto del patrón sobre la calidad físico-química y nutricional de la fruta en algunas de las variedades de mayor interés comercial en la actualidad, mandarinas ‘Clemenules’ y ‘Tango’ y, naranjas sanguinas ‘Tarocco Rosso’ y ‘Moro’.

En ‘Clemenules’, variedad de mandarina más representativa en el área mediterránea, se llevó a cabo la evaluación de la calidad de la fruta de árboles injertados sobre ocho patrones en tres momentos de cosecha, realizando los estudios en dos campañas. Entre los patrones evaluados, Forner-Alcaide 13 y C-35 destacaron por adelantar el cambio de color, lo que es de gran interés desde el punto de vista comercial. Por otra parte, Forner-Alcaide V17 destacó por mantener niveles óptimos de acidez hasta el final de la campaña y además también presentó el mayor contenido en vitamina C, flavonoides, glucosa y fructosa. Carrizo Citrange también indujo altas concentraciones de sacarosa y vitamina C en la fruta.

‘Tango’ es una mandarina de reciente introducción en el área mediterránea, con gran interés por su periodo de recolección, que comienza cuando finaliza el de las clementinas. En esta Tesis se abordó el estudio de los cambios en la calidad fisicoquímica, nutricional y sensorial de la mandarina ‘Tango’ injertada sobre dos patrones (Carrizo Citrange y Forner-Alcaide 5) durante el periodo de cosecha en las dos áreas principales de producción de Andalucía. Los resultados revelaron que la calidad de la fruta se vio influenciada por la localización de las parcelas, lo que se relacionó sobre todo con la composición de la textura del suelo. En ambas localizaciones, Forner-Alcaide 5 fue el patrón que indujo mayor contenido en acidez, sólidos solubles totales, sacarosa, vitamina C y ácido cítrico en la fruta. Las determinaciones físico-químicas, junto

con la evaluación sensorial permitieron establecer el momento óptimo de recolección dependiendo de las diferentes condiciones estudiadas.

También se ha incluido un estudio del comportamiento postcosecha de esta variedad, ya que no existían datos en nuestras condiciones de cultivo. La mandarina 'Tango' presentó síntomas externos de daños por frío a partir de los 20 días almacenada a 1°C y 5°C. Se realizó un estudio micro-estructural para caracterizar la alteración provocada por las bajas temperaturas. Los frutos del patrón Forner-Alcaide 5 presentaron una menor incidencia de los daños por frío. El almacenamiento a 9°C no comprometió la calidad externa o interna de esta variedad.

Dentro del grupo de naranjas, en los últimos años existe una creciente demanda por las naranjas sanguinas para su consumo en fresco. Para evaluar el efecto del patrón sobre naranjas sanguinas se tomaron dos variedades de referencias, 'Moro' y 'Tarocco Rosso' injertadas sobre ocho patrones. La calidad interna se vio influenciada por el momento de cosecha, lo que fue más evidente en la variedad 'Moro'. En ambas variedades el patrón afectó a los cambios en los parámetros de calidad estudiados durante la maduración. En 'Moro', se observó una reducción en el color del zumo debido a la degradación de las antocianinas, siendo los patrones C-35, Macrophylla y Volkameriana, los que mostraron un mayor descenso. Esta degradación de antocianinas se relacionó con el cambio de la temperatura experimentada durante el periodo de recolección. En 'Tarocco Rosso' no se evidenció una degradación de antocianinas, lo que sugiere que esta variedad es menos sensible a los cambios de temperatura ambiental. En esta variedad los patrones Forner-Alcaide 5 y Forner-Alcaide 13 produjeron la fruta con mayor contenido en anticianinas y azúcares.

Además se llevó a cabo el estudio de la aptitud a la frigoconservación de dos variedades de sanguinas, en este caso 'Tarocco Rosso' y 'Sanguinelli'. Mientras que la calidad interna no se vio afectada por el almacenamiento a ninguna de las temperaturas ensayadas (1°C, 5°C y 9°C) durante 45 días, la fruta presentó síntomas de daños por frío a 1°C. 'Sanguinelli' presentó mayor incidencia que 'Tarocco Rosso'. Ambas variedades se pueden almacenar entre 5 y 9°C durante 30 días en el caso de 'Sanguinelli' y hasta 45 en el caso de 'Tarocco Rosso'.

RESUM

La citricultura s'enfronta constantment a escenaris ambientals canviants que provoquen diferents estressos biòtics i abiòtics que poden dificultar la producció o afectar la qualitat dels fruits. El patró sobre el qual s'empelta una varietat específica és una important eina per a millorar la seua adaptabilitat agronòmica en cada àrea de cultiu.

En la present Tesi s'ha dut a terme l'estudi de l'efecte del patró sobre la qualitat físic-química i nutricional de la fruita en algunes de les varietats de major interés comercial en l'actualitat, mandarines ‘Clemenules’ i ‘Tango’ i, taronges sanguines ‘Tarocco Rosso’ i ‘Moro’.

En ‘Clemenules’, varietat de mandarina més representativa en l'àrea mediterrània, es va dur a terme l'avaluació de la qualitat de la fruita d'arbres empeltats sobre huit patrons en tres moments de collita, realitzant els estudis en dues campanyes. Entre els patrons avaluats, Forner-Alcaide 13 i C-35 van destacar per avançar el canvi de color, la qual cosa és de gran interés des del punt de vista comercial. D'altra banda Forner-Alcaide V17 va destacar per mantindre nivells òptims d'acidesa fins al final de la campanya i a més també va presentar el major contingut en vitamina C, flavonoides, glucosa i fructosa. Carrizo Citrange també va induir altes concentracions de sacarosa i vitamina C en la fruita.

‘Tango’ és una mandarina de recent introducció en l'àrea mediterrània, amb gran interés pel seu període de recol·lecció, que comença quan finalitza el de les clementines. En la present Tesi es van estudiar els canvis en la qualitat físic-química, nutricional i sensorial de la mandarina Tango empeltada sobre dos patrons (Carrizo Citrange i Forner-Alcaide 5) durant el període de collita en les dues àrees principals de producció d'Andalusia. La qualitat de la fruita es va veure influenciada per la localització de les parcel·les, la qual cosa es va relacionar sobretot amb la composició de la textura del sòl. En totes dues localitzacions, Forner-Alcaide 5 va ser el patró que va induir major contingut en acidesa, sòlids solubles totals, sacarosa, vitamina C i àcid cítric en la fruita. Les determinacions físic-químiques juntament amb l'avaluació sensorial van permetre establir el moment òptim de recol·lecció depenent de les diferents condicions estudiades.

També s'ha inclòs un estudi del comportament postcollita d'aquesta varietat, ja que no existien dades en les nostres condicions de cultiu. La mandarina ‘Tango’ va presentar símptomes externs de danys per fred a partir dels 20 dies emmagatzemada a 1°C i 5°C. Es va realitzar un estudi micro-estructural per a caracteritzar l'alteració provocada per les baixes temperatures. Els fruits del patró Forner-Alcaide 5 van presentar una menor incidència dels danys per fred. L'emmagatzematge a 9°C no va comprometre la qualitat externa o interna d'aquesta varietat.

Dins del grup de taronges, en els últims anys existeix una creixent demanda per les taronges sanguines per al seu consum en fresc. Per a avaluar l'efecte del patró sobre taronges sanguines es van prendre dues varietats de referències, ‘Moro’ i ‘Tarocco Rosso’ empeltades sobre huit patrons. La qualitat interna es va veure molt influenciada pel moment de collita, la qual cosa va ser més evident en la varietat ‘Moro’. En totes dues varietats el patró va afectar els canvis en els paràmetres de qualitat estudiats durant la maduració. En ‘Moro’, es va observar una reducció en el color del suc degut a la degradació de les antocianines, sent els patrons C-35, Macrophylla i Volkameriana, els que van mostrar un major descens. Aquesta degradació de antocianines es va relacionar amb el canvi de la temperatura experimentada durant el període de recol·lecció. En ‘Tarocco Rosso’ no es va evidenciar una degradació de antocianines, la qual cosa suggereix que aquesta varietat és menys sensible als canvis de temperatura ambiental. En aquesta varietat els patrons Forner-Alcaide 5 i Forner-Alcaide 13 van produir la fruita amb major contingut en antocianos i sucres.

A mes s'aporta l'estudi de l'aptitud a la frigoconservació de dues varietats de sanguines, en aquest cas ‘Tarocco Rosso’ i ‘Sanguinelli’. Mentre que la qualitat interna no es va veure afectada per l'emmagatzematge a cap de les temperatures assajades (1°C, 5°C i 9°C) durant 45 dies, la fruita va presentar símptomes de danys per fred a 1°C. `Sanguinelli’ va presentar major incidència que ‘Tarocco Rosso’. Totes dues varietats es poden emmagatzemar entre 5°C i 9°C durant 30 dies en el cas de ‘Sanguinelli’ i fins a 45 en el cas de ‘Tarocco Rosso’.

INDEX

I. INTRODUCTION

III.1 'CLEMENULES' MANDARINS

<u>CHAPTER I</u>

<u>CHAPTER II</u>

ABREVIATIONS

	Rootstocks
FA13	Forner-Alcaide 13 [Citrus reshni x Poncirus trifoliate (L.) Raf no 13]
FA5	Forner-Alcaide 5 [Citrus reshni x Poncirus trifoliate (L.) Raf no 5]
CC	Citrange Carrizo [*Citrus sinensis* (L.) Osb.×*Poncirus trifoliate* (L.) Raf.]
C35	Citrange C-35 [*Citrus sinensis* (L.) Osb.x*Poncirus trifoliata* (L.) Raf.]
CL	Cleopatra mandarin (*Citrus reshni* Hort. Ex Tan)
M	Macrophylla [*Citrus macrophylla* Wester]
CT	'Swingle' Citrumelo [*Citrus paradisi* (L.) × *Poncirus trifoliate* (L.) Raf.]
VK	Volkameriana (*Citrus volkameriana* Ten. and Pasq.)
V17	Forner-Alcaide V17 [*Citrus volkameriana* Ten & Pasq. x *Poncirus trifoliata* (L.) Raf. no V17]
FA 2324	Forner-Alcaide 2324 (Hybrid of 'Troyer' citrange and Cleopatra mandarin
FA 418	Forner-Alcaide 418 [Troyer citrange × common mandarin (*C. deliciosa* Ten.]
TC	'Troyer' citrange [*Citrus sinensis* (L.) Osb.×*Poncirus trifoliate* (L.) Raf.]
SO	Sour Orange (*Citrus aurantium* L.)
	General
IVIA	Instituto Valenciano de Investigaciones Agrarias
CTV	Citrus Tristeza Virus
TSS	Total Soluble Solids (°Brix)
TA	Titratable Acidity (g of citric acid/100 mL juice)
Vit. C	Vitamin C (Ascorbic Acid)
AA	Antioxidant Activity
MI	Maturity Index
CCI	Citrus Color Index
L	Luminosity Axis (0=Black; 100=White)
a	A axis (-=Green; +=Red)

b	B axis (-=Blue; +=Yellow)
HPLC-DAD	High-Performance Liquid Chromatography Diode-Array Detection
HPLC-MS	High-Performance Liquid Chromatography Mass Spectrometry
DTT	DL-dithiothreitol
LSD	Least Significant Difference
ROS	Reactive Oxygen Species
EC1-5	Electrical Conductivity (µS/cm at 20°C)
H	Harvest
FEDER	Fondo Europeo de Desarrollo Económico y Regional
	Introduction
IAA	Indole-3-Acetic Acid
GA3	Gibberellic Acid
ZT	Zeatin
ABA	Abscisic Acid
SERB	Stem-end Rind Breakdown
EO	Essential Oil
SC	‘Swingle’ Citrumelo [*Citrus paradisi* (L.) × *Poncirus trifoliate* (L.) Raf.]
	Chapter I
	Mineral Nutrients
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Zn	Zinc
	Chapter II
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
A0	DPPH Absorbance
Ax	Juice Absorbance
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
AAe	Ascorbic Acid equivalent (mM)
TCA	Tricarboxylic Acid
	Chapter III

Cryo-SEM	Cryo Scanning Electron Microscopy
	Chapter IV
LDL	Low-Density Lipoprotein
R_T	Retention Time
$[MH]^+$	Atomic mass-to–charge ratio in Positive Ion Mode (m/z)
UV-Vis	Ultraviolet Visible
PCA	Principal Component Analysis
UVB	Ultraviolet B-Rays
Dp-3-glu	Delphinidin 3-glucoside
Cy-3-glu	Cyanidin 3-glucoside
Cy-3,6”mal-glu	Cyanidin 3-(6”-malonyl)-glucoside
Cy-3,6”diox-glu	Cyanidin 3-(6”-dioxalyl)-glucoside
HES	Hesperidin
NAR	Narirutin
DID	Didymin
SUCR	Sucrose
GLU	Glucose
FRU	Fructose
CHLOR	Chlorogenic
FER	Ferulic
SIN	Sinapic
PC1	Principal Component 1
PC2	Principal Component 2
Q	Quartile
	Chapter V
CI	Chilling Injury
IA	Index of Alterations

I. INTRODUCTION

INTRODUCTION

1. Citrus fruit production

Citrus is one of the main fruit crops in the world whose production is estimated at 124.2 million tonnes, according to the latest results reported by the Food Agriculture Organization (FAO, 2017). Oranges are the most widely produced citrus fruit worldwide (50.5% of the total), followed by mandarins (33.7%), lemons (8.4%) and grapefruit (7.4%) (USDA, 2020).

The first worldwide producer is China (32.7 mill tonnes), followed by Brazil (16.6 mill tons), India (9.8 mill tonnes) and the USA (7.8 mill tons). Spain is the fifth producer country in the world with 6.9 million tonnes and the first producer in the Mediterranean Region, followed by Egypt and Turkey (4.9 and 3.7 mill tonnes, respectively) (FAO, 2017).

In Spain, citrus production is based mainly on oranges (3.31 mill tonnes; 50%) and mandarins (1.82 mill tonnes; 30%), followed by lemons (1.06 mill tons; 17%) (Fig. 1). Although grapefruits are also grown, their production is still very low, close to 0.06 mill tonnes (MAPA, 2019). Of the total citrus production, more than 85% is destined for the fresh consumption market.

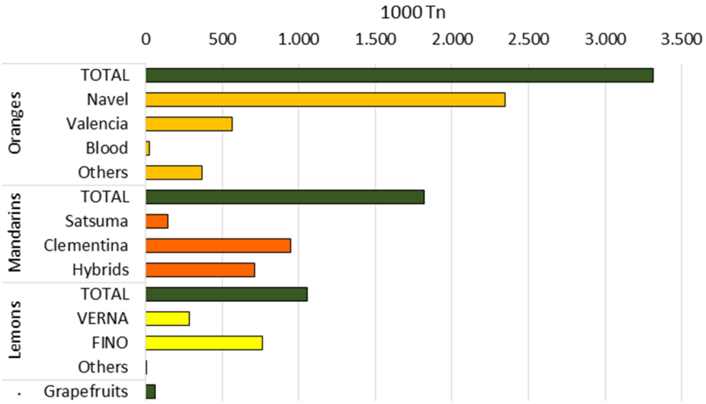


Figure 1: Citrus production in Spain, forecast for season 2019/2020 (MAPA)

Spain is considered the world leader in foreign citrus trade with almost 50% of its harvest exported. The rest, approximately 30%, goes to its domestic market and 20% to the juice industry (Anuario MAPAMA, 2018). The main destination of Spanish citrus fruit is EU countries, with 90% of total citrus exports (FEPEX, 2020). Nevertheless, in recent years, Spanish citrus exports have substantially grown to countries like Norway, Switzerland, Canada and China (USDA, 2019).

Citrus production in Spain is concentrated mainly in the Mediterranean Region. Valencia is the Spanish Community with the largest citrus production area, 54.7% of the total and 160,345 hectares. Andalusia is the second community with the largest citrus area (83,921 hectares), which is the equivalent to 28.6%. The third community is the Murcia Region with 40,112 hectares (13.7%), and the fourth is Cataluña with 8,913 hectares (3%) (MAPA, 2019).

2. Importance of the varieties under study

Among oranges, the main varieties cultivated in Spain belong to the Navel and Valencia groups. Nevertheless, the importance of blood oranges is significantly increasing, as in other Mediterranean countries. In Spain, blood orange production has gone from 17,265 tonness in 2014 to 29,467 tonnes in 2018, where the main producer area is Andalusia, followed by the Valencian Community and the Murcia Region (Fig. 2) (MAPA, 2018). Three main groups appear in blood orange cultivars: 'Tarocco', 'Sanguinelli' and 'Moro'.

Nowadays, consumers are interested in fruit that support and promote health, and citrus fruit is recognized as an important component of human diet because it provides a range of key nutrients that are important for human nutrition since they possess antioxidant properties (Barreca, Bellocco, Leuzzi & Gattuso, 2017; Ahmed & Azmat, 2019). In this context blood oranges are characterized by their high content of anthocyanins, water-soluble polyphenolic compounds which, apart from conferring fruit its characteristic red color, are related to human health properties thanks to their antioxidant activity (Habibi, Ramezanian, Guillén, Serrano & Valero, 2020). These anthocyanins exert potential action against certain diseases, and reduce the risk of several cancer types, heart disease and low-density lipoprotein (LDL) cholesterol accumulation (Hou, 2003). The presence of anthocyanins depends on variety, maturity stage and growth conditions, such as orchard location, temperature or irradiation (Rapisarda, Bellomo & Intelisano, 2001; Crifò, Puglisi, Petrone, Recupero & Lo Piero, 2011).

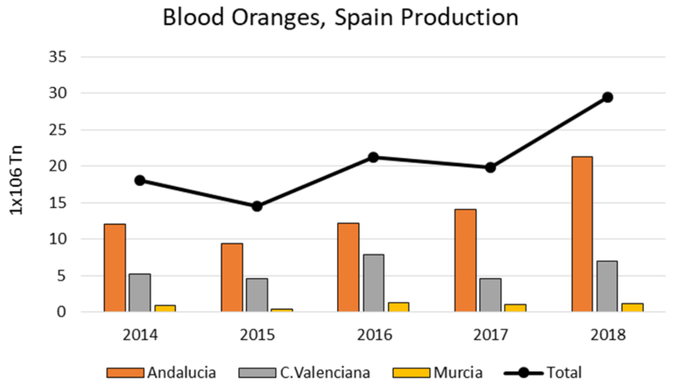


Figure 2: Blood orange production in Spain (2014-2018). (MAPA)

Despite blood oranges being mainly consumed as juice, the demand for fresh fruit consumption is increasing. So besides internal fruit color, attention must be paid to other quality attributes required by consumers. Of these parameters, firmness can be a limiting factor to commercialize pigmented oranges because they usually have low firmness values compared to blond oranges, especially when the postharvest fruit is submitted to long-term shipping or prolonged storage (Pallottino, Menesatti, Lanza, Strano, Antonucci & Moresi, 2012). Another attribute to take into account is sensitivity to low temperatures. Some blood orange varieties are sensitive to cold stress and exhibit chilling injury symptoms when stored at temperatures below 8°C, depending on cultivars and storage temperatures (Pratella, 1969).

In the past decade, the mandarin trade (clementines, satsumas and hybrids) has increased at the expense of fresh oranges. This is because consumer preferences have shifted toward small-sized, easy-peeler and seedless fruit, with hedonistic properties like sweetness and smell (Di Vita, Borrello, Vecchio, Gulisano & D'Amico, 2020).

Among mandarins, the clementine group is the most important with the highest production in the Valencian Community (74% of total production; Fig. 3). 'Clemenules' are the most representative cultivar of clementines (De-Miguel, Caballero & Fernández-Zamudio, 2019). As with most clementine varieties, 'Clemenules' is considered an early mandarin (García-Sanchez et al., 2016), and is harvested from early September to late December. As most mandarins in the Mediterranean Region reach internal maturity before full coloration takes place, clementines are usually submitted to degreening ethylene treatment at the beginning of the season to enhance their external color. Citrus fruit respond to exogenous ethylene application by degrading chlorophylls and synthesizing the carotenoids present in citrus peel (Li, Xie, Liu, Chen, & Yin, 2019; Sumiasih, Poerwanto, Efendi, Agusta, & Yuliani, 2018).

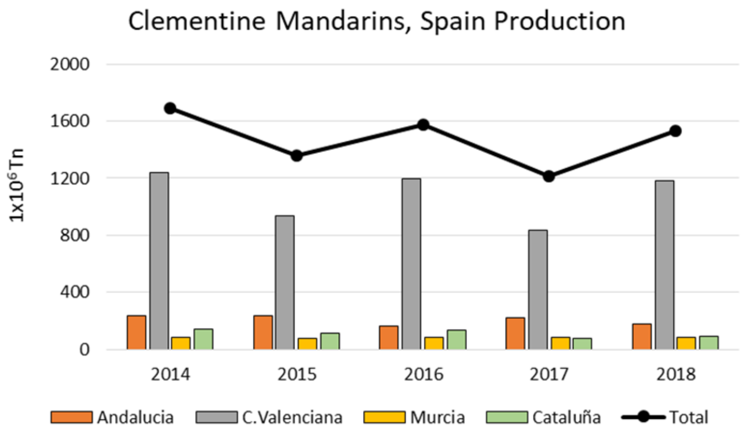


Figure 3: Clementine mandarin production in Spain (2014-2018). (MAPAMA)

Regarding other mandarins, some hybrids like 'Nova' and 'Ortanique' have been introduced into Spanish citriculture to meet the late-season mandarin demand of international markets. This allows the harvesting period to continue until May. These hybrids are also self-incompatible, but pollen and ovules are viable and cross-pollinate with clementines to produce fruit with seeds in both cultivar groups. Nevertheless, seedlessness is one of the most important characteristics for mandarins on the fresh fruit market because consumers do not accept seeded fruit.

Lack of seedless mandarin production after February, and the steady increase in both the consumption and demand of these sweet fruits, lead to new high-quality mid- and late-season mandarin varieties with seedless fruit to be introduced in order to supply the market, mainly from February to May.

This is why the ‘Tango’ cultivar is being introduced as an interesting alternative to be commercialized when the clementine season ends. The ‘Tango’ mandarin is a recent cultivar that was developed at the University of California, Riverside, from an irradiated bud of the diploid mandarin cultivar ‘W. Murcott’ (Roose & Williams, 2007). As the ‘Tango’ cultivar produces seedless mandarins of high organoleptic quality, this cultivar is very much in demand worldwide. Despite in Spain the main alternative cultivars are grown in the Valencian Community (Fig. 4), ‘Tango’ mandarins are grown mainly in Andalusia. The ‘Tango’ harvest season begins at the end of December and may continue until the end of February. However, no information is available about the evolution of quality parameters during this period, and the optimal time to harvest fruit remains to be determined.

Introducing a variety into a new cultivation area requires studying postharvest fruit behavior. Cold storage is necessary to extend the commercial period of fruit, and also while transporting fruit to national and international markets. To date, information about the sensitivity of ‘Tango’ mandarins to low temperature remains limited.

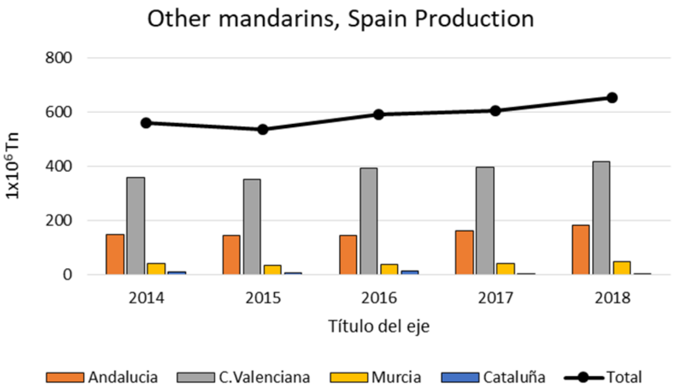


Figure 4: The production of other mandarins, including hybrid mandarins, in Spain (2014-2018). (MAPAMA)

3. Importance of rootstock in citriculture

In today's citriculture, trees are almost always propagated by grafting the scion (the fruiting cultivar) onto a second citrus cultivar, which will form the lower trunk and root system. The second cultivar is termed the rootstock, which functions as the root system of the grafted plant (Mudge, Janick, Scofield & Goldschmidt, 2009). In most cases, the rootstock cultivar has been created or selected, and evaluated over time for the specific purpose of being used as a rootstock. The rootstocks currently used have appeared naturally or are the result of citrus rootstock breeding programs that started more than 100 years ago (Bowman & Joubert, 2020).

One main advantage of using rootstock in citrus trees is that it offers the chance to rapidly obtain the original fruiting tree with identical genetic and the same fruit characteristics in unlimited quantities. Thus rootstocks are a relevant tool because there are some citrus types, like certain mandarin trees, that are unable to grow from seed. Moreover, rootstock confers citrus trees the ability to grow under different biotic (*Phytophthora*, CTV, nematodes, HLB, etc.) and abiotic (salinity, water deficit, flooding, cold, calcareous soils, etc.) stress (Filho, Espinoza-Nunez, Stuchi, & Ortega, 2007). In the present day, such stresses increase due to global climate change with rising temperatures, which affect water availability or predispose citrus trees to new diseases. In this context, the evaluation of suitable rootstocks with the ability to adapt to changing climate has been recently addressed in different crops, including citrus fruit (Shafqat, Jaskani, Maqbool, Khan & Ali).

Rootstocks also offer citriculture benefits that can lead to significant economical savings. In this way, dwarfing or semi-dwarfing rootstocks can reduce the size of the variety grafted onto them. This can lower pruning costs, phytosanitary and harvesting treatments, and can even induce greater productive efficiency in grafted varieties (Forner-Giner, Alcaide, Primo-Millo & Forner, 2003; Forner-Giner, Rodriguez-Gamir, Martínez-Alcántara, Quiñones, Iglesias, Primo-Millo & Forner, 2014). Rootstock can also influence the appearance of physiological disorders, which cause vast economic losses (Koepke & Dhingra, 2013).

In addition, the use of rootstock affects both external and internal quality parameters (Zhang, Breksa, Mishchuk, & Slupsky, 2011; Legua, Bellver, Forner & Forner-Giner, 2011). Indeed, the nutritional quality of citrus fruit juice has been reported to be directly linked with rootstock effects on the plant-water relations that regulate sucrose transport (Barry, Castle, & Davies, 2004). In the fruits destined for fresh consumption, quality characteristics, such as juice content, the TSS/TA ratio or nutritional composition, are very important. Nevertheless, as the rootstock effect on fruit quality is scion-dependent, it renders studying the rootstock effect on each cultivar of interest necessary. Nowadays, despite it being well-known that rootstock has an effect on fruit quality, very few studies have paid attention to its impact on organoleptic characteristics and bioactive compounds in certain citrus cultivars (Legua, Forner, Hernandez & Forner-Giner, 2014). More of these studies would allow rootstocks to be selected according to functional citrus fruit characteristics, which would have an impact with increased quality. An extensive review as an article on the effect of rootstock on the main quality parameters is included in the following section.

The widespread use of rootstocks in citriculture began in many countries in the mid-1800s (Castle, 2010), where sour orange (*Citrus aurantium* L., F1 hybrid between mandarin and pummelo) and other natural citrus rootstocks were used. However, the emergence of disasters that affected the citrus industry accelerated the search for rootstocks that allowed citrus trees to adapt to this changing situation. *Citrus tristeza virus* (CTV) was one of the main precursors to mobilize the sector to search for solutions. Sour orange, the most widespread rootstock in the world, is sensitive to CTV, a virus that has caused the death of millions of trees around the world. In 1957, it began to affect citrus fruit in the Mediterranean Region. From that time, the possibility of fixing a problem with a specific rootstock or combining complementary traits of various rootstocks made citrus rootstock breeding an important research activity worldwide (Forner-Giner, Continella & Grosser, 2020).

Citrus rootstocks are selected because they are naturally-occurred species (Rough lemon, Cleopatra mandarin, Rangpur lime, Trifoliate orange or Sweet orange) or hybrids from controlled crosses to view the worldwide use as citrus rootstocks (Carrizo/Troyer Citrange, Macrophylla or 'Swingle' Citrumelo) (Webber et al., 1967). More recently, the rootstocks obtained by advanced hybrids, somatic hybrids, the product of induced mutation or from any other type of genetic manipulation, have been evaluated to include higher germplasm diversity and other types of genetic combinations (Bowman & Joubert, 2020). Of these rootstocks, Carrizo Citrange is the most widely used in the main citrus-producing countries like Spain, South Africa, Australia, California and Italy (Bowman & Joubert, 2020). However, Trifoliate orange is preferred in other countries like Japan, China, Argentina and Uruguay, and those with cold environments. Other rootstocks are selected for specific cultivars like Macrophylla, which is often used for lemon production (Forner-Giner et al., 2020).

Currently, many rootstock breeding programs are underway in different citrus-producing areas, such as Florida (Grosser, Chandler, LinG & Barthe, 2011; Grosser, Barthe, Castle, Gmitter & Lee, 2015; Bowman and McCollum, 2018a,b,c), Brazil (Fadel, Stuchi, Couto, Ramos & Mourão Filho, 2018), Australia (Smith, 2017; Sykes, 2011a,b), Italy (Reforgiato Recupero, Russo, Recupero, Zurru, Deidda & Mulas, 2009) and China (Guo, Cheng & Deng 2002; Liu & Deng, 2007).

In Spain, the Valencian Institute for Agricultural Research (IVIA) began a citrus breeding program in 1974 to obtain citrus rootstocks by hybridization. The main problems that the Spanish citrus industry had to deal with have been salinity, iron chlorosis, CTV, flooding and water stress (Forner-Giner, Primo-Millo & Forner., 2009; Llosá, Bermejo, Cano, Quiñones & Forner-Giner, 2009; Martínez-Alcántara, Rodriguez-Gamir, Martínez-Cuenca, Iglesias, Primo-Millo & Forner-Giner, 2012; Rodríguez-Gamir, Primo-Millo, Forner & Forner-Giner., 2010).

In the breeding program developed at the IVIA, more than 500 hybrid rootstocks have been evaluated to select the most promising rootstocks. Forner-Alcaide 13 (FA13) and Forner-Alcaide no. 5 (FA5) are hybrids between Cleopatra mandarin [*Citrus reshni* hort. ex Tanaka] and *Poncirus trifoliata* (L.) Raf. These rootstocks were commercially released by the European Union in 2005 (FA5) and 2008 (FA13) (Forner-Giner et al., 2009). These rootstocks are resistant to CTV and *Phytophtora* spp., and are tolerant to salinity (Forner-Giner, Legaz, Primo-Millo & Forner, 2011). FA5 is also resistant to citrus nematode (*Tylenchulus semipenetrans* Cobb.), and is tolerant to calcareous soils (Forner, Forner-Giner & Alcaide, 2003) and to iron chlorosis (Llosá et al., 2009; González-Mas, Llosa, Quijano & Forner-Giner, 2009). More recently, Forner-Alcaide no. V17 (V17) was nationally registered in 2017 (MAPA, 2017). V17 is a hybrid between *Citrus Volkameriana* x *P. trifoliate*.

In the following section a review as an article about the effect of rootstock on the main fruit quality parameters is presented.

REFERENCES

Ahmed, W., & Azmat, R. (2019). Citrus: An Ancient Fruits of Promise for Health Benefits. In Citrus Health Benefits and Production Technology, 1st ed.; Sajjid, M., Amanullah, Eds.; IntechOpen; London, United Kingdom, 19-30. https://doi.org/10.5772/intechopen.79686

Barreca, D., Bellocco, E., Leuzzi, U., & Gattuso, G. (2014). First evidence of C- and O-glycosyl flavone in blood orange (Citrus sinensis (L.) Osbeck) juice

and their influence on antioxidant properties. *Food Chemistry*, *149*, 244-252.
https://doi.org/10.1016/j.foodchem.2013.10.096

Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. *Journal of the American Society for Horticultural Science, 129(6),* 881-889.
https://doi.org/10.21273/JASHS.129.6.0881

Bowman, K.D., & Joubert, J. (2020). Citrus rootstocks. In The Genus Citrus, 1st ed.; Talón, M., Caruso, M., Gmitter, F.G., Eds.; Woodhead Publishing: Duxford, United Kingdom, 105-127.
https://doi.org/10.1016/B978-0-12-812163-4.00006-1

Bowman, K.D., & McCollum, G.M., 2018a. Notice to Fruit Growers and Nurserymen Relative to the Naming and Release of the US SuperSour 1 Citrus Rootstock. U.S. Department of Agriculture, Beltsville, MD.

Bowman, K.D., & McCollum, G.M., 2018b. Notice to Fruit Growers and Nurserymen Relative to the Naming and Release of the US SuperSour 2 Citrus Rootstock. U.S. Department of Agriculture, Beltsville, MD.

Bowman, K.D., & McCollum, G.M., 2018c. Notice to Fruit Growers and Nurserymen Relative to the Naming and Release of the US SuperSour 3 Citrus Rootstock. U.S. Department of Agriculture, Beltsville, MD.

Castle, W.S. (2010). A career perspective on citrus rootstocks, their development, and commercialization. HortScience, 45(1), 11-15.
https://doi.org/10.21273/HORTSCI.45.1.11

Crifò, T., Puglisi, I., Petrone, G., Reforgiato Recupero, G., & Lo Piero, A.R. (2011). Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. *Gene, 476(1-2),* 1-9.
https://doi.org/10.1016/j.gene.2011.02.005

De-Miguel, M.D., Caballero, P., & Fernández-Zamudio, M.A. (2019). Varietal Change Dominates Adoption of Technology in Spanish Citrus Production. *Agronomy, 9(10),* 631. https://doi.org/10.3390/agronomy9100631

Di Vita, G., Borrello, M., Vecchio, R., Gulisano, G., & D'Amico, M. (2020). Purchasing Drivers of Fresh Citrus Fruits in Urban Italy: Is It All about Taste? *Nutrients, 12(4),* 979. https://doi.org/10.3390/nu12040979

Fadel, A.L., Stuchi, E.S., Couto, H.T.Z., Ramos, Y.C., & Mourão Filho, F.A.A., (2018). Trifoliate hybrids as alternative rootstocks for 'Valencia' sweet orange under rainfed conditions. *Scientia Horticulturae, 235,* 397–406. https://doi.org/10.1016/j.scienta.2018.01.051

Federación Española de Asociaciones de Productores Exportadores de Frutas, Hortalizas, Flores y Plantas vivas (FEPEX), (2020). Exportación/Importación Española de Frutas y hortalizas. Consulta: 091020. https://www.fepex.es/datos-del-sector/exportacion-importacion-espa%C3%B1ola-frutas-hortalizas

Filho Mourão, F.D.A.A., Espinoza-Núñez, E., Stuchi, E.S., & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. *Scientia Horticulturae, 114(1*), 45-49. https://doi.org/10.1016/j.scienta.2007.05.007

Food and Agriculture Organization of the United Nations (FAO), (2017). Citrus Fruit - Fresh and Processed Statistical Bulletin 2016. Market and Policy Analysis of Raw Materials, Horticulture and Tropical (RAMHOT) Products Team. Rome

Forner, J.B., Forner-Giner, M., & Alcaide, A. (2003). Forner-Alcaide 5 and Forner-Alcaide 13: two new citrus rootstocks released in Spain. *HortScience, 38(4),* 629-630. https://doi.org/10.21273/HORTSCI.38.4.629

Forner-Giner, M.A., Alcaide, A., Primo-Millo, E., & Forner, J.B. (2003). Performance of 'Navelina' orange on 14 rootstocks in Northern Valencia (Spain). *Scientia Horticulturae, 98(3),* 223-232. https://doi.org/10.1016/S0304-4238(02)00227-3

Forner-Giner, M.A., Primo-Millo, E., & Forner, J.B. (2009). Performance of Forner-Alcaide 5 and Forner-Alcaide 13, hybrids of Cleopatra mandarin x Poncirus trifoliate, as salinity-tolerant citrus rootstocks. *Journal of the American Pomological Society, 63(2),* 72.

Forner-Giner, M.A., Legaz, F., Primo-Millo, E., & Forner, J. (2011). Nutritional responses of citrus rootstocks to salinity: performance of the new hybrids Forner-Alcaide 5 and Forner-Alcaide 13. *Journal of plant nutrition, 34(10),* 1437-1452. https://doi.org/10.1080/01904167.2011.585202

Forner-Giner M.A., Continella A., Grosser J.W. (2020) Citrus Rootstock Breeding and Selection. In: The Citrus Genome, 1st Ed.; Gentile A., La Malfa S., Deng Z., eds; Woodhead Publishing: Duxford, United Kingdom, Compendium of Plant Genomes. Springer, Cham, Switzerland, 49-74. https://doi.org/10.1007/978-3-030-15308-3_5

Forner-Giner, M.A., Rodriguez-Gamir, J., Martínez-Alcántara, B., Quiñones, A., Iglesias, D.J., Primo-Millo, E., & Forner, J. (2014). Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). Scientia Horticulturae, 179, 376-387. https://doi.org/10.1016/j.scienta.2014.07.032

Garcia-Sanchez, F., Simon-Grao, S., Gimeno, V., Galvez-Sola, L., Lidon, V., Simon, I., Hernández, F., Martínez-Nicolás, J.J., & Carbonell-Barrachina, A.A. (2016). Phytochemical properties and volatile composition profile of nine early maturing mandarins cultivated in south-east Spain. *Journal of Agriculture, Science and Technology, 18,* 1367-1380.

González-Mas, M.C., Llosa, M.J., Quijano, A., & Forner-Giner, M.A. (2009). Rootstock effects on leaf photosynthesis in ‘Navelina’ trees grown in calcareous soil. *HortScience, 44(2),* 280-283. https://doi.org/10.21273/HORTSCI.44.2.280

Grosser, J.W., Chandler, J.L., LinG, P., & Barthe, G.A. (2011). New somatic hybrid rootstock candidates for tree-size control and high juice quality. *Procedings of the Florida State Horticultural Society, 124,* 131-135.

Grosser, J.W., Barthe, G.A., Castle, B., Gmitter, F.G., & Lee, O. (2015). The development of improved tetraploid citrus rootstocks to facilitate advanced production systems and sustainable citriculture in Florida. *Acta Horticulturae, 1065,* 319-327. https://doi.org/10.17660/ActaHortic.2015.1065.38

Guo, W.W., Cheng, Y.J., & Deng, X.X. (2002). Intergeneric somatic hybrids of Citrus reticulata with Poncirus trifoliata for complementary rootstock improvement and their identification by AFLP analysis. *Plant Cell Reports, 20,* 829–834.

Habibi, F., Ramezanian, A., Guillén, F., Martínez-Romero, D., Serrano, M., & Valero, D. (2020). Susceptibility of Blood Orange Cultivars to Chilling Injury Based on Antioxidant System, Physiological and Biochemical Responses at Different Storage Temperatures. *Preprints,* 2020090255. https://doi.org//10.20944/preprints202009.0255.v1

Hou, D.X. (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. Current Molecular Medicine, 2003, 3(2), 149–159. https://doi.org/10.2174/1566524033361555

Koepke, T., & Dhingra, A. (2013). Rootstock scion somatogenetic interactions in perennial composite plants. *Plant cell reports, 32(9),* 1321-1337. https://doi.org/10.1007/s00299-013-1471-9

Legua, P., Bellver, R., Forner, J., & Forner-Giner, M.A. (2011). Plant growth, yield and fruit quality of 'Lane Late' navel orange on four citrus rootstocks. *Spanish Journal of Agricultural Research, 9(1),* 271-279.

Legua, P., Forner, J.B., Hernandez, F.C.A., & Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. *Scientia Horticulturae, 174,* 60-64. https://doi.org/10.1016/j.scienta.2014.05.004

Li, S.J., Xie, X.L., Liu, S.C., Chen, K.S., & Yin, X.R. (2019). Auto-and mutual-regulation between two CitERFs contribute to ethylene-induced citrus fruit degreening. *Food Chemistry, 299,* 125163. https://doi.org/10.1016/j.foodchem.2019.125163.

Liu, Y.Z., & Deng, X.X. (2007). Citrus breeding and genetics in China. *The Asian and Australasian Journal of Plant Science and Biotechnology, 1,* 23–28.

Llosá, M.J., Bermejo, A., Cano, A., Quiñones, A., & Forner-Giner, M.A., (2009). The citrus rootstocks Cleopatra mandarin, Poncirus trifoliata, Forner-Alcaide 5 and Forner-Alcaide 13 vary in susceptibility to iron deficiency chlorosis. *Journal of the American Pomological Society, 63(4),* 160–167.

Martínez-Alcántara, B., Rodriguez-Gamir, J., Martínez-Cuenca, M.R., Iglesias, D.J., Primo-Millo, E., & Forner-Giner, M.A. (2013). Relationship between hydraulic conductance and citrus dwarfing by the Flying Dragon rootstock (Poncirus trifoliata L. Raft var. monstruosa). *Trees, 27(3),* 629-638. https://doi.org/10.1007/s00468-012-0817-1

Ministerio de Agricultura, Pesca y Alimentación (MAPA), 2017. Spanish Office of Plant Varieties; National and community catalogs. https://www.mapa.gob.es/app/regVar/ResBusLicencias.aspx?id=ES&TxtLicenciatario=BENIPLANT+S.L.

Ministerio de Agricultura, Pesca y Alimentación (MAPA), 2020. Avance del Anuario de Estadística 2019. Madrid. https://www.mapa.gob.es/estadistica/pags/anuario/2019-Avance/ CAPITULOS_TOTALES /AE19-C07.pdf

Ministerio de Agricultura, Pesca y Alimentación (MAPA), September 2019. Aforo Nacional de Cítricos. Campaña 2019/2020.

Mudge, K., Janick, J., Scofield, S. & Goldschmidt, E.E. (2009) A history of grafting. *Horticultural Reviews. (American Society of Horticultural Science), 35*, 437–493.

Pallottino, F., Menesatti, P., Lanza, M.C., Strano, M. C., Antonucci, F., & Moresi, M. (2012). Assessment of quality-assured Tarocco orange fruit sorting rules by combined physicochemical and sensory testing. *Journal of the Science of Food and Agriculture, 93(5), 1176*-1183. https://doi.org/10.1002/jsfa.5871

Pratella, G., Tonini, G., and Cessari, A. (1969) Postharvest disease problems of Italian citrus fruit. Paper presented at: First Int. Citrus Symp. (University of California at Riverside).

Rapisarda, P., Bellomo, S.E., & Intelisano S. (2001). Storage temperature effects on blood orange fruit quality. *Journal of Agricultural and Food Chemistry, 49(7),* 3230-3235. https://doi.org/10.1021/jf010032l

Reforgiato Recupero, G., Russo, G., Recupero, S., Zurru, R., Deidda, B., & Mulas, M. (2009). Horticultural evaluation of new Citrus latipes hybrids as rootstocks for citrus. *HortScience 44,* 595–598. https://doi.org/10.21273/HORTSCI.44.3.595

Rodríguez-Gamir, J., Primo-Millo, E., Forner, J.B., & Forner-Giner, M.A. (2010). Citrus rootstock responses to water stress. *Scientia Horticulturae, 126(2),* 95-102. https://doi.org/10.1016/j.scienta.2010.06.015

Roose, M., & Williams, T. (2007). Mandarin variety named ‘Tango’. US Patent Application № 11/220,875 11 /220,875

Shafqat, W., Jaskani, M.J., Maqbool, R., Khan, A.S., & Ali, Z. (2019). Evaluation of citrus rootstocks against drought, heat and their combined stress based on growth and photosynthetic pigments. *International Journal of Agriculture and Biology, 22(5),* 1001-1009. https://doi.org/10.17957/IJAB/15.1160

Smith, M. (2017). CT13004 Queensland Citrus Improvement Scheme: Finding Better Rootstocks for Australia. https://www.citrusaustralia.com.au/wp-content/uploads/Smith-Malcolm-Imperial-Rootstock.pdf.

Sumiasih, I.H., Poerwanto, R., Efendi, D., Agusta, A., & Yuliani, S. (2018). β-Cryptoxanthin and Zeaxanthin pigments accumulation to induce orange color on citrus fruits. IOP Conference Series: Materials Science and Engineering, 299, 012074. https://doi.org/10.1088/1757-899X/299/1/012074.

Sykes, S.R. (2011a). Chloride and sodium excluding capacities of citrus rootstock germplasm introduced to Australia from the People’s Republic of China. *Scientia Horticulturae, 128(4),* 443–449. https://doi.org/10.1016/j.scienta.2011.02.012

Sykes, S.R. (2011b). Characterisation of citrus rootstock germplasm introduced as seeds to Australia from the People's Republic of China. *Scientia Horticulturae, 127(3),* 298–304. https://doi.org/10.1016/j.scienta.2010.10.015

United States Department of Agriculture (USDA), 2019. EU-28: Citrus Annual. Madrid

United States Department of Agriculture (USDA), July 2020. Citrus: World Market and Trade. Foreign Agriculture Service. Office of Global Analysis.

Webber, H.J. (1967). History and development of the citrus industry. In The citrus industry, 1st Ed.; Reuther, W., Webber, H.J., Batchelor, L.D., eds., University of California, Berkeley, 1-39.

Zhang, X., Breksa III, A.P., Mishchuk, D.O., & Slupsky, C.M. (2011). Elevation, rootstock, and soil depth affect the nutritional quality of mandarin oranges. *Journal of agricultural and food chemistry, 59(6),* 2672-2679. https://doi.org/10.1021/jf104335z

Effect of Rootstock on Citrus Fruit Quality. A Review

Julia Morales [a], Almudena Bermejo [b], Pilar Navarro [a], Ana Quiñones[c] and Alejandra Salvador [a]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[c]Centro de Desarrollo de Agricultura Sostenible, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

Reference: *Food Reviews International*
(Under Review, minor revision: 08-09-2020).

Abstract

Citrus rootstocks are a relevant tree part that contributes to crops adapting to biotic and abiotic conditions, it becoming a key factor to face the current era of climate change. Although the emphasis of most studies on rootstocks has been placed on the yield and optimization of the citrus fruit grown in different environments, other studies have paid attention to the effect of rootstock on fruit quality. Hence the quality of citrus fruit is becoming increasingly more relevant as consumers demand high internal and external qualities to eat citrus fruit fresh. To better understand how rootstock influences citrus fruit quality, the literature that focuses on physico-chemical parameters, nutritional compounds and physiological disorders has been revised. This review points out the influence that the external aspects surrounding plants has on fruit quality, such as the rootstock/scion interaction, the water uptake capacity of roots, the modification of the photosynthetic rate or availability of nutrients minerals.

Keywords. Nutritional quality, sugars, organic acids, vitamin C, phenolic compound, volatiles, fruit color, rind thickness, peel disorders, juice content

1. Introduction

Rootstock plays a very important role in commercial citrus production. Selecting rootstock acts as an important tool to adapt crops to adverse environmental conditions, or to biotic (diseases, insect-pests) and abiotic (drought, salinity, water logging, alkalinity, cold) stresses (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007; Yıldırım, Yıldırım, San & Ercişli, 2016). Optimum rootstock selection is crucial as the scion/rootstock interaction influences tree vigor, increases yield, reduces the juvenile period or enhances fruit quality characteristics. These conditions also vary depending on each specific region (Castle, 2010; Hussain, Curk, Anjum, Pailly & Tison, 2013; Sharma, Dubey, Awasthi & Kaur, 2016). The effect of rootstock on cultivar performance has been reported to be due mainly to water relations, mineral nutrition and hormonal contribution (Sharma et al., 2016; Lado, Gambetta & Zacarias, 2018).

The aim of rootstock breeding programs worldwide is to diversify scion-rootstock combinations to help to increase yields and fruit quality, and to extend the harvest season. Therefore, new rootstocks that are tolerant to salinity, iron chlorosis, water stress and flooding conditions, or resist diseases such as citrus tristeza virus (CTV), are being developed (Reforgiato Recupero, Russo, Recupero, Zurru, Deidda & Mulas, 2009; Legua, Forner, Hernandez & Forner-Giner, 2014; Fu, Chai, Ding & Pan, 2016; Continella et al., 2018). In addition, rootstock is a key for facing new citriculture challenges, such as optimizing crops in organic farming vs. conventional farming to respect the environment more or to cope with new diseases appearing, such as huanglongbing disease, which has emerged in recent years (Albrecht, McCollum & Bowman, 2012; Klein, Shalev, Cohen & Sachs, 2017). On the other hand the rootstock becomes a key factor to face the current era of climate change. Global climate changes affect water availability and increase in temperature and are responsible for drought and heat stress which alter the plant morphology, physiology and genetic expression of plants. In this context the evaluation of suitable rootstocks with ability to adapt to changing climate is being recently addressed in different crops including citrus fruit (Ollat, Touzard & van Leeuwen, 2016; Shafqat, Jaskani, Maqbool, Khan & Ali, 2019;

Jiménez, Fattahi, Bedis, Nasrolahpour-moghadam, Irigoyen & Gogorcena, 2020).

For the fresh fruit market, citrus fruit should offer excellent fruit quality, which includes the content of both primary (sugars, organic acids, and vitamins) and secondary (flavonoids and carotenoids) metabolism compounds, with special attention being paid to physiological disorders that may compromise fruit commercialization. The influence of rootstock on fruit quality has been widely addressed by different authors. Nevertheless, the most studies are focused on a specific cultivar grafted onto different rootstocks. For this reason, a compilation of the results of different variables (metabolism compounds, physicochemical properties and the incidence of physiological disorders) are needed to understand the effect of rootstock on fruit quality, and this information can be useful for selecting the optimum scion/rootstock combination to meet consumers' product quality demands.

This paper reviews information on the effect of rootstocks on the main fruit quality parameters, and the factors that may cause these effects.

2. External Color

Fruit coloration is one of the most important attributes to determine citrus fruit quality. In citrus peel, color is due to the accumulation of three main classes of pigments: chlorophylls, carotenoids and anthocyanins (Rodrigo, Alquézar, Alós, Lado & Zacarías, 2013). Both content and composition in rind and pulp are specific of different varieties (Kato, 2012; Rodrigo et al., 2013).

Many studies have addressed the effect of rootstock on citrus fruit color. Machado, Costa, Teixeira & Costa (2015) found that rootstock impacted color development in 'Ruby Red' grapefruit. Indeed, while Cleopatra fruit developed a yellowish external color, Citrumelo fruit displayed a pinkish peel color. However, these differences were observed only at the beginning of the season. In the same study, rootstock was seen to not affect 'Star Ruby' grapefruit color. More recently, Emmanoulidou & Kyriacou (2017) reported that the differences observed in the color of the 'Lane Late' and 'Delta' cultivars grafted onto five different rootstocks were associated with maturity state, and not with the effect of rootstock.

By comparing the physico-chemical properties of ‘Lane Late’ Navel orange trees on the most widely used commercial rootstocks in Spain, plus eight new hybrids, Legua, Forner, Hernández & Forner-Giner (2013) found that the highest color index was exhibited by the fruit from Cleopatra and hybrid rootstocks 020324, while the lowest color index was for the fruit from hybrid rootstocks 030131 and 030127. Another study on the performance of Navel oranges grafted onto two new dwarfing citrus rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418), compared to Carrizo citrange, reported that FA-418 had a lower color index than FA-517 and Carrizo citrange (Forner-Giner et al., 2014). Nevertheless, when evaluating three different lemon cultivars (‘Fino 49, ‘Fino de Elche’ and ‘Verna 50’) on three rootstocks (Forner-Alcaide 5, Forner-Alcaide 418 and Forner-Alcaide 2324), the fruit from Forner-Alcaide-418 also exhibited the highest external color index (Legua, Martínez-Cuenca, Bellver & Forner-Giner, 2018).

This difference in citrus peel color could be due to the influence of rootstock on the accumulation of pigments, which has also been addressed in different studies. With ‘Owari’ mandarin grafted onto two rootstocks, Cleopatra fruit had higher levels of carotenoids β-cryptoxanthin and violaxanthin (in flavedo and albedo tissue) than those of rootstock Troyer citrange (Cano & Bermejo, 2011). Although most studies have reported the effect of rootstock on external color, some authors have also evaluated this effect on internal color, above all in Blood orange cultivars. Continella et al. (2018) analyzed the effect of eight rootstocks on ‘Tarocco Scirè’ sweet blood orange. They reported that the fruit budded onto rootstock Bitters had the highest external color, while Citrumelo fruits had the lowest. Moreover, the anthocyanin content measured in juice followed the same pattern. Conversely, Incesu, Çimen, Yesiloglu & Yilmaz (2013) observed that the influence of six rootstocks on ‘Moro blood’ oranges was different between peel and pulp color because red color rind was higher in the fruits from Carrizo citrange and Troyer citrange, while the fruit from rootstocks Yuzu and Cleopatra had the highest color in pulp.

Abiotic conditions can also influence the effect of rootstock on citrus color. While comparing the performance of 'Clemenules' mandarin on rootstocks Carrizo citrange and Cleopatra, Navarro, Gómez-Gómez, Pérez-Pérez & Botia (2010a) found no differences in external fruit color. Nevertheless, the same study carried out under deficit irrigation conditions revealed that external coloration delay was more evident in Cleopatra fruits than in those on rootstock Carrizo citrange (Navarro, Pérez-Pérez, Romero & Botía, 2010b).

Color breakdown from green to orange is the result of chloroplast to chromoplast conversion, which involves chlorophyll degradation and carotenoid accumulation (Huff, 1984; Alós, Cercós, Rodrigo, Zacarías & Talón, 2006). This change is known to be influenced by environmental and stress conditions, nutrient availability and hormonal action. Reduced nitrogen content and a rise in sucrose levels have been reported to promote color changes in the flavedo of citrus fruits (Huff, 1983; Iglesias, Tadeo, Legaz, Primo-Millo & Talon, 2001).

Rising sugar levels have been positively related to color break in citrus peel (Goldschmidt & Koch, 1996; Iglesias et al., 2001). These observations are consistent with the known effects of soluble sugars, especially hexoses, on the down-regulation of the genes encoding chlorophyll and photosynthetic enzymes (Pourtau, Jennings, Pelzer, Pallas & Wingler, 2006; Rolland, Baena-González & Sheen, 2006). As indicated below in the Sugars and Organic Acids section, rootstock has a major effect on levels of sugars in citrus fruit. Legua, Bellver, Forner & Forner-Giner (2011a) reported in 'Lane Late' oranges that Cleopatra fruit, with a higher color index than for the fruit of Macrophylla and Volkameriana, also had higher sucrose contents in juice. Nevertheless in a study about 'Navelina' orange grafted onto 14 rootstocks, Cleopatra fruit exhibited the lowest external color but, in this case, fruit also gave the lowest TSS:TA ratio (Forner-Giner, Alcaide, Primo-Millo & Forner, 2003). Despite the few studies available, it has been proved that rootstock can modify nitrogen uptake and translocation to the main sink (fruit) and, therefore, the amount in citrus peel. Hass (1948) found different amounts of N in dry matter in 'Valencia' orange peel as regards which rootstocks were grafted. The fruit from rootstocks Sour oranges and Rough lemon had the highest total N content, while those from trifoliate orange had the smallest amount. However, no studies were found in which variations in N due to rootstock have been related directly to fruit color change.

3. Peel Thickness

Peel is the first fruit barrier against abiotic and biotic factors during preharvest and postharvest periods that can be involved in fruit quality losses. Thus peel thickness is one of the most important factors for the incidence of peel disorders. Thin rinds are prone to split and are sensitive to peel disorders, which can occur during shipping and storage (Al-Jaleel, Zekri & Hammam, 2005). So peel thickness is a characteristic that is usually taken into account when studying the effect of rootstock on citrus fruit quality.

Table 1 shows some of the most relevant studies in which the effect of rootstock on rind thickness has been evaluated.

The results obtained in different studies reveal that the effect of rootstock on peel thickness largely depends on the evaluated cultivar and rootstocks were compared in each case (Table 1). Even so, some rootstocks frequently have the same effect on skin thickness in different varieties. In the reviewed literature, rootstocks Macrophylla, Volkameriana and Rough lemon induced the thickest peel (Zekri & Al-Jaleel, 2004; Al-Jaleel et al., 2005; Legua et al., 2011a; Berdeja-Arbeu, Méndez, Velázquez, Huerta, Capurata & Martínez, 2016), while rootstock Cleopatra led to the thinnest peel (Forner-Giner et al., 2003; Zekri & Al-Jaleel, 2004; Al-Jaleel et al., 2005; Al-Obeed, Harhash & Sourour, 2005; Legua et al., 2011a).

It is also noteworthy that, besides rootstock, biotic and abiotic factors can affect rind thickness. Despite some studies offering repetitive results for different seasons (Le, 2016), in other studies the differences found among rootstocks for one season were not observed for the following season, such as grapefruit ‘Marsh’ and ‘Rio Red’ (Al-Obeed et al., 2005; Yildiz, Kaplankiran, Demirkeser, Toplu & Uysal-Kamiloglu, 2014).

Citrus peel thickness is known to be highly dependent on mineral nutrition. An increase in P supply has been reported to lead to a higher leaf P content, which causes reduced rind thickness. Conversely, an increase in leaf K content

Table 1. Effect of rootstock on rind thickness of citrus fruits.

Cultivar (Country)	Root. No.	Effect of rootstock		Reference
		Thickest	**Thinnest**	
Orange				
‘Navelina’ (Spain)	14	FA 418	Carrizo citrange Cleopatra FA13	*Forner-Giner et al., 2003*
‘Olinda Valencia’ ‘Washington Navel’ (Saudi Arabia)	9	Macrophylla Rough lemon	Cleopatra Amblycarpa Sour Orange	*Zekri & Al-Jaleel, 2004*
‘Lane Late’ (Spain)	4	Macrophylla Volkameriana	Gou Tou Cleopatra	*Legua et al., 2011a*
‘Queen’ (Iran)	6	No differences (VK, TC, CC, SO, SC, CL)		*Shafieizargar et al., 2012*
Mandarin				
‘Nova’ (Cyprus)	11	Palest. Sweet lime Volkameriana	Troyer citrange	*Georgiou, 2000*
‘Nova’ ‘Robinson’ (Turkey)	3	No differences (TC, CC, SO)		*Demirkeser et al., 2009*
‘Duong’ (Vietnam)	3	Matt Orange	Tau lemon Carrizo citrange	*Le, 2016*
Grapefruit				
‘Piemonte’ (Brazil)	14	Volkameriana	Cleopatra Sunki Riverside TSCK x CTSW-028	*França et al., 2018*
‘Marsh’ (Saudi Arabia)	7	Shaub Rough lemon	Volkameriana	*Al-Obeed et al., 2005*
‘Rio Red’ (Turkey)	7	1st Season Volkameriana 2nd Season No differences	Calamondin Smooth Flat Seville	*Yilidiz et al., 2014*
‘Henderson’ (Turkey)	5	No differences (VK, TC, CC, SO, SCT)		*Yeşiloğlu et al., 2014*
Lemon				
‘Allen Eureka’ (Saudi Arabia)	7	Macrophylla Volkameriana Rough lemon	Cleopatra Citrumelo Amblycarpa Sour Orange	*Al-Jaleel et al., 2005*
‘Fino 49’,‘Verna 50’ ‘Fino de Elche’ (Spain	3	FA2324	FA 418	*Legua et al., 2018*
Lime				
‘Mexican’ (Saudi Arabia)	7	Shaub Rough lemon	Cleopatra	*Al-Obeed et al., 2005*
‘Persa’ (Mexico)	8	Volkameriana Dulce de Palestina	Flying dragon	*Berdeja-Arbeu et al., 2016*

increases rind thickness and coarseness (Chapman, 1968). Although it is known that rootstock influences nutrient uptake (Legua et al., 2013; Zambrosi, Mesquita, Tanaka, Quaggio & Mattos, 2013; Yilmaz, Cimen, Incesu, Uysal Kamiloglu & Yesiloglu, 2018), very few studies report the influence of rootstock on peel thickness and its nutritional composition. In 'Nova' mandarin, this correlation was observed in the fruit from rootstock Troyer citrange with the thinnest peel, where tree leaves had the largest amounts of P and the least amounts of K (Georgiou, 2000). Nevertheless, when Dubey & Sharma (2016) compared four rootstocks for 'Kagzi Kalan' lemon, they found that the thickest peel was exhibited by the fruit from the trees grafted onto Karna Khatta, with the highest P content in leaves instead of the lowest as expected.

Recently, the influence of rootstock on the endogenous hormone metabolism that regulates peel thickness has been explored (Rong, Luo, Liao & Wang, 2019). 'Kiyomi' tangor fruits had higher rind thickness values in Citrus Junos than in Poncirus Trifoliata, which coincided with the highest levels of IAA, GA3 and ZT hormones in rootstock Citrus Junos. These hormones correlated directly with increased peel thickness. Regarding hormone ABA, despite the amount in Citrus Junos fruit being higher than in Poncirus Trifoliata fruit, no correlation with increased peel thickness was found.

4. Juice Content

Juice content is an important quality parameter for citrus fruit, and it has also been reported to be possibly affected by rootstock. As juice percentage is related to water uptake, greater absorption regulated by rootstock increases juice content. It is important to take account the ability of specific rootstocks to increase juice yield, especially in the growing regions where is experimenting drought stress due to climatic changes. Thus, rootstocks can be a useful tool to adapt citrus crops to the changing conditions.

Kumar, Chohan & Vij (1994) reported high juice content in the 'Early Gold' sweet oranges grafted onto Carrizo citrange, but with no significant differences to Volkameriana and Jatti Khatti, which had the lowest levels for the fruit from

Benton. In ‘Lane Late’ oranges, Legua et al. (2011a) also reported higher juice content in the fruit grafted onto Gou Tou Chen than on Macrophylla and Volkameriana. Another study evaluated ‘Lane Late’ oranges grafted onto 10 rootstocks, in which the fruit from the trees on hybrids 030118 and 030127 had the highest juice content, while the fruits from hybrid rootstocks Forner-Alcaide 418 and 030230 had the lowest juice content (Legua, Bellver, Forner & Forner-Giner, 2011b).

França, Amorim, Girardi, Passos & Soares Filho (2016) evaluated the performance of ‘Tuxpan Valencia’ sweet oranges grafted onto 14 rootstocks. These authors reported that the rootstocks which led to fruit with higher juice content were Riverside, Indio citrandarin, TSKFLxCTTR-017, TSKCxCTSW-028, Cravo Santa Cruz lime, Volkameriana, Sunki Tropical mandarin, TSKC x (LCR x TR)–001 and CLEO x CTCZ–226.

Recently Carvalho et al. (2019) studied different varieties of sweet orange (‘Valencia Tuxpan’, ‘Sincora’ and ‘Pineapple) grafted onto six rootstocks. They found that, except for ‘Sincora’ sweet oranges, Red Rough lemon induced lower juice content. Nevertheless, they pointed out that the effects of rootstock on fruit juice depended on annual climate variations and edaphic conditions, which have been related to tree water status. Similarly, Barry, Castle & Davies (2004) observed higher juice content in Carrizo citrange fruit than in Rough lemon fruit, which was also associated with a lower juice osmotic potential.

Martínez-Cuenca, Primo-Capella & Forner-Giner (2016) recorded that the larger the fruit, the thicker the rind and the less juice content. Accordingly, García-Sánchez, Pérez-Pérez, Botía & Martínez (2006) found that the fruit of ‘Clemenules’ mandarin grafted onto Carrizo citrange had a higher juice percentage and a lower peel percentage than those grafted onto Cleopatra. Emmanouilidou and Kyriacou (2017) reported that although thicker rind could predispose fruit to better postharvest performance, it also tended to negatively impact its juice content. Thus ‘Lane Late’ and ‘Delta’ sweet oranges from Volkameriana induced the lowest juice content, due largely to thickened rind. This has been corroborated by Legua et al. (2018) in ‘Fino 49’ lemon, who reported that hybrid rootstock FA 2324 induced thicker peel and lower juice content than rootstock FA 418.

We ought to take into account that juice content can lower due to disorders like granulation. As explained below, rootstock can influence the incidence of this disorder. Sau, Ghosh, Sarkar & Gantait (2018) worked with 'Nagpur' mandarin and observed a negative correlation, more juice content and less granulation. The fruit from rootstocks Kumquat and Rough lemon obtained the lowest percentage of granulation and the highest juice content. On the contrary, the fruit from rootstocks Karna Khatta, Rangpur lime, Lemon and Gandharaj exhibited the highest granulation incidence with the lowest juice content.

5. Sugars and Organic Acids

Many studies have addressed the effect of rootstock on sugars and acids, which are important factors that strongly influence the characteristic flavor of citrus fruit.

Although the effect on the content of sugars and acids depends on the rootstock/scion interaction, some rootstocks have been observed to exert the same effect on different cultivars. This happened with Sour orange, which has been reported to increment total soluble solids (TSS) and titratable acidity (TA) levels in different citrus species scions by delaying commercial maturity compared to other rootstocks. McCollum, Bowman & Castle (2002) studied the effect of different rootstocks on 'Marsh' grapefruit, and found the highest TSS content and acidity in the fruit grafted onto Sour orange. In their study, the Carrizo citrange fruit had the lowest acidity, but the lowest TSS content was found in the Smooth Flat Seville fruit. In 'Washington Navel' oranges, Hifny, Elrazik, Abdrabboh & Sultan (2012) obtained fruit with higher TSS and TA levels when they were grafted onto rootstock Sour Orange than onto rootstock Volkameriana. More recently, this effect has been reported in a study about six rootstocks on 'Lane Late' and 'Delta' oranges (Emmanouilidou & Kyriacou, 2017). In both cultivars, the fruit grafted onto rootstock Volkameriana had the lowest TSS and TA contents, while the fruit from Sour orange and Carrizo citrange had the highest levels. This was also reflected in organic acids and individual sugars. Nevertheless, Bassal (2009) found different results when evaluating four rootstocks for ‘Marisol’ clementine as the fruits grafted onto Carrizo citrange had higher TSS and TA than those from Sour orange.

In some studies, the effect of rootstock has been observed only on TSS, as in ‘Freemont’ tangerine, ‘Okitsu’ mandarin, ‘Clemenules’ mandarin or ‘Rhode Red Valencia’ orange (El-Shafee, 1999; Kaplankiran, Demirkeser & Yildiz, 2005; García-Sánchez et al., 2006; Yildiz, Hakan Demirkeser & Kaplankiran, 2013). Hussain et al. (2013) worked with ‘Clementine’ mandarin from nine rootstocks and found that the highest TSS values went to the fruit from rootstock Poncirus Trifoliate oranges and the lowest values were for the Carrizo citrange fruit. However, these authors found no significant differences in acidity content. In ‘Arrayana’ mandarin grafted onto six different rootstocks, once again no effect was found in fruit acidity, but the influence of rootstock was strong on TSS content (Chaparro-Zambrano, Velásquez-Ramírez & Ordúz-Rodríguez, 2017). The fruit from Sunki x English and Sunki x Jacobson exhibited the highest TSS values, with the lowest for the fruit from Volkameriana. Likewise, in ‘Ray Ruby’ grapefruit grafted onto seven rootstocks, McCollum & Bowman (2017) ran different assays and found that rootstock US-897 induced the highest TSS content, but found no major differences in fruit acidity.

Some authors have addressed the influence of rootstock on the main individual sugars and organic acids. In ‘Lane Late’ oranges, of the four evaluated rootstocks Macrophylla and Volkameriana gave fruit with the lowest citric acid levels, while malic acid was not affected by rootstock (Legua et al., 2011a). In the same study, the highest TSS content went to Macrophylla and Cleopatra, which differed from Volkameriana, while the highest sucrose levels were noted for Cleopatra fruit, and the highest glucose and fructose contents were for Macrophylla fruit. When a study on the effect of rootstock was carried out on ‘Clemenules’ mandarins, Legua et al. (2014) observed that of the 14 rootstocks, the highest sucrose level went to Cleopatra fruit and the lowest to the fruit from Forner-Alcaide 41. The Cleopatra rootstock also induced higher glucose and fructose levels, together with Macrophylla. However, Volkameriana was the rootstock that induced the biggest amount of the three main organic acids: citric, tartaric and ascorbic acid. Another study by Legua et al. (2013), which evaluated the effect of eight new rootstocks hybrids in ‘Lane late’ oranges by comparing them with the most widely used commercial rootstocks (Carrizo citrange and Cleopatra), reported that semi-dwarfing rootstock FA 418 induced higher sucrose and citric acid levels.

Saini, Capalash, Kaur & Singh (2019) also observed significant differences in the content of individual sugars and total sugars of the ‘Kinnow’ mandarin grafted onto different six rootstocks. The highest concentration of total and individual sugars was observed in the fruit grafted onto Cleopatra mandarin and Shekwasha, whereas the lowest ones were recorded for Sour orange and Rough lemon-2. The differences in TSS in fruit were attributed to yield and tree vigor. In that study, a significant effect of rootstock on organic acids was reported. Higher levels of citric acid and total organic acids were induced by Cleopatra mandarin, followed by Sour orange, and the lowest by Rough lemon. In oranges 'Delta' and 'Lane Late', Emmanouilidou & Kyriacou (2017) reported the influence of rootstock on organic acids by showing that the fruit from Carrizo citrange and Sour orange had the highest levels. Specifically, Volkameriana increased the malate levels in both orange scions. In that study, the effect of rootstock on sugar levels was significant for ‘Lane Late’, with the lowest levels of hexoses, sucrose and total sugars obtained for Volkameriana, and the highest for Cleopatra. In ‘Delta’ the same effect was found, but only for fructose and glucose.

The influence of rootstock on sugars and acid content has been related to the inherent rootstock differences that affect plant water relations. These differences include root distribution, water uptake ability, hydraulic conductivity and leaf or stem water potentials (Albrigo & Carter, 1977; Castle & Krezdorn, 1977; Syvertsen & Albrigo, 1980; Bevington & Castle, 1982; Syvertsen & Graham, 1985). The ‘Valencia’ sweet oranges grafted onto Carrizo citrange had more TSS than the fruit from Rough lemon, which was explained by Rough lemon experiencing less water stress than Carrizo citrange due to the larger root distribution of Rough lemon and greater hydraulic conductivity (Barry et al., 2004).

The influence of rootstock on the content of sugars and acids in fruit has also been related to the differences it induces in the leaf photosynthetic rates of scion and photosynthetic product distribution (Morinaga & Ikeda, 1990). The better the scion-rootstock's photosynthetic capacity, the more carbohydrate compounds transported from leaves to fruits (Jover et al., 2012). Moreover, accumulation of sugars in fruit has been related to vascular resistance to sucrose transport at the rootstock's budding union. So the reduced translocation of photoassimilates from leaves to roots limits root development, and also contributes to these compounds being more available in the scion, which results in increased carbon transport toward fruit (Forner-Giner et al., 2014; Martínez-Cuenca et al., 2016).

6. Phenolic Compounds

Citrus fruits contain phenolic compounds, especially flavonoids and phenolic acids. In recent years, more attention has been paid to the phenolic compounds of citrus fruits because many epidemiological studies have indicated that eating polyphenol-rich foods is associated with a reduced risk of cardiovascular diseases and certain cancer forms. It has been suggested that these compounds play an important role in the antioxidant capacity of citrus fruits (Sdiri, Salvador, Farhat, Navarro & Besada, 2014). Moreover, the presence of phenolics contributes to the sensory quality of fruit and juice through their effect on color, bitterness, astringency and flavor (Sousa, da Rocha, Cardoso, Silva & Zanoni, 2004). Among phenolic compounds, special attention has been paid to flavonoid compounds given their potential beneficial effects for human health.

In most species, abiotic stress induces the expression of flavonoid biosynthetic genes and their subsequent accumulation because flavonoids are involved in the regulation of environmental stress responses (Caldwell, Britz, & Mirecki, 2005; Dempsey, Vlot, Wildermuth & Klessig, 2011).

To explain the effect of rootstock on phenolic content, different theories have been proposed and are based on tree vigor, water stress, uptake and the transport of water and minerals and mobile signals (Lockard, Schneider &

Kemp, 1982; Tavarini et al., 2011; Agut, Gamir, Jaques & Flors, 2016; Yildim et al., 2016).

Tavarini et al. (2011) reported that rootstocks affect internal fruit quality through the interaction of water with nutrient availability in soil, which may consecutively affect the synthesis of phytochemicals. Thus reduced water supply may also lead to high levels of phytochemicals, such as phenolic compounds. In response to soil flooding, tolerant citrus rootstocks have been reported to have more flavonoids in leaves than sensitive ones (Djoukeng, Arbona, Argamasilla & Gomez-Cadenas, 2008), which apparently constitutes an adaptive response (Arbona, Manzi, Zandalinas, Vives-Peris, Pérez-Clemente & Gómez-Cadenas, 2017). Souza et al. (2017) evaluated the interaction of 'Valencia Delta' sweet orange with two rootstocks that displayed different tolerances to drought stress. These authors found that the fruit from the tolerant rootstock to long drought stress had flavonoid biosynthesis pathway genes that were overexpressed. The effect of being able to tolerate combined heat and drought conditions has also been studied, where Carrizo citrange was selected as being tolerant and Cleopatra was the sensitive rootstock. Rootstock Cleopatra displays greater activation of flavonoid biosynthesis in leaves, which could mitigate the greater oxidative damage observed in this genotype (Zandalinas, Sales, Beltrán, Gómez-Cadenas & Arbona, 2017).

There have also been reports that phenolic metabolism can be modified by rootstock due to either wounding or stress inflicted by grafting and graft union formation (Koepke & Dhingra, 2013). Emmanouilidou and Kyriacou (2017) evaluated the performance of oranges 'Delta' and 'Lane Late' grafted onto six rootstocks, and found that fruit phenolic content was higher in the least compatible Gou Tou and lower in the highly productive Volkameriana.

Mashayekhi et al. (2013) showed the highest phenol and flavonoid contents in the flavedo of 'Mars' oranges grafted onto Rough lemon compared to rootstocks Carrizo citrange and Cleopatra. In 'Clemenules' mandarin, Volkameriana induced the highest fruit phenolic content of the 14 analyzed rootstocks (Legua et al., 2014). A different scion-dependent effect of rootstock on phenolic content was reported by Sharma et al. (2016). By using the same nine rootstocks, 'Marsh seedless' grapefruit had higher phenolic content grafted onto Sour orange, and the highest values in 'Redblush' grapefruit were for the fruit grafted onto Billikhichli. Legua, Hernández & Forner-Giner (2017) evaluated the effect of three rootstocks on the fruit quality of clementines 'Clemenrubí' and 'Orogrós' and found significant differences in flavonoid content among rootstocks. Rootstock Forner-Alcaide 5 produced the highest flavonoid content, while Carrizo citrange and C-35 citrange had similar values. Recently, Zouaghi, Najar & Abderrabba (2018) observed how 'Maltese' oranges grafted onto Volkameriana and Sour orange had the highest flavonoid content compared to the fruit of six other evaluated rootstocks.

The effect of rootstock on the main individual citrus flavonoids has also been recorded (Cano & Bermejo, 2011; Aghajanpour, Ghasemnezhad, Faghih, & Rastegar, 2015). In general, it has been reported that the dominant flavanone glycosides in Sweet orange rootstock are hesperidin and narirutin, whereas in Sour oranges the two predominant flavanone glycosides are neohesperidin and naringin as reflected in different studies on specific cultivars (Peterson et al., 2006). Hemmati, Ghasemnezhad, Moghaddam & Ebrahimi (2018) studied four orange scions grafted onto four rootstocks, and found that the highest hesperidin content was in the peel of the four scions grafted onto rootstock Shelmahalleh, followed by Citrumelo. Nevertheless, the same study indicated that the highest naringin content was detected in the peel and pulp of all the cultivars grafted onto Sour orange. Naringin has been suggested to be a proprietary constituent of rootstock Sour orange (Peterson et al., 2006). Another study addressed the effect of six rootstocks on 'Kinnow' mandarin (Saini et al., 2019). Sour orange induced the largest amount in flavanones (hesperidin, naringin, narirutin, naringenin and neoeriocitrin) and dihydroxy-B-flavanols (rutin and quercetin). Finally, Rough lemon-2 had a suppressing effect on large amounts of phenolic compounds, which led to a marked reduction in these metabolites compared to other rootstocks.

Similarly, Babazadeh-Darjazi (2018) reported how Flying dragon rootstock induced higher levels of naringenin and total flavonoids than five other studied rootstocks. These authors considered the fact that naringenin chalcone is necessary for the synthesis of flavonoids, and assumed a specialized function for this molecule that could be better performed by Flying dragon. In 'Daisy' mandarins, Feng, Niu, Suh, Hung & Wang (2018) observed how the highest narigenin content was found in Rough lemon fruit when referring to other rootstocks like Carrizo citrange and Trifoliate orange. Hesperitin and eriodyctol were also present in large amounts in Rough lemon fruit, as explained by these compounds deriving from naringenin. However, another study conducted in two grapefruit cultivars revealed that Sour orange had the highest total phenol content in 'Marsh Seedless' fruit and the highest naringenin content in 'Rough lemon' fruit (Mallick, Dubey, Singh & Sharma, 2019). In pigmented citrus cultivars, also called blood oranges, it has been demonstrated that rootstock affects anthocyanin biosynthesis and its accumulation (Continella et al., 2018).

7. Vitamin C

Vitamin C is considered one of most important nutrients in citrus fruit, and is a water-soluble antioxidative component and an excellent reducing agent (Gadjeva, Kuchukova & Georgieva, 2005). Vitamin C content in Citrus sp. depends on species and cultivar (Wang, Chuang, & Ku, 2007; Cano, Medina & Bermejo, 2008; Sdiri, Bermejo, Aleza, Navarro & Salvador, 2012), but also on the other factors like maturity stage, climate and other different agronomic factors (Lee & Kader 2000; Rapisarda, Bianco, Pannuzzo & Timpanaro, 2008).

It has been stated that rootstock affects vitamin C production in citrus fruit, as reported by Magwaza, Mditshwa, Tesfay & Opara, (2017) who collected relevant data about the preharvest factor affecting vitamin C.

Some of the most relevant studies to have addressed the effect of rootstock on vitamin C content in citrus are shown in Table 2.

A positive effect of Volkameriana on Vitamin C has been reported in some oranges (New Hall', 'Navelina', 'Lane Late', 'Spring', 'Fisher' and 'Fukumoto', Washington Navel), mandarins ('Clemenules') and grapefruits ('Marsh' and 'Ruby Red') (Legua et al., 2014; Ramin & Alirezanezhad, 2005; Hikal, 2014; Nasser, Bondok, Shaltout & Mansour, 2014). Nevertheless in other studies on 'Washington Navel' oranges and 'Balady' and 'Fremont' mandarins, which compared the effect of Volkameriana and Sour orange on vitamin C, the fruit grafted onto Sour orange had higher vitamin C levels than those from

Volkameriana (Hifny et al., 2012; Khalifa & Hamdy, 2015). Similarly, with oranges ‘Delta’ and ‘Lane Late’, the fruit from Volkameriana exhibited the lowest vitamin C content than the fruit from five other rootstocks (Emmanouilodou & Kyriacou, 2017).

Table 2. Effect of rootstock in vitamin C in citrus fruit juice.

Cultivar (Country)	**Root. No.**	**Effect of rootstock**		**Reference**
		Highest Vit. C	**Lowest Vit. C**	
Orange				
‘Washington Navel’ (Egypt)	2	Sour orange	Volkameriana	*Hifny et al., 2012*
‘Washington Navel’ (Egypt)	4	Volkameriana	Sour orange Troyer citrange Rangpur lime	*Hikal, 2014*
‘New Hall’, ‘Navelina’, ‘Lane Late’, ‘Spring’, ‘Fisher’ and ‘Fukumoto’ Navel (Egypt)	2	Volkameriana	Sour orange	*Nasser et al., 2014*
‘Lane Late’ (Spain)	6	Cleopatra	Forner-Alcaide 41	*Cardeñosa et al., 2015*
‘Delta’ and ‘Lane Late’ (Cyprus)	6		Volkameriana	*Emmanouilidou and Kyriacou, 2017*
Mandarin				
‘Marisol’ (Egypt)	4	Cleopatra Carrizo citrange Citrumelo	Sour orange	*Bassal, 2009*
‘Clemenules’ (Spain)	14	Volkameriana		*Legua et al., 2014*
‘Balady’ and ‘Fremont’ (Egypt)	2	Sour orange	Volkameriana	*Khalifa & Hamdy, 2015*
‘Nagpur’ (India)	7	Kumquat		*Sau et al, 2018*
‘Kinnow’ (India)	6	Rough lemon	Shekwasha Pectinifera	*Saini et al., 2019*
Grapefruit				
‘Marsh’ ‘Ruby Red’ (Iran)	8	C.Amblycarpa Volkameriana Cleopatra Sour orange		*Ramin& Alirezanezhand, 2005*
‘Marsh Seedless’ ‘Redblush’ (India)	8	Attani-2		*Sharma et al., 2016*
Lemon				
‘Kagzi Kalan’	8	RLC-4 Troyer citrange	Karna Khatta	*Dubey & Sharma, 2016*

Cleopatra is another rootstock that some studies have reported as being able to induce incremental vitamin C content. The ‘Lane Late’ oranges from Cleopatra exhibited the highest vitamin C levels of the fruit from six other evaluated rootstocks (Cardeñosa, Barros, Barreira, Arenas, Moreno-Rojas & Ferreira, 2015). Similar results were found by Bassal (2009), who evaluated vitamin C content in ‘Marisol’ clementines grafted onto four different rootstocks, and found that Cleopatra induced the largest amount, but observed no significant differences with Carrizo citrange and Swingle citrumelo. Ramin & Alirezanezhand (2005) also reported that Cleopatra is one of the rootstocks that led to fruit having the highest vitamin C content of eight evaluated rootstocks.

Sharma et al. (2016) also reported differences in vitamin C in grapefruits ‘Marsh Seedless’ and ‘Redblush’ when evaluating the fruit from eight rootstocks. They found that Attani-2 induced higher contents. In ‘Kagzi Kalan’ lemons, the effect of rootstock on vitamin C has also been recorded (Dubey & Sharma, 2016).

It is known that rootstock interferes with the nutritional status of citrus trees due to differences in physical root systems and in water and mineral uptake (Georgiou, 2002; Romero et al., 2006). The mineral uptake ability of rootstocks can be reflected in vitamin C levels in fruit. Regarding macronutrients, the presence of high P and N contents can lower the amount of vitamin C (Lee & Kader, 2002; Dou, Jones, Obreza, & Rouse, 2005), and a considerable amount of K can increase the amount of vitamin C (Patil & Alva, 2002; Dou et al., 2005). In terms of micronutrients, Zn can increase vitamin C concentrations (Eman, El-moneim, El Migeed, Omayma & Ismail, 2007). However, no studies have been found in which the relation between the influence of rootstock on vitamin C content and the mineral uptake ability of rootstocks has been specifically addressed.

8. Antioxidant Activity

The antioxidant activity (AA) of citrus fruit is an important nutritional claim. Like other parameters, AA has been related to the effect of rootstock. Recently in ‘Sweetie’ grapefruit grafted onto four different rootstocks, the least AA was found in the fruit budded onto rootstock Citrumelo in organic and conventional cultivation terms (Klein et al., 2017).

In citrus fruit, AA has been related mainly to the presence of total phenolic compounds and vitamin C (Raveh, Saban, Zipi & Beit-Yannai, 2009; Zou, Xi, Hu, Nie & Zhou, 2016). In ‘Lane late’ sweet orange grafted onto six different rootstocks, Cleopatra fruit had the greatest AA concomitantly with the highest vitamin C level. Likewise, the lowest levels of both parameters were observed in the fruit from Forner-Alcaide 41 compared to the other five studied rootstocks (Hamilton, Gundel, Helander & Saikkonen, 2012). However, another study about the effect of rootstock on amounts of total phenolic compounds, vitamin C and AA in ‘Kinnow’ mandarins grafted onto six rootstocks reported the greatest AA in Sour orange and the least AA in Rough lemon-2, both of which were attributed to the levels of phenolic compounds observed in these fruits (Saini et al., 2019).

Antioxidant activity increases by reactive oxygen species (ROS) in response to abiotic and biotic stress (Hamilton et al., 2012). So rootstock also influences this parameter as it is used as a tool to tolerate these different stresses in plant trees. By studying the effect of three rootstocks on the AA of ‘Thomson’ navel oranges at low temperature, the Trifoliate orange fruit had the highest AA level. It was concluded that this rootstock could improve tolerance to freezing stress (Tajvar, Ghazvini, Hamidoghli & Sajedi, 2011).

Antioxidant activity is due to a lipophylic fraction, such as tocopherols and carotenoids, and also to a hydrophilic fraction, such as total phenolic compounds or vitamin C. Legua et al. (2014) reported that both fractions can be affected by rootstocks. In ‘Clemenules’ mandarin grafted onto 14 different rootstocks, the biggest lipophilic fraction was presented in the fruit juice from Carrizo citrange. No differences were observed with the fruit from Citrumelo, Macrophylla and Forner-Alcaide 5, with the smallest fraction found in the fruit from Forner-Alcaide 2324. The effect of rootstock differs for the hydrophylic fraction. The biggest fraction was detected in the fruit grafted onto Forner-Alcaide 41, with no significant differences to the fruit from Citrumelo, Volkameriana, Forner-Alcaide 418 and Forner-Alcaide 21. The smallest fraction was noted in the juice fruit from rootstocks Forner-Alcaide 5, Forner-Alcaide 13 and C-35.

With other crops like peach, a relation between rootstock vigor and AA has been reported. Whereas semivigorous rootstocks induce high AA, less vigorous rootstocks exhibit the least AA (Orazem, Stampar & Hudina, 2011). This fact has been not addressed in citrus.

9. Fruit Disorders

Rootstock has been reported to influence the development of citrus disorders. The main effects on physiological disorders are described in Table 3.

Albedo breakdown (also referred to as ‘creasing’) is one of the most prominent pre-harvest rind disorders to limit the suitability of mandarins and oranges for fresh fruit markets. Albedo breakdown is characterized by the outer colored portion of flavedo folding inwardly into channels to form the underlying albedo tissue as adjacent albedo cells separate (Alirezanezhad & Ramin, 2006). This disorder has been reported to be influenced by scion, rootstock, rind thickness and Ca levels (Treeby, Storey & Bevington, 1995; Storey, Treeby & Milne, 2002; Storey & Treeby, 2002; Treeby, Henriod, Bevington, Milne & Storey, 2007).

The effect of rootstock on albedo breakdown has been related to water uptake capacity. Treby et al. (2007) demonstrated the major influence of rootstock genotype on the response to irrigation management. A study carried out during two seasons established that the response to albedo breakdown incidence in ‘Bellamy Navel’ oranges depended on rootstock. Rootstocks Troyer, Carrizo citrange and Trifoliate orange induced higher percentages of affected fruit than Cleopatra and Sweet oranges. Under deficit irrigation conditions, while fruit budded onto Sweet orange halved the incidence of this disorder, and those from rootstocks Troyer citrange and Carrizo citrange exhibited a major reduction, and the fruit from Cleopatra and Trifoliate orange displayed no effect.

Table 3. Effect of rootstock on peel disorders of citrus fruit.

Cultivar (Country)	Root. No.	Rootstock's Effect		Reference
		High disorder	Low disorder	
Albedo Breakdown				
'Navelate' Sweet orange (Spain)	3	Carrizo citrange	Sour orange	*Agustí et al., 2003*
'Bellamy' Navel orange	5	Normal Conditions Carrizo citrange* Troyer citrange* Trifoliate orange *: <50% Reduction after Deficit Irrigation **: 50% Reduction after Deficit Irrigation	 Cleopatra** Sweet orange	*Treeby et al., 2007*
'Chislett' Navel orange	4	Carrizo citrange C-35	Cleopatra FA5	*Mesejo et al., 2016*
'Nova' mandarin	2	Carrizo citrange	FA	
'ClemenRuby' mandarin (Spain)	2	Carrizo citrange	Poncirus Trifoliate	
Oleocellosis				
'Hamlin' Sweet orange (China)	3	Rangpur lime	Lichi 16-6 Trifoliate Gou Tou Cheng	*Zheng et al., 2011* *Zheng et al., 2018*
Peel Pitting				
'Nadorcott' mandarin (South Africa)	2	Rough lemon	Carrizo citrange	*Cronjé, 2013*
'Valencia' orange (Australia)	3	Symons Sweet orange	Rangpur lime Emperor	*El-Zeftawi et al., 1989*
'Nova' and 'Fremont' mandarin (Turkey)	3	Carrizo citrange	Sour orange	*Özdemir et al., 2016* *Özdemir et al., 2019*
Stem-End Rind Breakdown				
'Marsh' grapefruit (Florida)	6	Sour orange		*McCollum et al., 2002*
'Valencia' orange	5	US-952	Gou Tou	*Ritenour et al., 2004a*
'Ray Ruby' and 'Oroblanco' grapefruit (Florida)	4 9	No differences		
Chilling Tolerance				
'Clementine' mandarin (France)	2	Tetraploid Carrizo citrange	Diploid Carrizo citrange	*Oustric et al., 2017*
Decay				
'Washington' orange (Egypt)		Sour orange	Volkameriana	*Hifny et al.,2012*
'Marsh' and 'Ruby Red' grapefruit (Iran)	8	King Mandarin	Volkameriana	*Alirezanezhad & Ramin, 2006*
Granulation (Pulp)				
'Kinnow' mandarin (Pakistan)	9	Volkameriana	Rough lemon citrange (x3)	*Ahmed et al., 2006*
'Hamlin' orange (Oman)	6	Cleopatra Volkameriana	Acid lime Sour orange	*Al-Hosni et al., 2008*

Agusti, Almela, Juan, Mesejo & Martinez-Fuentes (2003) evaluated the influence of three rootstocks on the rind breakdown of ‘Navelate’ sweet oranges. Carrizo citrange induced the highest rind breakdown level, followed by Cleopatra and Sour orange. This fact correlated with fruit-tree water relations due to the average xylem vessel diameter of the peduncles fruit on Carrizo citrange being 6% and 17% larger than those on Cleopatra and Sour orange, respectively. Later the effect of rootstock on the size of xylem vessel diameter in the peduncles zone was correlated with breakdown incidence (Mesejo, Reig, Martínez-Fuentes, Gambetta, Gravina & Agustí, 2016). In that study, ‘Chislett’ navel orange exhibited a high fruit split percentage when fruit were grafted onto Carrizo citrange and C-35 than for the fruit grafted onto rootstocks Cleopatra and Forner-Alcaide 5. Similarly, ‘Nova’ mandarin had a higher incidence when budded onto Carrizo citrange than onto Forner-Alcaide 5, and the same applied to ‘Clemenruby’ mandarin when grafted onto Carrizo citrange than onto Poncirus trifoliata. In all cases, the higher percentages of damage due to rootstocks coincided with a larger vessels diameter.

Oleocellosis (or oil spotting) is a physiological disorder that occurs after the peel oil gland ruptures, which causes visible pitting due to the released oil that is phytotoxic to pericarp cells (Shomer & Erner, 1989; Chikaizumi, 2000; Montero, Schwarz, dos Santos, dos Santos & Bender, 2012). Zheng et al. (2011; 2018) studied tolerance of on-tree oleocellosis in ‘Hamlin’ sweet oranges grafted onto three different rootstocks. They observed that the fruits grafted onto Rangpur lime exhibited the lowest oleocellosis incidence, which was related to the ability to adjust the water potential and antioxidant enzyme activities in trees. The peel of the fruit grafted onto Rangpur lime maintained a higher water potential and relative water content.

Peel pitting is a common postharvest citrus disorder that can be manifested at chilling or non-chilling temperatures. Postharvest peel pitting at non chilling temperatures is a physiological disorder that affects the fruit of several citrus cultivars worldwide. This disorder affects subepidermal cells on fruit surfaces (Agustí, Almela, Juan, Alférez, Tadeo & Zacarías, 2001; Lafuente & Sala, 2002; Alférez, Agustí & Zacarías, 2003; Alférez, Zacarías & Burns, 2005). Previous reports have related peel water status to peel pitting, and have suggested that variation in water relations within fruit peel can contribute to this disorder (Alférez, Alquezar, Burns & Zacarías, 2010). This postharvest disorder has been reported to be affected by rootstock. In 'Nadorcott' mandarin, and greater susceptibility of the fruit from Rough lemon rootstocks compared to those from Carrizo citrange was found. This fact was related to the lesser ability of the fruit rind from Rough lemon to prevent water loss (Cronjé, 2013).

As for peel pitting appearing as a response to chilling temperatures, El-Zeftawi, Peggie & Minnis (1989) stated that 'Newton late Valencia' oranges, stored at 5-15°C for 18 days, had a higher peel disorder incidence when grafted onto rootstock Symons Sweet orange than those grafted onto rootstocks Rangpur lime or Emperor. Özdemir, Didin, Candir, Kaplankiran & Yildiz (2019) studied 'Nova' mandarin and corroborated the influence of rootstock on peel pitting during storage at 4°C and 6°C. The fruit budded onto Troyer and Carrizo citrange exhibited higher percentages of fruit with this disorder than the fruit budded onto Sour orange. 'Fremont' mandarin grafted onto Carrizo citrange also displayed a higher incidence of physiological disorders than when grafted onto Sour orange after cold storage (Özdemir et al., 2016). With grapefruit cultivars, higher incidence of chilling injury was observed in 'Marsh' grapefruit stored at 5°C when fruit were grafted onto Sour orange than when grafted onto five other rootstocks (McCollum et al., 2002). Recently, Oustric et al. (2017) studied the effect of tetraploid and diploid rootstocks on the chilling tolerance of common clementine, and reported better chilling resistance for the leaves of clementines grafted onto tetraploid rootstocks compared to diploid ones, which was related to the antioxidant system. Hence it would be interesting to study how this effect would be reflected on fruit quality.

Rootstock has also been reported to affect the development of stem-end rind breakdown (SERB) (Agustí, 1999; Ritenour, Stover, Boman, Dou, Bowman & Castle, 2004a). This disorder has been related to water peel status, and the water stress that leads to SERB in citrus fruit may regulate phospholipase gene expression and involves ABA signaling (Romero, Gandía & Alférez, 2013). Ritenour et al. (2004a) examined the rootstock effect on SERB incidence, and observed how 'Valencia' Navel oranges consistently showed considerable SERB damage on the fruit grafted onto rootstock US-952, but less damage on the fruit from rootstock Gou Tou. However, these authors did not report any rootstock effect on grapefruits 'Ray Ruby' and 'Oroblanco'.

The influence of rootstock on citrus fruit decay has also been reported. Alirezanezhad & Ramin (2006) evaluated eight different rootstocks in grapefruits 'Marsh' and 'Ruby Red' and observed the highest decay percentage in King Mandarin fruit and the lowest incidence in Volkameriana fruit. The decay incidence in 'Washington' Navel oranges was also lower in the fruit grafted onto Volkameriana than on those grafted onto Sour orange (Hifny et al., 2012). In other studies, no influence of rootstock has been observed on the decay incidence (Soleimani, Tabil, Mirmajidi, & Shafieezargar, 2007; Özdemir et al., 2019).

Regarding internal quality, granulation is one of the most important pre-harvest disorders to seriously affect citrus commercialization. It is a disorder of juice sacs of citrus fruit when they become hard, dry and enlarged, take a grayish color and have only a little free juice (Agustí, 1999). In most cases, a positive relation between the granulation incidence and rootstock vigor has been reported, and this effect has been related to the vigor that rootstock confers (Kumar et al., 1994). Kotsias (2004) reported that 'Valencia' oranges grafted onto Poncirus Trifoliate, a dwarfing rootstock, showed less granulation than the fruit grafted onto Citrus Aurantium, a vigorous rootstock. In 'Kinnow' mandarin, the granulation incidence was very high in the fruit from the trees grafted onto vigorous rootstock Sohsarkar, but was very low in the fruit from the trees grafted onto Troyer citrange, a dwarfing rootstock (Sharma & Saxena, 2004). Those authors also found that pectinesterase and diastase enzyme activities, which are closely related to granulation, were less marked in granulated fruit than in unaffected fruit, and were significantly influenced by rootstocks.

Al-Hosni, Mustafa, Al-Busaidi, Al-Jabri & Al-Azri (2008) also stated that the number of granulated fruits in 'Hamlin' oranges was affected by rootstock selection. The most affected fruit was that grafted onto Cleopatra followed by Volkameriana, while rootstocks Acid lime and Sour orange induced lower fruit damage incidence. 'Kinnow' mandarin gave a higher granulation incidence when grafted onto Volkameriana than onto rootstocks Rough lemon and three citrange ones (Ahmed, Pervez, Amjad, Khalid, Ayyub & Nawaz, 2006). Nevertheless, it is noteworthy that besides the influence of rootstock, the incidence of this disorder depended on other factors like cultivar, agroclimatic conditions and genetic differences (Sharma & Saxena, 2004).

10. Volatile Compounds

Essential oil (EO) is the volatile liquid fraction that is usually distilled by steam stripping. EO contains mixtures of terpenic hydrocarbons and oxygenated compounds, such as aldehyde, alcohol, ketone and ester derivatives, which are responsible for fruit aroma. The principal component in all citrus fruit EOs is the monoterpene limonene (Palma, Cruz, Cruz, Bugayong, & Castillo, 2019).

Regarding 'Page' mandarin grafted onto eight rootstocks, Babazadeh-Darjazi (2009) stated that juice and peel oil from the fruit grown on Citrumelo Swingle and Yuzu had the highest aldehydes content, which is one of the most important fractions of citrus volatile compounds.

Benjamin, Tietel & Porat (2013) carried out an in-depth study into the influence of five rootstocks on the volatile composition of pulp fruit of mandarins 'Or' and 'Odem', 'Valencia' oranges and 'Redson' grapefruits. The results revealed that despite the wide variability in volatile compounds due to the grafting combination, the fruit grown onto rootstock Volkameriana had the lowest levels of volatile compounds. Regarding mandarins, 'Or' from Sour orange had the highest levels of aldehydes nonanal and decanal, and 'Odem' from rootstock US-812 presented the most aroma volatiles. In the last cultivar, these differences were due to the levels of linalool (alcohol), perillaldehyde and dodecanal (aldehydes), β-pinene, limonene and γ-terpinene (monoterpenes). The 'Redson' grapefruit from Sour orange had larger amounts of 19 volatile compounds than rootstocks Volkameriana and Macrophylla. In 'Valencia' oranges, not all the volatile compounds were affected by rootstock in the same way. While carvone compound levels were higher in the Sour orange fruit than in those from rootstocks Volkameria and x639, the levels of α-pinene, sabinene, β-pinene, limonene (terpenes) and copaene (sesquiterpene) were higher in the fruit from rootstock x639 than in those from rootstocks Sour orange and Volkameriana.

Saini et al. (2019) studied four different rootstocks and found that most volatile compounds were differently affected depending on rootstock. The juice of the ‘Kinnow’ mandarin from Pectinifera had the highest total volatile contents because this rootstock induced the highest limonene levels. Nevertheless, Shekwasha was the rootstock that induced the highest levels of β-pinene, dodecyl aldehyde, octanal, α-terpineol, terpinen-4-ol, perialdehyde, nonanal, isoleucine, linalool and hexanal, while rootstock Sour orange induced the highest trans-β-ionone concentrations. Even the Cleopatra rootstock, which induced the lowest levels of total volatile compounds, led to the highest ethyl acetate levels.

Apart from the influence of the amount of volatile compounds on fruit, rootstock has also been described as having an effect on the presence or absence of certain volatile compounds. This was the case of ‘Persian’ lime grafted onto five rootstocks, in which β-myrcene was found only in the fruit from rootstocks Sour orange and Flying dragon. Likewise, β-thujene and dodecane were detected only in Volkameriana and C-35, respectively. Linalool was present in the fruit from all the rootstocks, except in the Sour orange fruit (Raddatz-Mota, Franco-Mora, Mendoza-Espinoza, Rodríguez-Verástegui, de León-Sánchez & Rivera-Cabrera, 2019).

11. Conclusion

Rootstocks significantly impact the internal and external quality parameters of citrus fruit. Nevertheless, the influence of a specific rootstock on citrus quality is highly dependent on the cultivar, and also on climate conditions and cultural practices. They all need to be taken into account when making decisions about the rootstock to be used in each specific case.

The influence of the rootstock on fruit quality has been linked with the water uptake capacity of roots. Nevertheless, there are few studies that address in depth the absorption of nutrients from the soil by rootstocks. The knowledge about the nutrients translocation thought the rootstock-cultivar graft union and its effect on fruit quality is crucial to select the optimal rootstock in soils with a certain mineral composition.

On the other hand, many studies have addresed the influence of rootstock on endogenous production of primary and secondary metabolites such as sugars, acids, volatiles and vitamins, which in turn are involve on the fruit quality. Nevertheless, few attempts have made to elucidate the mechanism underlying rootstock-induced phenotypic changes. The molecular mechanism related to fruit quality affected by rootstock is unclear and it becomes essential due to increasing consumer demand for high quality fruit.

Metabolomics and transcriptomics tools are needed to explore the candidate genes involved in the metabolic processes affected by rootstock. Knowledge of changes in gene expression caused by the rootstock-scion interaction can would provide useful information to develop or select genotypes for future rootstock breeding programs.

12. Acknowledgments

This study has been supported by Instituto Valenciano de Investigaciones Agrarias and co-financed by European Social Fund.

13. References

Aghajanpour, S.M., Ghasemnezhad, A., Faghih, N.M., & Rastegar, M. (2015). Study the effect of rootstock on the fruit hesperidin and naringin contents of five commercial mandarins. Plant Production Technology, 14(2), 119-126.

Agustí, M. (1999). Preharvest factors affecting postharvest quality of citrus fruit. Advances in postharvest diseases and disorders control of citrus fruit. Trivandrum: Research Signpost, 1-34.

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F.R., & Zacarias, L. (2001). Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Annals of Botany, 88(3), 415-422. https://doi.org/10.1006/anbo.2001.1482

Agusti, M., Almela, V., Juan, M., Mesejo, C., & Martinez-Fuentes, A. (2003). Rootstock influence on the incidence of rind breakdown in 'Navelate' sweet orange. The journal of horticultural science and biotechnology, 78(4), 554-558. https://doi.org/10.1080/14620316.2003.11511662

Agut, B., Gamir, J., Jaques, J.A., & Flors, V. (2016). Systemic resistance in citrus to Tetranychus urticae induced by conspecifics is transmitted by grafting and mediated by mobile amino acids. Journal of Experimental of Botany, 67(19), 5711-5723. https://doi.org/10.1093/jxb/erw335

Ahmed, W., Pervez, M.A., Amjad, M., Khalid, M., Ayyub, C.M., & Nawaz, M.A. (2006). Effect of stionic combination on the growth and yield of kinnow mandarin (Citrus reticulata blanco). Pakistan journal of botany, 38(3), 603.

Albrecht, U., McCollum, G., & Bowman, K.D. (2012). Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (Citrus sinensis [L.] Osbeck) trees. Scientia Horticulturae, 138, 210-220. https://doi.org/10.1016/j.scienta.2012.02.027

Albrigo, L.G. & Carter, R.D. (1977). Citrus Science and Technology. Nagy, S.

Alférez, F., Agusti, M., & Zacarías, L. (2003). Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biology and Technology, 28(1), 143-152. https://doi.org/10.1016/S0925-5214(02)00120-5

Alférez, F., Alquezar, B., Burns, J.K., & Zacarías, L. (2010). Variation in water, osmotic and turgor potential in peel of ‘Marsh’grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology, 56(1), 44-49. https://doi.org/10.1016/j.postharvbio.2009.12.007

Alférez, F., Zacarias, L., & Burns, J.K. (2005). Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. Journal of the American Society for Horticultural Science, 130(2), 225-231. https://doi.org/10.21273/JASHS.130.2.225

Al-Hosni, A.S., Mustafa, S., Al-Busaidi, K., Al-Jabri, M., & Al-Azri, H. (2008). Effects of different citrus rootstocks on growth, yield, quality and granulation of 'Hamlin' orange in Oman. In IX International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems 903, 563-568.

Alirezanezhad, R.A. & Ramin, A. (2006). Effect of eight citrus rootstocks on storage life of grapefruit cultivars Marsh and Ruby Red. Iranian Journal of Agricultural Sciences, 37(3), 447-455.

Al-Jaleel, A., Zekri, M., & Hammam, Y. (2005). Yield, fruit quality, and tree health of ‘Allen Eureka’ lemon on seven rootstocks in Saudi Arabia. Scientia horticulturae, 105(4), 457-465. https://doi.org/10.1016/j.scienta.2005.02.008

Al-Obeed, R.S., Harhash, M.M., & Sourour, M.M. (2005). Performance of" Marsh" grapefruit and" Mexican" lime trees on seven rootstocks in Saudi Arabia. Journal of Advanced Agriculture Research, 10(1), 165-179.

Alós, E., Cercós, M., Rodrigo, M.J., Zacarías, L., & Talón, M. (2006). Regulation of color break in citrus fruits. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening

retardants. Journal of Agricultural and Food Chemistry, 54(13), 4888-4895.
https://doi.org/10.1021/jf0606712

Arbona, V., Manzi, M., Zandalinas, S. I., Vives-Peris, V., Pérez-Clemente, R. M., & Gómez-Cadenas, A. (2017). Physiological, metabolic, and molecular responses of plants to abiotic stress. In Stress Signaling in Plants: Genomics and Proteomics Perspective, 1st Ed.; Sarwat, M., Ahmad, A., Abdin, M.Z, & Ibrahim, M.M. Eds.; Volume 2. Springer, Cham, Switzerland, 1-35.
https://doi.org/10.1007/978-3-319-42183-4_1

Babazadeh-Darjazi, B. (2018). The effect of rootstocks on the peel phenolic compounds, cartenoids, chlorophylls and ethylene of Younesi tangerine (Citrus reticulata). Plant Physiology, 8(2), 2371-2379.
https://doi.org/10.22034/IJPP.2018.539177

Babazadeh-Darjazi, B., Rustaiyan, A., Talaei, A., Khalighi, A., Golein, B., Hayatbakhsh, E., & Taghizad, R. (2009). The effects of rootstock on the volatile flavour components of page mandarin [(C. reticulata var. Dancy C. paradisi var Dancan) C. clementina] juice and peel. Iranian Journal of Chemistry and Chemical Engineering, 28(2), 99-111.

Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. Journal of the American Society for Horticultural Science, 129(6), 881-889.
https://doi.org/10.21273/JASHS.129.6.0881

Bassal, M.A. (2009). Growth, yield and fruit quality of ‘Marisol’ clementine grown on four rootstocks in Egypt. Scientia Horticulturae, 119(2), 132-137.
https://doi.org/10.1016/j.scienta.2008.07.020

Benjamin, G., Tietel, Z., & Porat, R. (2013). Effects of rootstock/scion combinations on the flavor of citrus fruit. Journal of agricultural and food chemistry, 61(47), 11286-11294. https://doi.org/10.1021/jf402892p

Berdeja-Arbeu, R., Méndez, L.A., Velázquez, D.M., Huerta, G.V., Capurata, R.E.O., & Martínez, A.I. (2016). Calidad de fruta de lima 'Persa' en diferentes portainjertos en Veracruz, México. Acta Agrícola y Pecuaría, 2(1), 17-22.

Bevington, K.B., & Castle, W.S. (1982). Development of the root system of young Valencia orange trees on rough lemon and Carrizo citrange rootstocks. In Procedings of Florida State of Horticultural Society, 95, 33-37.

Caldwell, C.R., Britz, S.J., & Mirecki, R.M. (2005). Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [Glycine max (L.) Merrill] grown in controlled environments. Journal of agricultural and food chemistry, 53(4), 1125-1129. https://doi.org/10.1021/jf0355351

Cano, A., & Bermejo, A. (2011). Influence of rootstock and cultivar on bioactive compounds in citrus peels. Journal of the Science of Food and Agriculture, 91(9), 1702-1711. https://doi.org/10.1002/jsfa.4375

Cano, A., Medina, A., & Bermejo, A. (2008). Bioactive compounds in different citrus varieties. Discrimination among cultivars. Journal of Food Composition and Analysis, 21(5), 377-381. https://doi.org/10.1016/j.jfca.2008.03.005

Cardeñosa, V., Barros, L., Barreira, J.C., Arenas, F., Moreno-Rojas, J.M., & Ferreira, I.C. (2015). Different Citrus rootstocks present high dissimilarities in their antioxidant activity and vitamins content according to the ripening stage. Journal of plant physiology, 174, 124-130. https://doi.org/10.1016/j.jplph.2014.10.013

Carvalho, L.M., de Carvalho, H.W., de Barros, I., Martins, C.R., Soares Filho, W. D.S., Girardi, E.A., & Passos, O.S. (2019). New scion-rootstock combinations for diversification of sweet orange orchards in tropical hardsetting soils. Scientia Horticulturae, 243, 169-176. https://doi.org/10.1016/j.scienta.2018.07.032

Castle, W.S. (2010). A career perspective on citrus rootstocks, their development, and commercialization. HortScience, 45(1), 11-15. https://doi.org/10.21273/HORTSCI.45.1.11

Castle, W.S., & Krezdorn, A.H. (1977). Soil water use and apparent root efficiencies of citrus trees on four rootstocks. Journal American Society for Horticultural Science, 102, 403-406.

Chaparro-Zambrano, H.N., Velásquez-Ramírez, H.A., & Ordúz-Rodríguez, J.O. (2017). Evaluation of 'Arrayaría' tangerine (Citrus reticulata Blanco) grafted onto different rootstocks in tropical lowlands of Colombian Orinoquia, 2005-2011 (second cycle). Agronomía Colombiana, 35(1), 29-34. http://dx.doi.org/10.15446/agron.colomb.v35n1.60082

Chapman, H.D. (1968). The mineral nutrition of citrus. In The Citrus Industry, vol. 2, 1st Ed.; Reuther, W., Webber, H.J., Batchelor, L.D., Eds.; University of California, Division of Agricultural Sciences, Berkeley, CA, USA, 127-189.

Chikaizumi, S. (2000). Mechanisms of Rind-oil Spot Development in 'Encore' (Citrus nobilis Lour. × C. deliciosa Ten.) Fruit. Journal of the Japanese Society for Horticultural Science, 69(2), 149-155. https://doi.org/10.2503/jjshs.69.149

Continella, A., Pannitteri, C., La Malfa, S., Legua, P., Distefano, G., Nicolosi, E., & Gentile, A. (2018). Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange. Scientia horticulturae, 230, 62-67. https://doi.org/10.1016/j.scienta.2017.11.006

Cronjé, P.J.R. (2013). Postharvest rind disorders of 'Nadorcott' mandarin are affected by rootstock in addition to postharvest treatments. In II All Africa Horticulture Congress, 1007, 111-117. https://doi.org/10.17660/ActaHortic.2013.1007.9

Demirkeser, T.H., Kaplankıran, M., Toplu, C., & Yıldız, E. (2009). Yield and fruit quality performance of Nova and Robinson mandarins on three rootstocks in Eastern Mediterranean. African Journal of Agricultural Research, 4(4), 262-268. https://doi.org/10.5897/AJAR. 9000766

Dempsey, D.M.A., Vlot, A., Wildermuth, M.C., & Klessig, D.F. (2011). Salicylic acid biosynthesis and metabolism. The Arabidopsis book/American Society of Plant Biologists, 9, e-156, 1-24. https://doi.org/10.1199/tab.0156

Djoukeng, J. D., Arbona, V., Argamasilla, R., & Gomez-Cadenas, A. (2008). Flavonoid profiling in leaves of citrus genotypes under different environmental situations. Journal of Agricultural and Food Chemistry, 56(23), 11087-11097. https://doi.org/10.1021/jf802382y

Dou, H., Jones, S., Obreza, T., & Rouse, B. (2005). Influence of various phosphorus and potassium rates on juice vitamin C, [beta]-carotene, lycopene and sugar concentrations of flame grapefruit. In Proceedings of the Florida State Horticultural Society, 118, 372-375.

Dubey, A.K., & Sharma, R.M. (2016). Effect of rootstocks on tree growth, yield, quality and leaf mineral composition of lemon (Citrus limon (L.) Burm.). Scientia horticulturae, 200, 131-136. https://doi.org/10.1016/j.scienta.2016.01.013

El-Shafee, E. M. H. (1999). Studies on the effect of some citrus rootstocks on growth and productivity of some mandarin cultivars. Thesis in Pomologycal Faculty of Agriculture, Minufiya University, Egypt.

El-Zeftawi, B.M., Peggie, I.D., & Minnis, D.C. (1989). Postharvest treatments, storage temperature and rootstocks in relation to storage disorders and fruit quality of 'Valencia' oranges. Journal of Horticultural Science, 64(3), 373-378. https://doi.org/10.1080/14620316.1989.11515967

Eman, A.A., Abd El-moneim, A., El Migeed, M.A., Omayma, A., & Ismail, M.M. (2007). GA3 and zinc sprays for improving yield and fruit quality of Washington Navel orange trees grown under sandy soil conditions. Research Journal of Agriculture and Biological Sciences, 3(5), 498-503.

Emmanouilidou, M.G., & Kyriacou, M.C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of 'Lane Late' and 'Delta' sweet orange. Scientia horticulturae, 225, 112-121. https://doi.org/10.1016/j.scienta.2017.06.056

Feng, S., Niu, L., Suh, J.H., Hung, W.L., & Wang, Y. (2018). Comprehensive metabolomics analysis of mandarins (citrus reticulata) as a tool for variety, rootstock, and grove discrimination. Journal of agricultural and food chemistry, 66(39), 10317-10326. https://doi.org/10.1021/acs.jafc.8b03877

Filho, F.D.A.A., Espinoza-Núñez, E., Stuchi, E.S., & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. Scientia Horticulturae, 114(1), 45-49.

https://doi.org/10.1016/j.scienta.2007.05.007

Forner-Giner, M.A., Alcaide, A., Primo-Millo, E., & Forner, J.B. (2003). Performance of 'Navelina' orange on 14 rootstocks in Northern Valencia (Spain). Scientia Horticulturae, 98(3), 223-232. https://doi.org/10.1016/S0304-4238(02)00227-3

Forner-Giner, M.A., Rodriguez-Gamir, J., Martínez-Alcántara, B., Quinones, A., Iglesias, D.J., Primo-Millo, E., & Forner, J. (2014). Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). Scientia Horticulturae, 179, 376-387. https://doi.org/10.1016/j.scienta.2014.07.032

França, N.D.O., Amorim, M.D.S., Girardi, E.A., Passos, O.S., & Soares Filho, W.D.S. (2016). Performance of 'Tuxpan Valencia' sweet orange grafted onto 14 rootstocks in northern Bahia, Brazil. Revista Brasileira de Fruticultura, 38(4), e-684(1-9) https://doi.org/10.1590/0100-29452016684

França, N.D.O., Girardi, E.A., Amorim, M.D.S., Gesteira, A.D.S., Passos, O.S., & Soares Filho, W.D.S. (2018). Plant growth, yield and fruit quality of 'Piemonte' tangor grafted onto 14 rootstocks on the northern coast of the state of Bahia, Brazil. Revista Brasileira de Fruticultura, 40(4), e-784 (1-8). https://doi.org/10.1590/0100-29452018784

Fu, L., Chai, L., Ding, D., & Pan, Z. (2016). A novel citrus rootstock tolerant to iron deficiency in calcareous soil. Journal of the American Society for Horticultural Science, 141(2), 112-118. https://doi.org/10.21273/JASHS.141.2.112

Gadjeva, V., Kuchukova, D., & Georgieva, R. (2005). Vitamin combinations reduce oxidative stress and improve antioxidant status in patients with iron deficiency anemia. Comparative Clinical Pathology, 14(2), 99-104. https://doi.org/10.1007/s00580-005-0560-8

García-Sánchez, F., Pérez-Pérez, J.G., Botía, P., & Martínez, V. (2006). The response of young mandarin trees grown under saline conditions depends on the rootstock. European Journal of Agronomy, 24(2), 129-139. https://doi.org/10.1016/j.eja.2005.04.007

Georgiou, A. (2000). Performance of 'Nova' mandarin on eleven rootstocks in Cyprus. Scientia Horticulturae, 84(1-2), 115-126. https://doi.org/10.1016/S0304-4238(99)00120-X

Georgiou, A. (2002). Evaluation of rootstocks for 'Clementine' mandarin in Cyprus. Scientia Horticulturae, 93(1), 29-38. https://doi.org/10.1016/S0304-4238(01)00311-9

Goldschmidt, E.E., & Koch, K.E. (1996). Citrus. In Photoassimilate Distribution in Plants and Crops, 1st Ed.; E. Zamski, E., & SchaVer, A.A., Eds.; Marcel Dekker Inc., New York, USA, 797–824.

Haas, A.R.C. (1948). Effect of the rootstock on the composition of citrus trees and fruit. Plant Physiology, 23(3), 309-330. https://doi.org/10.1104/pp.23.3.309

Hamilton, C.E., Gundel, P.E., Helander, M., & Saikkonen, K. (2012). Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Diversity, 54(1), 1-10. https://doi.org/10.1007/s13225-012-0158-9

Hemmati, N., Ghasemnezhad, A., Moghaddam, J.F., & Ebrahimi, P. (2018). Variation in the content of bioflavonoids of orange as affected by scion, rootstock, and fruit part. Acta Physiologiae Plantarum, 40(5), 83 (1-8). https://doi.org/10.1007/s11738-018-2648-1

Hifny, H.A., Elrazik, A.M., Abdrabboh, G.A., & Sultan, M.Z. (2012). Effect of some citrus rootstocks on fruit quality and storability of 'Washington' navel orange under cold storage conditions. American-Eurasian Journal of Agricultural & Environmental Sciences, 12(10), 1266-1273. https://doi.org/10.5829/idosi.aejaes.2012.12.10.251212

Hikal, A.R.F. (2014). Effect of different rootstocks on vegetative growth, fruiting, fruit quality and fruit storage on trees of 'Washington' navel orange. Journal of Plant Production, 5(2), 347-355. https://doi.org/10.21608/JPP.2014.53650

Huff, A. (1983). Nutritional control of regreening and degreening in citrus peel segments. Plant Physiology, 73(2), 243-249. https://doi.org/10.1104/pp.73.2.243

Huff, A. (1984). Sugar regulation of plastid interconversions in epicarp of citrus fruit. Plant physiology, 76(2), 307-312. https://doi.org/10.1104/pp.76.2.307

Hussain, S., Curk, F., Anjum, M.A., Pailly, O., & Tison, G. (2013). Performance evaluation of common clementine on various citrus rootstocks. Scientia Horticulturae, 150, 278-282. https://doi.org/10.1016/j.scienta.2012.11.010

Iglesias, D.J., Tadeo, F.R., Legaz, F., Primo-Millo, E., & Talon, M. (2001). In vivo sucrose stimulation of colour change in citrus fruit epicarps: interactions between nutritional and hormonal signals. Physiologia Plantarum, 112(2), 244-250. https://doi.org/10.1034/j.1399-3054.2001.1120213.x

Incesu, M., Çimen, B., Yesiloglu, T., & Yilmaz, B. (2013). Rootstock effects on yield, fruit quality, rind and juice color of Moro blood orange. Journal of food, Agriculture & Environment, 11(3&4), 867-871.

Jiménez, S., Fattahi, M., Bedis, K., Nasrolahpour-moghadam, S., Irigoyen, J.J., & Gogorcena, Y. (2020). Interactional Effects of Climate Change Factors on the Water Status, Photosynthetic Rate, and Metabolic Regulation in Peach. Frontiers in Plant Science, 11, 43. https://doi.org/10.3389/fpls.2020.00043

Jover, S., Martínez-Alcántara, B., Rodríguez-Gamir, J., Legaz, F., Primo-Millo, E., Forner, J., & Forner-Giner, M.A. (2012). Influence of rootstocks on photosynthesis in Navel orange leaves: effects on growth, yield, and carbohydrate distribution. Crop science, 52(2), 836-848. https://doi.org/10.2135/cropsci2011.02.0100

Kaplankiran, M., Demirkeser, T.H., & Yildiz, E. (2005). The Effects of Some Citrus Rootstocks on Fruit Yield and Quality for Okitsu Satsuma during the Juvenility Period in Dörtyol (Hatay, Turkey) Conditions. Proceeding of 7th International Congress of Citrus Nurserymen, 17-21.

Kato, M. (2012). Mechanism of carotenoid accumulation in citrus fruit. Journal of the Japanese Society for Horticultural Science, 81(3), 219-233. https://doi.org/10.2503/jjshs1.81.219

Khalifa, S.M., & Hamdy, A.E. (2015). Effect of some citrus rootstocks on yield and fruit quality of two mandarin varieties. In Sixth International Scientific Agricultural Symposium" Agrosym 2015", Jahorina, Bosnia and Herzegovina, October 15-18, 2015. Book of Proceedings, 182-190. University of East Sarajevo.

Klein, J.D., Shalev, Y.R., Cohen, S., & Sachs, M. (2017). Rootstocks for the grapefruit hybrid “Sweetie” (‘Oroblanco’) under organic and conventional management. Scientia Horticulturae, 222, 12-16. https://doi.org/10.1016/j.scienta.2017.05.002

Koepke, T., & Dhingra, A. (2013). Rootstock scion somatogenetic interactions in perennial composite plants. Plant cell reports, 32(9), 1321-1337. https://doi.org/10.1007/s00299-013-1471-9

Kotsias, D. (2004). Influence of Citrus aurantium (L.) and Poncirus trifoliata (L.) Raf. rootstocks and nutrient sprays on granulation of Valencia sweet orange [C. sinensis (L.) Osback] fruits. European Journal of Horticultural Sciences, 69(6), 244-249.

Kumar, H., Chohan, G.S., & Vij, V.K. (1994). Studies on tree survival, growth, yield and fruit quality of pineapple cv. of sweet orange on different rootstocks. Journal of Research Punjab Agricultural Research, 31(1), 27-31.

Lado, J., Gambetta, G., & Zacarias, L. (2018). Key determinants of citrus fruit quality: Metabolites and main changes during maturation. Scientia Horticulturae, 233, 238-248. https://doi.org/10.1016/j.scienta.2018.01.055

Lafuente, M.T., & Sala, J.M. (2002). Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rind staning of 'Navelina' orange (Citrus sinensis L. Osbeck) fruit. Postharvest Biology and Technology, 25(1), 49-57. https://doi.org/10.1016/S0925-5214(01)00162-4

Le, K.T. (2016). Early performance of duong mandarin (Citrus reticulata blanco) on three rootstock under acid sulfate soil fields at Mekong Delta of Vietnam. International Journal on Advanced Science, Engineering and Information Technology, 6(1), 10-15.

Lee, S.K., & Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest biology and technology, 20(3), 207-220. https://doi.org/10.1016/S0925-5214(00)00133-2

Legua, P., Bellver, R., Forner, J., & Forner-Giner, M.A. (2011a). Plant growth, yield and fruit quality of'Lane Late' navel orange on four citrus rootstocks. Spanish Journal of Agricultural Research, 9(1), 271-279.

Legua, P., Bellver, R., Forner, J.B., & Forner-Giner, M.A. (2011b). Trifoliata hybrids rootstocks for 'Lane Late' navel orange in Spain. Scientia Agricola, 68(5), 548-553. https://doi.org/10.1590/S0103-90162011000500006

Legua, P., Forner, J.B., Hernández, F., & Forner-Giner, M.A. (2013). Physicochemical properties of orange juice from ten rootstocks using multivariate analysis. Scientia Horticulturae, 160, 268-273.

https://doi.org/10.1016/j.scienta.2013.06.010

Legua, P., Forner, J.B., Hernandez, F.C.A., & Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. Scientia Horticulturae, 174, 60-64. https://doi.org/10.1016/j.scienta.2014.05.004

Legua, P., Hernández, F., & Forner-Giner, M.A. (2017). Influence of Citrus Rootstocks in Bioactive Compounds of Clementines. Journal of Food and Nutrition Research, 5(8), 545-552. https://doi.org/10.12691/jfnr-5-8-3

Legua, P., Martinez-Cuenca, M.R., Bellver, R., & Forner-Giner, M.A. (2018). Rootstock's and scion's impact on lemon quality in southeast Spain. International Agrophysics, 32(3), 325-333. https://doi.org/10.1515/intag-2017-0018

Lockard, R.G., Schneider, G.W., & Kemp, T.R. (1982). Phenolic compounds in two size-controlling apple rootstocks. Journal of American Society of Horticultural Science, 107(2), 183-186.

Machado, F.L.D.C., Costa, J.D.P.D., Teixeira, A.D.S., & Costa, J.M.C.D. (2015). The influence of rootstock and time of harvest on the fruit quality during storage of in two grapefruit cultivars. Acta Scientiarum. Agronomy, 37(3), 339-346. https://doi.org/10.4025/actasciagron.v37i3.16970

Magwaza, L.S., Mditshwa, A., Tesfay, S.Z., & Opara, U.L. (2017). An overview of preharvest factors affecting vitamin C content of citrus fruit. Scientia Horticulturae, 216, 12-21. https://doi.org/10.1016/j.scienta.2016.12.021

Mallick, M., Dubey, A.K., Singh, S.K., & Sharma, R.M. (2019). Tree morphology, yield and fruit quality of grapefruit cultivars on different rootstocks in Inceptisol. Indian Journal of Horticulture, 76(3), 405-410.

https://dx.doi.org/10.5958/0974-0112.2019.00065.3

Martínez-Cuenca, M.R., Primo-Capella, A., & Forner-Giner, M.A. (2016). Influence of Rootstock on Citrus Tree Growth: Effects on Photosynthesis and Carbohydrate Distribution, Plant Size, Yield, Fruit Quality, and Dwarfing Genotypes. Plant Growth, 16, 107-129. https://dx.doi.org/10.5772/64825

Mashayekhi, K., Sadeghi, H., Akbarpour, V., Atashi, S., Mousavizadeh, S.J., Abshaei, M., & Nazari, Z. (2013). Effect of some citrus rootstocks on the amount of biochemical composition of Parson Brown and Mars Oranges in Jiroft. Journal of Horticultural Science, 27, 9-17.

McCollum, G., & Bowman, K.D. (2017). Rootstock effects on fruit quality among 'Ray Ruby' grapefruit trees grown in the Indian River District of Florida. HortScience, 52(4), 541-546. https://doi.org/10.21273/HORTSCI11435-16

McCollum, T.G., Bowman, K.D., & Castle, W.S. (2002). Effects of rootstock on fruit quality and postharvest behavior of 'Marsh' grapefruit. In Procedings of Florida State of Horticultural Society, 115, 44-46.

Mesejo, C., Reig, C., Martínez-Fuentes, A., Gambetta, G., Gravina, A., & Agustí, M. (2016). Tree water status influences fruit splitting in Citrus. Scientia Horticulturae, 209, 96-104. https://doi.org/10.1016/j.scienta.2016.06.009

Montero, C.R.S., Schwarz, L.L., dos Santos, L.C., dos Santos, R.P., & Bender, R.J. (2012). Oleocellosis incidence in citrus fruit in response to mechanical injuries. Scientia horticulturae, 134, 227-231. https://doi.org/10.1016/j.scienta.2011.10.026

Morinaga, K., & Ikeda, F. (1990). The effects of several rootstocks on photosynthesis, distribution of photosynthetic product, and growth of young satsuma mandarin trees. Journal of the Japanese Society for Horticultural Science, 59(1), 29-34.

https://doi.org/10.2503/jjshs.59.29

Nasser, M.A., Bondok, A.Z., Shaltout, A.D., & Mansour, N. (2014). Evaluation of Some New Navel Orange Cultivars Budded on Sour Orange and Volkamer Lemon Rootstocks. Egyptian Journal of Horticulture, 41(2), 239-262.

Navarro, J.M., Gómez-Gómez, A., Pérez-Pérez, J.G., & Botia, P. (2010a). Effect of saline conditions on the maturation process of Clementine Clemenules fruits on two different rootstocks. Spanish journal of agricultural research, (2), 21-29.

Navarro, J.M., Pérez-Pérez, J.G., Romero, P., & Botía, P. (2010b). Analysis of the changes in quality in mandarin fruit, produced by deficit irrigation treatments. Food Chemistry, 119(4), 1591-1596. https://doi.org/10.1016/j.foodchem.2009.09.048

Ollat, N., Touzard, J.M., & van Leeuwen, C. (2016). Climate change impacts and adaptations: New challenges for the wine industry. Journal of Wine Economics, 11(1), 139-149. https://doi.org/10.1017/jwe.2016.3

Orazem, P., Stampar, F., & Hudina, M. (2011). Quality analysis of 'Redhaven' peach fruit grafted on 11 rootstocks of different genetic origin in a replant soil. Food Chemistry, 124(4), 1691-1698. https://doi.org/10.1016/j.foodchem.2010.07.078

Oustric, J., Morillon, R., Luro, F., Herbette, S., Lourkisti, R., Giannettini, J., Berti, L., & Santini, J. (2017). Tetraploid Carrizo citrange rootstock (Citrus sinensis Osb. × Poncirus trifoliata L. Raf.) enhances natural chilling stress tolerance of common clementine (Citrus clementina Hort. ex Tan). Journal of plant physiology, 214, 108-115. https://doi.org/10.1016/j.jplph.2017.04.014

Özdemir, A. E., Toplu, C., Çandır, E., Kaplankıran, M., Yıldız, E., Kamiloğlu, M., Yucel, F.; Kıvrak, M.; Demirkeser, O., & Ünlü, M. (2016). Cold storage of

'Fremont' mandarins grown on Carrizo Citrange and sour orange rootstocks. Bahçe, 45, 384-389.

Özdemir, A.E., Didin, Ö., Candir, E., Kaplankiran, M., & Yildiz, E. (2019). Effects of rootstocks on storage performance of Nova mandarins. Turkish Journal of Agriculture and Forestry, 43(3), 307-317.

Palma, C.E., Cruz, P.S., Cruz, D.T.C., Bugayong, A.M.S., & Castillo, A.L. (2019). Chemical composition and cytotoxicity of Philippine calamansi essential oil. Industrial Crops and Products, 128, 108-114. https://doi.org/10.1016/j.indcrop.2018.11.010

Patil, B.S., & Alva, A.K. (2002). Functional components in citrus: alteration by mineral elements. In Proceedings of the Symposium on Fertilizing Crops for Functional Food, Vol. 7, 1-4.

Peterson, J.J., Dwyer, J.T., Beecher, G.R., Bhagwat, S.A., Gebhardt, S.E., Haytowitz, D.B., & Holden, J.M. (2006). Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature. Journal of Food Composition and Analysis, 19, S66-S73. https://doi.org/10.1016/j.jfca.2005.12.006

Pourtau, N., Jennings, R., Pelzer, E., Pallas, J., & Wingler, A. (2006). Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in Arabidopsis. Planta, 224(3), 556-568. https://doi.org/10.1007/s00425-006-0243-y

Raddatz-Mota, D., Franco-Mora, O., Mendoza-Espinoza, J.A., Rodríguez-Verástegui, L.L., de León-Sánchez, F.D., & Rivera-Cabrera, F. (2019). Effect of different rootstocks on Persian lime (Citrus latifolia T.) postharvest quality. Scientia Horticulturae, 257, 108716. https://doi.org/10.1016/j.scienta.2019.108716

Ramin, A.A., & Alirezanezhad, A. (2005). Effects of citrus rootstocks on fruit yield and quality of Ruby Red and Marsh grapefruit. Fruits, 60(5), 311-317.

https://doi.org/10.1051/fruits:2005037

Rapisarda, P., Bianco, M.L., Pannuzzo, P., & Timpanaro, N. (2008). Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [Citrus sinensis (L.) Osbeck]. Postharvest biology and technology, 49(3), 348-354.
https://doi.org/10.1016/j.postharvbio.2008.02.002

Raveh, E., Saban, T., Zipi, H., & Beit-Yannai, E. (2009). Influence of rootstock and scion on antioxidant capacity of juice from new pomelo and mandarin varieties. Journal of the Science of Food and Agriculture, 89(11), 1825-1830.
https://doi.org/10.1002/jsfa.3639

Reforgiato Recupero, G., Russo, G., Recupero, S., Zurru, R., Deidda, B., & Mulas, M. (2009). Horticultural evaluation of new citrus latipes hybrids as rootstocks for citrus. HortScience, 44(3), 595-598.
https://doi.org/10.21273/HORTSCI.44.3.595

Ritenour, M.A., Stover, E., Boman, B.J., Dou, H., Bowman, K.D., & Castle, W.S. (2004a). Effect of rootstock on stem-end rind breakdown and decay of fresh citrus. HortTechnology, 14(3), 315-319.
https://doi.org/10.21273/HORTTECH.14.3.0315

Rodrigo, M.J., Alquézar, B., Alós, E., Lado, J., & Zacarías, L. (2013). Biochemical bases and molecular regulation of pigmentation in the peel of Citrus fruit. Scientia Horticulturae, 163, 46-62.
https://doi.org/10.1016/j.scienta.2013.08.014

Rolland, F., Baena-Gonzalez, E., & Sheen, J. (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. Annual Review of Plant Biology, 57, 675-709.
https://doi.org/10.1146/annurev.arplant.57.032905.105441

Romero, P., Gandía, M., & Alférez, F. (2013). Interplay between ABA and phospholipases A2 and D in the response of citrus fruit to postharvest dehydration. Plant physiology and biochemistry, 70, 287-294. https://doi.org/10.1016/j.plaphy.2013.06.002

Romero, P., Navarro, J.M., Pérez-Pérez, J., García-Sánchez, F., Gómez-Gómez, A., Porras, I., Martínez, V., & Botía, P. (2006). Deficit irrigation and rootstock: their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin. Tree physiology, 26(12), 1537-1548. https://doi.org/10.1093/treephys/26.12.1537

Rong, Y., Luo, J., Liao, L., & Wang, Z. (2019). Effects of endogenous hormone metabolism on pericarp thickness after grafting on different rootstocks of Citrus cv. 'Kiyomi tangor'. In AIP Conference Proceedings, AIP Publishing LLC. 2110 (1), 020037.

Saini, M.K., Capalash, N., Kaur, C., & Singh, S.P. (2019). Comprehensive metabolic profiling to decipher the influence of rootstocks on fruit juice metabolome of Kinnow (C. nobilis × C. deliciosa). Scientia Horticulturae, 257, 108673. https://doi.org/10.1016/j.scienta.2019.108673

Sau, S., Ghosh, S.N., Sarkar, S., & Gantait, S. (2018). Effect of rootstocks on growth, yield, quality, and leaf mineral composition of Nagpur mandarin (Citrus reticulata Blanco.), grown in red lateritic soil of West Bengal, India. Scientia Horticulturae, 237, 142-147. https://doi.org/10.1016/j.scienta.2018.04.015

Sdiri, S., Bermejo, A., Aleza, P., Navarro, P., & Salvador, A. (2012). Phenolic composition, organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid late-season mandarins. Food Research International, 49(1), 462-468. https://doi.org/10.1016/j.foodres.2012.07.040

Sdiri, S., Salvador, A., Farhat, I., Navarro, P., & Besada, C. (2014). Influence of postharvest handling on antioxidant compounds of Citrus fruits. In Citrus: molecular phylogeny, antioxidant properties and medicinal uses, 1st Ed.; Hayat, K., Eds; Nova Science Publishers, Inc, New York, USA, 73-94.

Shafieizargar, A., Awang, Y., Juraimi, A.S., & Othman, R. (2012). Yield and fruit quality of 'Queen' orange [citrus sinensis (L) Osb.] grafted on different rootstocks in Iran. Australian Journal of crop science, 6(5), 777-783.

Shafqat, W., Jaskani, M.J., Maqbool, R., Khan, A.S., & Ali, Z. (2019). Evaluation of Citrus Rootstocks against Drought, Heat and their Combined Stress Based on Growth and Photosynthetic Pigments. International Journal of Agriculture and Biology, 22(5), 1001-1009. https://doi.org/10.17957/IJAB/15.1160

Sharma, R.M., Dubey, A.K., Awasthi, O.P., & Kaur, C. (2016). Growth, yield, fruit quality and leaf nutrient status of grapefruit (Citrus paradisi Macf.): variation from rootstocks. Scientia Horticulturae, 210, 41-48. https://doi.org/10.1016/j.scienta.2016.07.013

Sharma, R.R., & Saxena, S.K. (2004). Rootstocks influence granulation in Kinnow mandarin (Citrus nobilis × C. deliciosa). Scientia horticulturae, 101(3), 235-242. https://doi.org/10.1016/j.scienta.2003.10.010

Shomer, I., & Erner, Y. (1989). The nature of oleocellosis in citrus fruits. Botanical Gazette, 150(3), 281-288.

Soleimani, M., Tabil, L., Mirmajidi, A., & Shafieezargar, A.R. (2007). Effects of rootstock and postharvest treatments on quality of grapefruit cultivars during storage. In 2007 ASAE Annual Meeting.American Society of Agricultural and Biological Engineers, 072506. https://doi.org/10.13031/2013.23390

Sousa, W.R., da Rocha, C., Cardoso, C.L., Silva, D.H.S., & Zanoni, M.V.B. (2004). Determination of the relative contribution of phenolic antioxidants in orange juice by voltammetric methods. Journal of Food Composition and Analysis, 17(5), 619-633. https://doi.org/10.1016/j.jfca.2003.09.013

Souza, J.D., de Andrade Silva, E.M., Coelho Filho, M.A., Morillon, R., Bonatto, D., Micheli, F., & da Silva Gesteira, A. (2017). Different adaptation strategies of two citrus scion/rootstock combinations in response to drought stress. PloS one, 12(5), e0177993. https://doi.org/10.1371/journal.pone.0177993

Storey, R., & Treeby, M.T. (2002). Cryo-SEM study of the early symptoms of peteca in 'Lisbon' lemons. The Journal of Horticultural Science and Biotechnology, 77(5), 551-556. https://doi.org/10.1080/14620316.2002.11511537

Storey, R., Treeby, M.T., & Milne, J. (2002). Crease: another Ca deficiency-related fruit disorder? The Journal of Horticultural Science and Biotechnology, 77(5), 565-571. https://doi.org/10.1080/14620316.2002.11511539

Syvertsen, J.P., & Albrigo, L.G. (1980). Some effects of grapefruit tree canopy position on microclimate, water relations, fruit yield, and juice quality. Journal of the American Society for Horticultural Science, 105(3), 454-459.

Syvertsen, J.P., & Graham, J.H. (1985). Hydraulic conductivity of roots, mineral nutrition, and leaf gas exchange of citrus rootstocks. Journal of the American Society for Horticultural Science, 110, 865-869.

Tajvar, Y., Ghazvini, R.F., Hamidoghli, Y., & Sajedi, R.H. (2011). Antioxidant changes of Thomson navel orange (Citrus sinensis) on three rootstocks under low temperature stress. Horticulture, Environment, and Biotechnology, 52(6), 576-580. https://doi.org/10.1007/s13580-011-0052-5

Tavarini, S., Gil, M. I., Tomas-Barberan, F.A., Buendia, B., Remorini, D., Massai, R., Degl'Innocenti, E., & Guidi, L. (2011). Effects of water stress and rootstocks on fruit phenolic composition and physical/chemical quality in Suncrest peach. Annals of Applied Biology, 158(2), 226-233. https://doi.org/10.1111/j.1744-7348.2010.00457.x

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., & Storey, R. (2007). Irrigation management and rootstock effects on navel orange [Citrus sinensis (L.) Osbeck] fruit quality. Agricultural Water Management, 91(1-3), 24-32.
https://doi.org/10.1016/j.agwat.2007.04.002

Treeby, M.T., Storey, R., & Bevington, K.B. (1995). Rootstock, seasonal, and fruit size influences on the incidence and severity of albedo breakdown in Bellamy navel oranges. Australian journal of experimental agriculture, 35(1), 103-108.
https://doi.org/10.1071/EA9950103

Wang, Y.C., Chuang, Y.C., & Ku, Y.H. (2007). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. Food chemistry, 102(4), 1163-1171.
https://doi.org/10.1016/j.foodchem.2006.06.057

Yeşiloğlu, T., Yılmaz, B., Çimen, B., & İncesu, M. (2014). Influences of rootstocks on fruit quality of ‘Henderson’ grapefruit. Türk Tarım ve Doğa Bilimleri Dergisi, 1(Özel Sayı-1), 1322-1325.

Yıldırım, F., Yıldırım, A. N., San, B., & Ercişli, S. (2016). The relationship between growth vigour of rootstock and phenolic contents in apple (malus× domestica). Erwerbs-Obstbau, 58(1), 25-29.
https://doi.org/10.1007/s10341-015-0253-7

Yildiz, E., Hakan Demirkeser, T., & Kaplankiran, M. (2013). Growth, yield, and fruit quality of ’Rhode Red Valencia’ and ’Valencia Late’ sweet oranges grown on three rootstocks in eastern Mediterranean. Chilean journal of agricultural research, 73(2), 142-146. http://dx.doi.org/10.4067/S0718-58392013000200009

Yildiz, E., Kaplankiran, M., Demirkeser, T.H., Toplu, C., & Uysal-Kamiloglu, M. (2014). Performance of ''Rio Red'' grapefruit on seven rootstocks in the eastern Mediterranean region of Turkey. Journal of Agricultural Science and Technology, 16(4), 897-908.

Yilmaz, B., Cimen, B., Incesu, M., Uysal Kamiloglu, M., & Yesiloglu, T. (2018). Rootstock influences on seasonal changes in leaf physiology and fruit quality of ‘Rio Red’ grapefruit variety. Applied Ecology and Environmental Research, 16(4), 4065-4080. http://dx.doi.org/10.15666/aeer/1604_40654080

Zambrosi, F.C.B., Mesquita, G.L., Tanaka, F.A.O., Quaggio, J.A., & Mattos Jr, D. (2013). Phosphorus availability and rootstock affect copper-induced damage to the root ultra-structure of Citrus. Environmental and experimental botany, 95, 25-33. https://doi.org/10.1016/j.envexpbot.2013.07.004

Zandalinas, S.I., Sales, C., Beltrán, J., Gómez-Cadenas, A., & Arbona, V. (2017). Activation of secondary metabolism in citrus plants is associated to sensitivity to combined drought and high temperatures. Frontiers in plant science, 7, 1954, 1-17. https://doi.org/10.3389/fpls.2016.01954

Zekri, M., & Al-Jaleel, A. (2004). Evaluation of rootstocks for Valencia and Navel orange trees in Saudi Arabia. Fruits, 59(2), 91-100. https://doi.org/10.1051/fruits:2004009

Zheng, Y., Deng, L., He, S., Zhou, Z., Yi, S., Zhao, X., & Wang, L. (2011). Rootstocks influence fruit oleocellosis in ‘Hamlin’ sweet orange (Citrus sinensis L. Osbeck). Scientia horticulturae, 128(2), 108-114. https://doi.org/10.1016/j.scienta.2011.01.005

Zheng, Y., Wang, Y., Yang, Q., Liu, Y., Xie, R., He, S., Deng, L.; Yi, S.; Lv, Q., & Ma, Y. (2018). Modulation of tolerance of "Hamlin" sweet orange grown on three rootstocks to on-tree oleocellosis by summer plant water balance supply. Scientia Horticulturae, 238, 155-162. https://doi.org/10.1016/j.scienta.2018.04.058

Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z. (2016). Antioxidant activity of Citrus fruits. Food chemistry, 196, 885-896. https://doi.org/10.1016/j.foodchem.2015.09.072

Zouaghi, G., Najar, A., & Abderrabba, M. (2018). Rootstocks effect on flavonoid content and antioxidant properties of Maltese (Citrus sinensis L.) ethanolic extracts. *Journal of New Sciences, 49,* 2956-2960.

II. OBJETIVES

GENERAL OBJECTIVE

To study the effect of rootstock on the fruit quality of cultivars with commercial interest under Mediterranean conditions: ‘Clemenules’ and ‘Tango’ mandarins, and ‘Tarocco Rosso’ and ‘Moro’ blood oranges.

SPECIFIC OBJECTIVES

To characterize the physico-chemical and nutritional quality of ‘Clemenules’ mandarins grafted onto eight rootstocks at three commercial harvest times during two seasons.

To evaluate the changes in the physico-chemical, sensorial and nutritional quality during the harvest and cold storage of ‘Tango’ mandarins from trees grafted onto Carrizo Citrange or Forner-Alcaide 5 under Mediterranean conditions.

To study the changes in the physico-chemical and nutritional quality of ‘Moro’ and ‘Tarocco Rosso’ blood oranges grafted onto eight rootstocks during harvest.

III. RESULTS

III.1. CLEMENULES MANDARINS

CHAPTER I

Rootstock Effect on Physico-Chemical and Nutritional Quality of Mandarin 'Clemenules' during the Harvest Season

Julia Morales [a], Almudena Bermejo [b], Pilar Navarro [a] and Alejandra Salvador [a]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

Reference: *Agronomy*, 2020, 10(9), 1350.

Abstract

This study reports the influence of eight rootstocks (‘Carrizo’ (CC), ‘C-35’ (C35) ‘Cleopatra’ (CL), ‘Volkameriana’ (VK), ‘Macrophylla’ (M), ‘Forner-Alcaide 5’ (FA5), ‘Forner-Alcaide 13’ (FA13) and ‘Forner-Alcaide V17’ (V17)) on the physico-chemical and nutritional quality of ‘Clemenules’ mandarins at three harvest times during two seasons. Fruit quality parameters were influenced by rootstocks, and this effect was mostly harvest time-dependent. External color was overall influenced by rootstocks upon the first harvest. FA13 and C35 induced a breakthrough in color change, while V17 brought about the greatest color increase. CL and V17 initially delayed the fruit maturity index. In the last harvest, while CL had one of the highest maturity indices, V17 exhibited the lowest as acidity was maintained as harvest advanced. The CC and M fruit had the highest and lowest sucrose content, respectively. The highest glucose and fructose contents in fruit were induced by V17, FA5 and CC. The highest citric acid content was displayed by V17 in all the harvests. The FA13, C35 and VK fruit exhibited the highest malic acid content. The effect that rootstock exert on ascorbic acid and flavonoid content depends on the studied season. The results reveal the importance of conducting studies during different seasons to obtain robust results.

Keywords: Ascorbic acid; flavonoids; organic acids; sugar; maturity index; firmness; color

1. Introduction

Rootstocks are frequently used in citriculture to improve crop adaptation to different biotic and abiotic conditions by regulating the uptake and movement of water and nutrients among plant organs (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007; Sharma, Dubey, Awasthi & Kaur, 2016; Yıldırım, Yıldırım, San & Ercişli, 2016). The rootstock can also influence fruit quality parameters (Castle, 1996) such as color (Machado, Costa, Teixeira & Costa, 2015; Legua, Martinez-Cuenca, Bellver, Forner-Giner, 2018) or maturity index (McCollum, Bowman & Castle, 2002; Bassal, 2009) and nutritional juice parameters, such as individual sugars and organic acids (Legua, Forner, Hernández & Forner-Giner, 2013; Saini, Capalash, Kaur & Singh, 2019), phenolic compounds (Legua, Forner, Hernández & Forner-Giner , 2014; Sharma et al., 2016) or vitamin C (Magwaza, Mditshwa, Tesfay & Opara, 2017). The effects of rootstock strongly depend on the rootstock-scion interaction.

Most studies that have addressed the effect of rootstock on citrus fruit quality have been conducted on a single point of maturity, and very few studies consider if these effects remain throughout the season (Cardeñosa, Barros, Barreira, Arenas, Moreno-Rojas & Ferreira, 2015; Emmanouilidou & Kyriacou, 2017). It is important to highlight this aspect for those varieties with a long harvest period. This is the case of early mandarins in the Mediterranean Region, where citrus fruit reaches commercial internal maturity before the color change from green to orange takes place. Thus fruit can be harvested at the beginning of the season, with no full external coloration, to be submitted to ethylene degreening treatment, which induces chlorophyll degradation and carotenoid biosynthesis (Yin et al., 2016; Sumiasih, Poerwanto, Efendi, Agusta & Yuliani, 2018). However, fruit can also be harvested later when it has completed typical external coloration halfway through or at the end of the season.

The effect of rootstock on internal quality parameters, such as acidity and sugar contents is crucial because it can not only determine harvest time, but can also influence the maintenance of these parameters throughout harvest. Moreover, the rootstock effect on external color can bring about advanced harvests, which is relevant at the beginning of the season, especially in early varieties.

‘Clemenules’ clementine (Citrus clementina Hort. ex Tan.) is considered one of the most important early mandarins that is harvested from early September to late December. Like most mandarins in the Mediterranean Region, this cultivar reaches internal maturity before full coloration. Thus at the beginning of the season, it is usually submitted to degreening ethylene treatment to enhance external color.

This study aims to characterize the physico-chemical and nutritional quality of ‘Clemenules’ mandarins grafted onto eight rootstocks at three commercial harvest times during two seasons.

2. Materials and Methods

2.1. Plant material

Experiments were conducted with ‘Clemenules’ mandarins taken from 12-year-old trees grafted onto eight different rootstocks: ‘Carrizo’ (CC) and ‘C-35’ (C35) citranges, ‘Cleopatra’ mandarin (CL), ‘Volkameriana’ (VK), ‘Macrophylla’ (M) and three hybrid selections, ‘Forner-Alcaide 5’ (FA5), ‘Forner-Alcaide 13’ (FA13); ‘Forner-Alcaide V17’ (V17), obtained from the rootstock breeding program carried out at the Instituto Valenciano of Investigaciones Agrarias (IVIA) (Table 1). They are cultivated in an experimental plot of Anecoop S. Coop. located in Museros, Valencia (39° 34′ 45.6′′ N, 0° 21′ 48.8′′ W) with sandy loam soil (sand 65.28%, silt 18% and clay 16.72%). This orchard had EC 1:5 of 0.344 dS m−1 (25 °C) and a pH of 7.8.

The study was carried out during two seasons (2016 and 2018) with fruit harvested at three different times. The first harvest was performed when the maturity index (MI) of fruit came close to 7, MI value established as the minimum to commercialize mandarin fruit (UNECE, 2017). The second and third harvests took place 30 and 60 days after the first harvest, respectively. The harvest dates were 28 September, 28 October and 28 November in the first season, and 20 September, 20 October and 20 November in the second season (2018).

Table 1. Description of the rootstocks tested for 'Clemenules' mandarin.

Rootstock	Abbreviation	Botanical Name
Forner-Alcaide 13	FA13	*Citrus reshni* x *Poncirus trifoliata* (L.) Raf. no 13
Forner-Alcaide 5	FA5	*Citrus reshni* x *Poncirus trifoliata* (L.) Raf. no 5
Citrange Carrizo	CC	*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.
C-35	C35	*Citrus sinensis* (L.) Osbeck *x Poncirus trifoliata* (L.) Raf. (Hybrid)
Cleopatra Mandarin	CL	*Citrus reshni* Hort. Ex Tan
Macrophylla	M	*Citrus macrophylla* Wester
Volkameriana	VK	*Citrus volkameriana* Ten. & Pasq.
Forner-Alcaide V17	V17	V17

At each harvest moment, 60 fruits were picked from three previously selected trees of each rootstock and transferred to the laboratory at IVIA where the following analyses were carried out: color, firmness, total soluble solids, acidity, maturity index, individual sugar concentrations, organic acid concentrations, flavonoid concentrations, ascorbic acid concentration.

2.2. External and internal quality parameters measurements

External fruit peel color was measured by a Minolta colorimeter (model CR-400, Minolta Co. Ltd., Osaka, Japan) with 15 fruits per lot by taking two measurements from the opposite equatorial sides of each fruit. The mean values for the 'L', 'a' and 'b' Hunter parameters were calculated with each fruit and expressed as the Citrus Color Index (CCI) (CCI = 1000a/Lb) (Jimenez-Cuesta, Cuquerella & Martínez-Jávega, 1981).

Firmness measurements were taken by a Universal Testing Machine (model 3343, Instron Limited, Buckinghamshire, UK) with 15 fruits per lot. The results were expressed as the percentage of millimeters of fruit deformation that resulted from a 10 N force on the longitudinal axis at constant speed.

In each fruit lot, three samples of five fruits each were squeezed using an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution, with phenolphthalein as the indicator, and expressed as g citric acid/100mL of juice. The total soluble solids content (TSS) in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd., Tokyo, Japan). Data were expressed as °Brix. MI was calculated as the TSS/TA ratio.

2.3. Biochemical measurements

Part of the juice from each sample, obtained as described above, was transferred after squeezing fruit into 2 mL eppendorf tubes and stored at −20°C for the biochemical analysis.

The extraction method and HPLC analysis of sugars were the same as a previously described procedure (Bermejo, Pardo, Morales & Cano, 2016). Fructose, glucose and sucrose sugars were identified by comparing their retention time to a standard (obtained from Sigma, Co., Barcelona, Spain). These sugars were quantified with an external calibration curve. The results were expressed as g/L.

The extraction and analysis of organic acids were carried out according to the previously described method (Bermejo et al., 2016). Compounds were analyzed by HPLC-DAD and HPLC-MS under electrospray ion negative conditions. An ICSep ICE-COREGEL 87H3 column (Transgenomic) was used in an isocratic mobile phase of 0.1% H_2SO_4 solution at the 0.6 mL/min flow rate. The injection volume was 5 µL. Standard compounds were supplied by Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g/L.

The main flavonoids were extracted following a previously described procedure (Bermejo, Pardo & Cano, 2012a), with some modifications to adapt the method to a microliter format (Bermejo et al., 2016). Narirutin, hesperidin and didymin were analyzed by HPLC-DAD and HPLC-MS under electrospray ion positive conditions according to formerly described method (Bermejo, Pardo & Cano, 2011). Standards were obtained from Extrasynthesis (Genay, France), Sigma (Sigma Co., Barcelona, Spain) and ChromaDex (Irvine, CA, USA). The results were expressed as mg/100 mL.

Total vitamin C was extracted with the previously reported method (Sdiri, Bermejo, Aleza, Navarro & Salvador, 2012). DL-dithiothreitol (DTT) was used as the reducing reagent of dehydroascorbic acid to ascorbic acid. Ascorbic acid quantification was performed by HPLC-DAD, as previously described (Bermejo et al, 2016). L-ascorbic acid was obtained from Sigma (Sigma Co., Barcelona, Spain) and DTT came from Fluka (Sigma Co., Barcelona, Spain). The results were expressed as mg/100 mL.

The HPLC analyses were performed with an Alliance liquid chromatographic system (Waters, Barcelona, Spain), equipped with a 2695 separation module, a 2996 photodiode array detector and a ZQ2000 mass detector. Samples were detected at 5°C. The column temperature was either 25°C or 35°C. The sugars analysis was carried out by a Waters 515 HPLC pump with 2414 refractive index detector and a 20 µL loop Rheodyne injector. Data were acquired and processed by the Empower 2 software (Waters, Spain). Grade solvents and Milli-Q water were used in all biocompound HPLC analyses.

2.4. Statistical analysis

Statistical procedures were performed using statistical software (Statgraphics Centurion XVII.II software; Manugistics, Rockville, NY, USA). All the data were subjected to an analysis of variance based on two factors (harvest time × rootstock) for each season. Moreover for each harvest, the rootstock effect was studied by one way Anova. The mean values were compared by the least significant difference test (LSD) ($p \leq 0.05$).

3. Results and discussion

3.1. Effect of rootstock on external color, firmness, total soluble solids and acidity

An effect of rootstock on the main pigments in citrus peel was reported previously by Cano & Bermejo (2011). In the present study the external color was influenced by both harvest and rootstock and the interaction between these two factors was significant (Table 2).

Table 2. *p*-values ($p \leq 0.05$) of rootstock (A), harvest (B) factors and rootstock-harvest interaction (AB) of the parameters measured of 'Clemenules' mandarins grafted onto eight different rootstocks. * Significant at $p \leq 0.05$.

	1st Season			**2nd Season**		
	A: Rootstock	**B: Harvest**	**AB**	**A: Rootstock**	**B: Harvest**	**AB**
Color	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
Firmness	0.0000*	0.0000*	0.0013*	0.0000*	0.0000*	0.0073*
TSS	0.0000*	0.0000*	0.2379	0.0000*	0.0001*	0.1971
Acidity	0.0000*	0.0000*	0.0001*	0.0000*	0.0000*	0.0001*
MI	0.0000*	0.0000*	0.0046*	0.0000*	0.0083*	0.0006*
Individual Sugas						
Sucrose	0.0094*	0.0000*	0.7684	0.0011*	0.0000*	0.0133*
Glucose	0.0000*	0.0000*	0.0038*	0.0000*	0.2197	0.1088
Fructose	0.0000*	0.0000*	0.3480	0.0000*	0.0594	0.3616
Organic Acids						
Citric Acid	0.0000*	0.0000*	0.0203*	0.0000*	0.0000*	0.0000*
Malic Acid	0.0000*	0.0000*	0.0080*	0.0000*	0.0001*	0.1018
Succinic Acid	0.0000*	0.0000*	0.0509	0.0001*	0.0000*	0.0602
Ascorbic Acid	0.0002*	0.0004*	0.1134	0.0000*	0.3626	0.7735
Flavonoids						
Hesperidin	0.1401	0.0719	0.1624	0.0000*	0.0000*	0.0503
Narirutin	0.0028*	0.4811	0.8620	0.0000*	0.0000*	0.1277
Didymin	0.0017*	0.1447	0.7960	0.2791	0.0000*	0.7972

During both studied seasons, upon the first harvest FA13 and C-35 fruit had the highest color index (Figure 1A), close to −12, while the other fruit obtained color index values from −18.0 to −15.5. At the second harvest, the major increase was exhibited by the V17 fruit, which had the highest values, −3.69 and 0.99 at first and second season, respectively. At this harvest, the lowest values were presented by the CL fruit. At the third harvest, the differences among rootstocks were less pronounced when color values came to 7.45–13.35 in the first season and 12.88–16.20 in the second one. C-35 fruit had the lowest values during both seasons. Similarly to what we herein observed, a major rootstock effect on the external color of ‘Ruby Red’ at the beginning of the season has been reported, with scarcely any differences as season advanced (Machado et al., 2015).

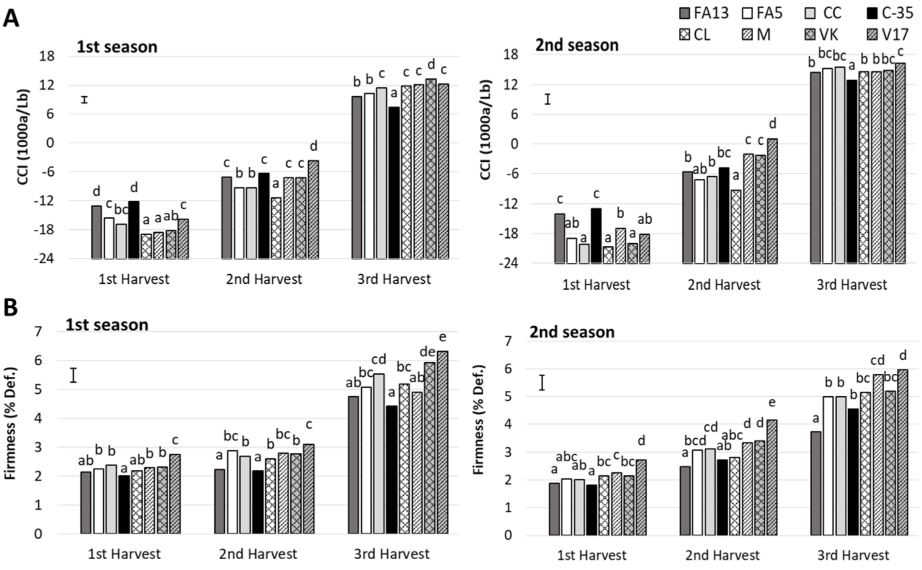


Figure 1. Citrus color index (CCI) **(A)** and firmness **(B)** of the ‘Clemenules’ mandarin grafted onto the eight studied rootstocks at three harvest times during two seasons. Vertical bar in each season represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction harvest-rootstock). Different letters above bars indicate significant differences among rootstocks at each harvest time ($p \leq 0.05$).

Firmness is an important fruit quality parameter, but is not included in the current citrus quality standards. Low fruit firmness can act as an important limitation for citrus fruit commercialization. Indeed, in citrus rootstock breeding programs, enhancing fruit firmness remains a challenge given its importance for fruit quality, especially when fruit is to be exported (Pallottino, Menesatti, Lanza, Strano, Antonucci & Moresi, 2012; Mazidi, Sadrnia & Khojastehpour, 2016). In the present study, an influence of both rootstock and harvest time on fruit firmness was detected during both seasons, existing a significant interaction between these two factors (Table 2). A rise in the deformation values with advanced harvest time was found, and this firmness loss was more marked during the third harvest (Figure 1B). The effects of rootstocks during both seasons were similar. FA13 and C-35 induced the highest firmness throughout the study while V17 obtained the lowest firmness values. Must be taken into account that all the fruit had commercial firmness values throughout the studied period, with deformation percentage values under 6.5%. Although very few references exist about limits of firmness values in citrus fruit for its fresh commercialization, there are reports in fruit to be exported about deformation values having to be lower than 10% in mandarins (Martínez-Jávega, Cuquerella, Salvador, Monterde & Navarro, 2004).

The rootstock influence on sugars and acid content has been related to the inherent rootstock effect on plant water relations (Albrigo & Carter, 1977; Castle & Krezdorn, 1977; Syversten & Graham, 1985). In the present study, TSS content increased with harvest time during both seasons (Figure 2A), as previously reported for most citrus fruit (Ladanyia, 2008). TSS content was strongly influenced by rootstocks and harvest time and no harvest-rootstock interaction was detected (Table 2). During the first season, the TSS content ranged was close to 11.0 in FA13, C-35, CL, M and VK with values close to 12.0 for FA5, CC, and V17. During the following harvests, the most important increase took place in the CC fruit, which had the highest values, 12.9 °Brix and 13.5 °Brix at second and third harvest, respectively. The lowest TSS values during the third harvest, close to 12.0 °Brix, were for M. During the second season, although the TSS values were lower than those recorded during the first one in all the harvests, the highest values were found for CC. The same effect of CC on TSS contents has been reported by Emmanouilidou & Kyriacou

(2017), who observed that ‘Lane Late’ and ‘Delta’ oranges grafted onto Carrizo Citrange had the highest TSS content than other four rootstocks.

During both seasons, at the first harvest, acidity ranged from values close to 2 g citric acid/100mL for CL to values near 1.5 in M (Figure 2B). The differences among rootstocks diminished as harvest advanced. The most marked descent in fruit acidity was detected in CL, between the first and second harvests. At third harvest and for both seasons, the V17 fruit maintained higher acidity levels than the other fruit, 1.01 g of citric acid/100mL. In other clementine mandarins, such as ‘Clémentine de corse’, a decrease of the acidity content with the fruit maturation was also observed (Julhia, Belmin, Meynard, Pailly & Casabianca, 2019).

The maturity index (MI) was represented in Figure 2C. All values were in agreement with other mandarin cultivars during harvest season such as ‘Fina’, ‘Loretina’ and ‘Arrufatina’ clementine mandarins (Bermejo & Cano, 2012b). In this study, an effect of rootstock was found, and the differences among rootstocks depended on harvest time, along with a significant harvest-rootstock interaction (Table 2). During both seasons for the first harvest, all the fruit exceeded the minimum commercial MI value of 7.0, except for CL and V17 due to high acidity. With MI above 7.0, clementine mandarins can be submitted to degreening treatment. Nevertheless, it is noteworthy that degreening treatment duration depends on the initial external color, and short duration is recommended to avoid any disorders associated with this treatment (Sdiri, Salvador, Farhat, Navarro & Besada, 2014). Thus according to external color, FA13 and C-35, with a CCI close to −12, could be subjected to shorter degreening treatments than the other fruit, which is relevant from a commercial point of view.

Although the CL fruit had the lowest MI values during the first and second harvests, this fruit in the third harvest gave one of the highest MI values. This was due to this fruit’s major acidity loss compared to other rootstocks. Conversely, the V17 fruit had the least MI increase as harvest advanced, with the lowest values that came close to 12.5 during the last harvest. This is related to acidity being maintained as harvest advanced. Previous studies also found the lowest MI values in V17 compared to other rootstocks (Legua et al., 2014). Nevertheless, it is noteworthy that this effect was observed only during the third harvest in the present study, without no major differences found to the other rootstocks studied during the first and second seasons.

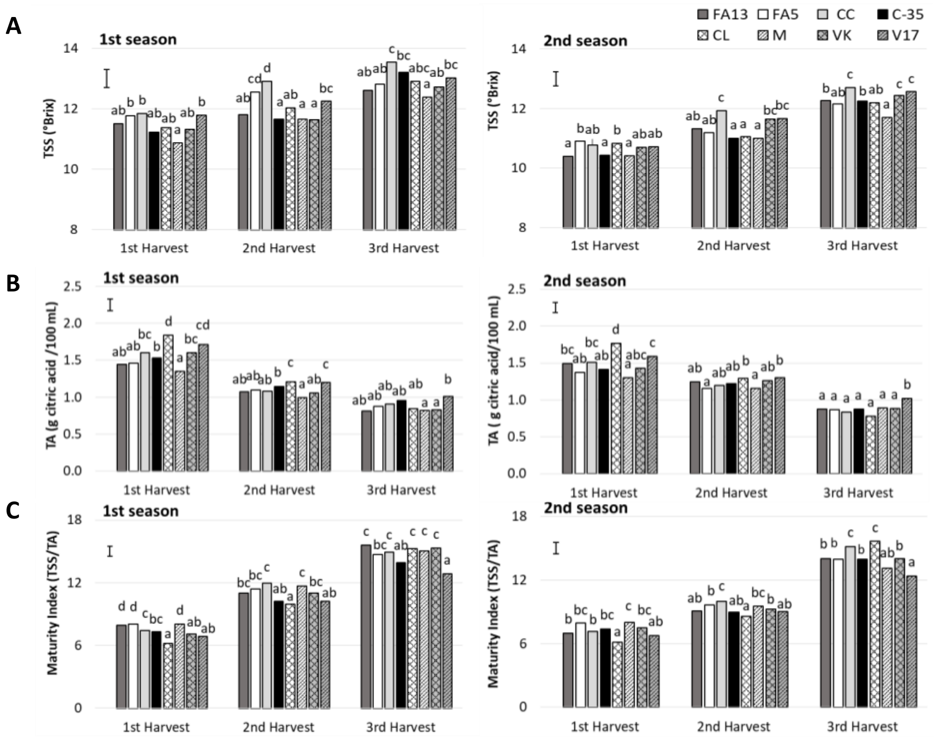


Figure 2. (A) Total soluble solids (TSS), **(B)** acidity (TA) and **(C)** Maturity Index (MI) of the 'Clemenules' mandarin grafted onto the eight studied rootstocks at three harvest times during two seasons. Vertical bar in each season represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction harvest-rootstock). Different letters above bars indicate significant differences among rootstocks at each harvest time ($p \leq 0.05$).

3.2. Rootstock effect on individual sugars, organic acids, vitamin c and flavonoids

Sugar composition was determined and three sugars were quantified (Figure 3). The predominant sugar was sucrose, followed by fructose and glucose, with values falling within the range reported previously for other clementine mandarin (Bermejo & Cano, 2012).

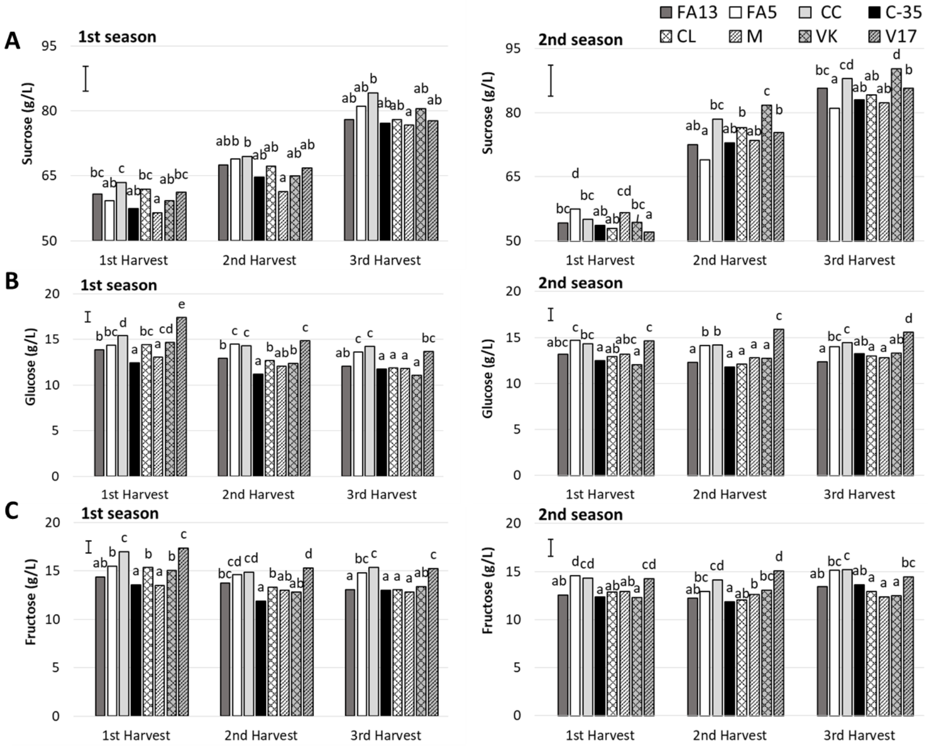


Figure 3. Individual sugars (**A:** sucrose; **B:** glucose; **C:** fructose) of the 'Clemenules' mandarin grafted onto the eight studied rootstocks at three harvest times during two seasons. Vertical bar in each season represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction harvest-rootstock). Different letters above bars indicate significant differences among rootstocks at each harvest time ($p \leq 0.05$).

During the first season, sucrose content rose from 56.49–63.52 g/L at first harvest to 76.69–84.23 g/L at third harvest. The differences among rootstocks were similar for the three harvests (no harvest-rootstock interaction was detected) (Table 2). The CC fruit had the highest sucrose content and the lowest values went to the M fruit. During the second season, sucrose values were higher and ranged from 52.05 to 57.45 g/L for the first harvest and from 81.01 to 90.30 g/L for the third. In this case, the rootstock influence was harvest-dependent. In general, the rootstocks that induced the highest sucrose content were CC and VK, and no differences with FA13 appeared during the third harvest. No remarkable differences were found among the remaining rootstocks.

Glucose content ranged from 11.06 to 17.42 g/L during both seasons. A general decrease as harvest advanced took place during the first season, while no harvest time influence was detected for the second season. Rootstock effect was observed during the two seasons with a harvest-rootstock interaction at the first season (Table 2). During both seasons, V17 was the rootstock that presented fruit with higher glucose contents, followed by FA5 and CC. No main differences were observed among the other rootstocks.

Similarly to that observed for glucose, during the first season fructose content tended to lower with harvest time, and its values ranged from 13.53–17.38 g/L at first harvest to 12.83–15.36 g/L at third, while no differences were shown for the second season as harvest advanced (values from 11.85 to 15.22 g/L). During both seasons, the highest fructose values were for the FA5, CC and V17 fruit. The other rootstocks showed no major differences between them.

The increase of TSS with harvest advance as described above was due to the increase of sucrose content. So, CC lead to fruit with the highest TSS and also induced the highest sucrose content. On the other hand, the higher increase of sucrose content respect to the other individual sugars with harvest advance has been previously reported in other mandarin cultivars (Fabroni, Romeo & Rapisarda, 2016).

Organic acid profile was determined and three acids (citric acid, malic acid and succinic acid) were quantified (Figure 4), all of which exhibited similar values to those reported in literature for clementine mandarins and other citrus fruits

(Bermejo & Cano, 2012b). The predominant organic acid was citric acid, followed by malic and succinic acid, respectively. Citric acid values (Figure 4A) for the first season fell within the 9.61–16.48 g/L range for the first harvest and the 4.63–7.50 g/L range for the last harvest. The drop in citric acid levels during ripening period has been also observed in 'Comune' and 'Tardivo' clementine mandarins (Distefano et al., 2009). These values were higher during the second season and ranged from 16.90–23.24 g/L to 7.85–10.32 g/L for the first and third harvest, respectively. During both seasons, an important rootstock effect was found and it depended on harvest time (Table 2). The differences among rootstocks decreased as harvest advanced, which coincided with the drop in acidity. The V17 fruit stood out for having the most citric acid, which was observed for both seasons. It was also outstanding that CL at first harvest and during both studied seasons, had similar values to V17, which coincided with the high titratable acidity values mentioned above. Previous studies have reported that FA13 displays the lowest citric acid levels in 'Clemenules' mandarins than other rootstocks (Legua et al., 2014). Nevertheless, in the present study, this was observed only during the first season, which indicates the importance of conducting studies at different maturity stages.

The second most relevant acid was malic acid (Figure 4B) which, unlike the other analysed organic acids, increased during the study period. During the first season, the effect of rootstock was harvest time dependent (Table 2). The amount of malic acid ranged from 1.33 to 1.71 g/L for the first harvest. In all cases, a gradual increase was observed during the following harvests until values ranged from 2.74 to 3.45 g/L in the third harvest. During the two first harvests, FA13 had the highest malic acid values. Nevertheless, at third harvest, when all the fruit significantly increased, the maximum values went to C35, with no differences with FA13 and VK. During the second season, a significant increase was observed from the first to the second harvest, with no major changes taking place during the last one. Similarly to that observed during the first season, FA13, C35 and VK had the highest values but in this case, this took place throughout the study period.

For succinic acid concentration (Figure 4C), major differences were observed between both studied seasons, and values were much lower during the first season, especially at first and second harvests. A marked rootstock influence was observed. During the second season, the succinic acid concentration for the first harvest ranged from values close to 2.50 g/L in CC and VK to values close to 1.65 g/L in FA13, FA5, C35, and CL. The values of the M and V17 fruit were close to 2.00 g/L. During the following harvests, the amount of succinic acid decreased in all cases, but differently depending on rootstocks. Thus in the second harvest, the M and V17 fruit had the maximum values close to 1.80 g/L, while the minimum ones were obtained by FA13 and C35 with values close to 1.15 g/L. At the third harvest, the reduction in this acid was marked, and the values of FA13 and C35 were very low, with 0.41 and 0.10 g/L respectively. The other fruit had values between 0.61 and 1.23 g/L with no major differences among them. The rootstock effect was less marked during the first season. Even so, similarly to that observed during the second season, FA13 and C35 induced a lower succinic acid concentration in fruit.

In previous studies an effect of rootstock in organic acids was reported when ‘Clemenules’ fruit were harvested at the end of the season (Legua et al, 2014). Nevertheless, the present study revealed that this effect can be harvest-dependent.

Citrus fruit is a well-known nutrient source of vitamin C in dietary intake, which is the major antioxidant compound found in such fruit (Yang et al., 2011). The range of the vitamin C levels obtained in the ‘Clemenules’ mandarin juices for all the rootstocks (Figure 4D) agree with the range previously reported for different citrus varieties (Bermejo & Cano, 2012b). The amounts of total ascorbic acid during the first season ranged from 46.92 to 35.98 mg/100 mL, which were bigger than for the second season, when values were between 40.53 and 34.07 mg/100 mL. During the first season, a slight decrease in ascorbic acid content was noted with harvest time, with no harvest effect detected during the second season (Table 2). A rootstock effect was found in the ascorbic acid content. The maximum ascorbic acid content was exhibited by CC, CL, M and V17 during the first season and by CC, VK and V17 during the second season. For both years, FA13 displayed the lowest ascorbic acid concentration. The study by Emmanouilidou & Kyriacou (2017) into ‘Lane late’ and ‘Delta’ oranges grafted onto five rootstocks found that VK also induced the highest vitamin C amount, followed by CC and CL, respectively.

The major flavonoid detected in the ‘Clemenules’ mandarin juices for all the rootstocks was the flavanone hesperidin, followed by narirutin and didymin, respectively, which agrees with the existing literature (Tripoli, La Guardia, Giammanco, Di Majo & Giammanco, 2007; Khan & Dangles, 2014).

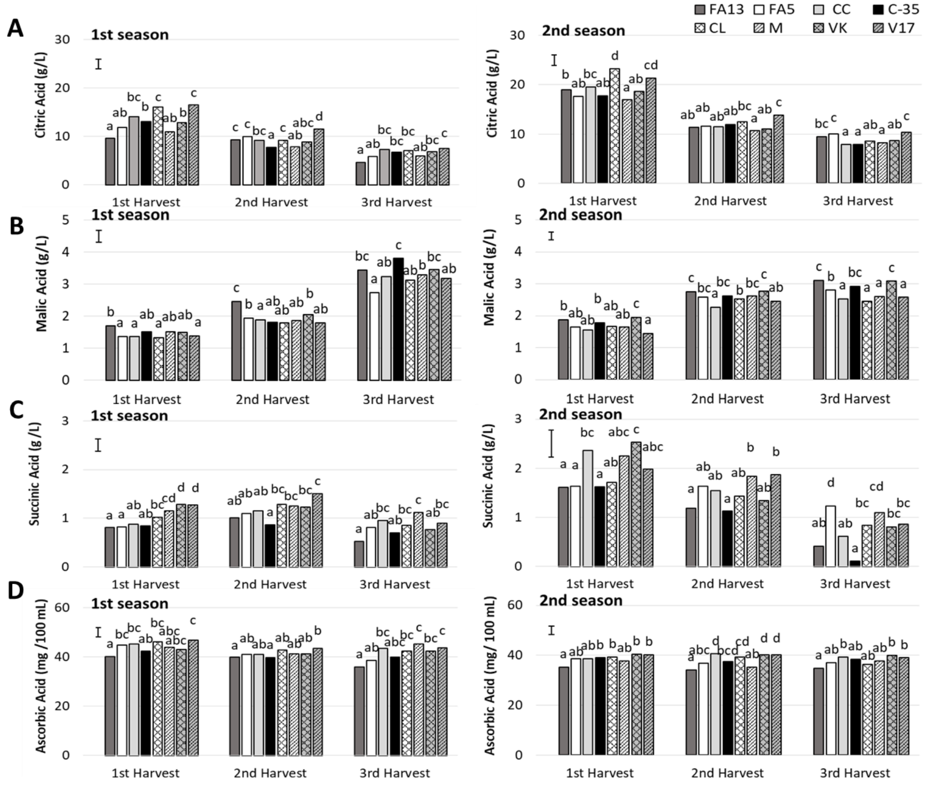


Figure 4. Organic acids (**A**: citric; **B**: malic; **C**: succinic) and vitamin C (**D**: ascorbic acid) of the ‘Clemenules’ mandarin grafted onto the eight studied rootstocks at three harvest times during two seasons. Vertical bar in each season represents the least significant difference (LSD) intervals (*p* ≤ 0.05) (interaction harvest-rootstock). Different letters above bars indicate significant differences among rootstocks at each harvest time (*p* ≤ 0.05).

Hesperidin was detected at concentrations between 11.98 and 14.14 mg/100 mL and from 11.52 to 15.32 mg/100 mL, during the first and second season, respectively (Figure 5A). For the first season, no changes were observed with harvest time, but a decrease was noted for the last harvest during the second season (Table 2). No significant differences were observed among rootstocks during the first season. Nevertheless, during the second one, M, VK and V17 induced higher hesperidin contents when fruit were picked during the second and third harvests.

Narirutin content (Figure 5B) during the first season still showed no significant changes with harvest time, but differences among rootstocks (Table 2). M, VK and V17 were the rootstocks that induced the maximum values, while FA13 stood out for having less narirutin content. During the second season, a gradual decrease in narirutin content took place during harvests in all cases, except for FA13 whose values were similar throughout the study period and came close to 1.10 mg/100 mL. The narirutin contents of the C35 and CL fruit dropped in the second harvest, and were the lowest values with 0.85 mg/100 mL in the third harvest.

Didymin was the minor determined flavanone (Figure 5C). For both seasons, its content ranged from 0.13 to 0.21 mg/100 mL. During the second season, a significant decrease was detected for the last harvest, which was not observed during the first season (Table 2). A rootstock effect appeared during the first season, which was due to the lowest values for CC when comparing with the fruit from the other rootstocks. No rootstock influence was observed during the second season.

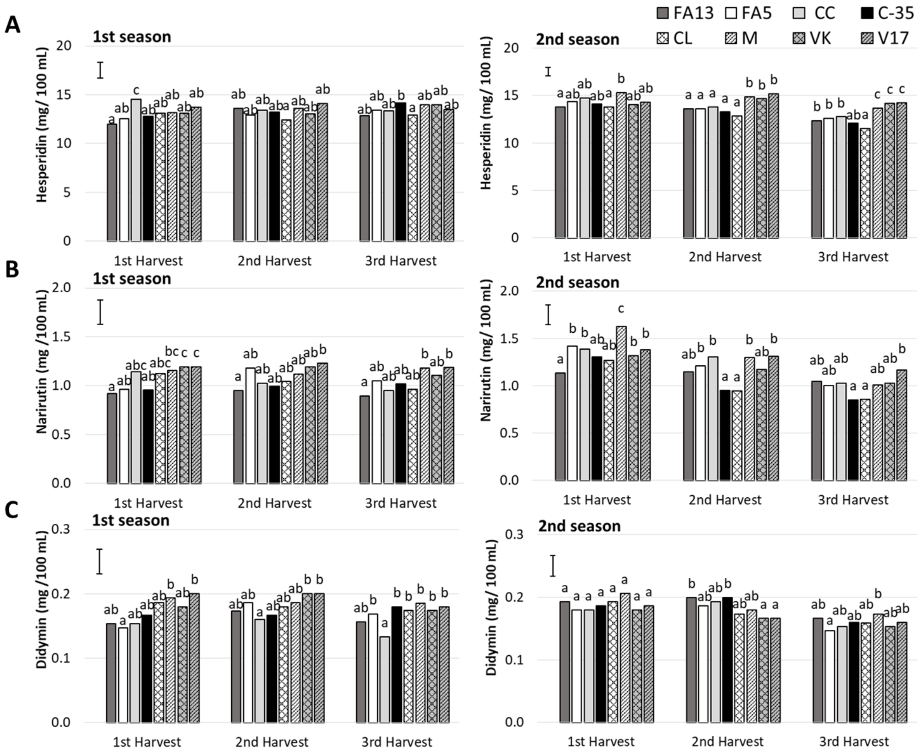


Figure 5. Flavonoids (**A**: hesperidin; **B**: narirutin; **C**: didymin) of the 'Clemenules' mandarin grafted onto the eight studied rootstocks at three harvest times (1st Harvest (MI = 7), 2nd Harvest (30 days after MI = 7) and 3rd Harvest (60 days after MI = 7)) during two seasons. Vertical bar in each season represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction harvest-rootstock). Different letters above bars indicate significant differences among rootstocks at each harvest time ($p \leq 0.05$).

4. Conclusions

The most remarkable result of this study was that the effect that rootstock could have on the different fruit quality parameters may be affected by their maturity stage. In order to obtain robust results, the importance of conducting studies in different seasons was clearly revealed.

The rootstock grafted onto 'Clemenules' mandarins can influence the optimal harvest time. This was the case of FA13 and C35, which induced a breakthrough in color change. This is an important aspect at the beginning of the season. Fruit can be harvested earlier and be submitted to shorter degreening treatment. FA13 and C35 induced the highest fruit firmness values. Regarding nutritional quality, FA13 and C35 induced the highest malic acid content, but the lowest succinic concentration. V17 stood out for being the rootstock that underwent the most marked color increase and the least acidity loss during harvest times, and was related to citric acid content. The lowest acidity loss exerted by V17 can be very useful to prolong the commercial period with fruit with high acidity at the end of the season. The fruit from this rootstock exhibited the highest glucose and fructose contents, as well as the highest vitamin C, hesperidin and narirutin contents. CC had the highest TSS content due to sucrose content. Similarly to V17, CC had high vitamin C content, but exhibited a tendency to present low flavonoid contents, particularly narirutin and didymin.

According to the results obtained in this study, the use of different rootstocks in the same agroclimatic location could allow an extension of the commercial season. In the case of 'Clemenules' mandarins, could be interesting to select FA13 and C35 to obtain fruit with high quality parameters earlier and V17 to have fruit with consumers acceptation at the end of this cultivar season.

Acknowledgements

The authors thank Anecoop S. Coop. for supplying the fruit herein used and its technical support. This study was co-financed by FEDER and European Social Funds.

References

Albrigo, L.G., & Carter, R.D. (1977). Carbohydrates: Composition, distribution, significance. In Citrus Science and Technology, 1st ed.; Nagy, S., Shaw, P.E., Matthew, K., Veldhuis, M.K., Eds.; Avi Publishing Company: Westport, CT, USA; California University: Oakland, CA, USA, Volume 2, pp. 74–93.

Bassal, M.A. (2009). Growth, yield and fruit quality of ‘Marisol’ clementine grown on four rootstocks in Egypt. *Scientia Horticulturae*, *119,* 132–137. https://doi.org/10.1016/j.scienta.2008.07.020

Bermejo, A., & Cano, A. (2012). Analysis of nutritional constituents in twenty citrus cultivars from the Mediterranean area at different stages of ripening. *Food and Nutrition Sciences*, *3,* 639–650. http://dx.doi.org/10.4236/fns.2012.35088

Bermejo, A., Pardo, J., & Cano, A. (2011). Influence of gamma irradiation on seedless citrus production: Pollen germination and fruit quality. *Food and Nutrition Sciences, 2*, 169–180. http://dx.doi.org/10.4236/fns.2011.23024

Bermejo, A., Pardo, J., & Cano, A. (2012). Murcott seedless: Influence of gamma irradiation on citrus production and fruit quality. *Spanish journal of agricultural research,* 10(3), 768–777. http://dx.doi.org/10.5424/sjar/2012103-460-11

Bermejo, A., Pardo, J.L., Morales, J., Cano, A. (2016). Comparative study of bioactive components and quality from juices of different mandarins: Discriminant multivariate analysis of their primary and secondary metabolites. *Agricultural Sciences, 7(6),* 341–351. http://dx.doi.org/10.4236/as.2016.76035

Cano, A., & Bermejo, A. (2011). Influence of rootstock and cultivar on bioactive compounds in citrus peels. *Journal of the Science of Food and Agriculture, 91,* 1702–1711. https://doi.org/10.1002/jsfa.4375

Cardeñosa, V., Barros, L., Barreira, J.C., Arenas, F., Moreno-Rojas, J.M., & Ferreira, I.C. (2015). Different Citrus rootstocks present high dissimilarities in their antioxidant activity and vitamins content according to the ripening stage. *Journal of plant physiology*, *174,* 124–130. https://doi.org/10.1016/j.jplph.2014.10.013

Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science, 23(4),* 383–394. https://doi.org/10.1080/01140671.1995.9513914

Castle, W.S., & Krezdorn, A.H. (1977). Soil water use and apparent root efficiencies of citrus trees on four rootstocks. *Journal American Society for Horticultural Science, 102,* 403–406.

Distefano, G., Las Casas, G., Caruso, M., Todaro, A., Rapisarda, P., La Malfa, S., Gentile, A., & Tribulato, E. (2009). Physiological and molecular analysis of the maturation process in fruits of clementine mandarin and one of its late-ripening mutants. *Journal of agricultural and food chemistry*, *57(17),* 7974–7982. https://doi.org/10.1021/jf900710v

Emmanouilidou, M.G., & Kyriacou, M.C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of ‘Lane Late’ and ‘Delta’ sweet orange. *Scientia Horticulturae, 225,* 112–121. https://doi.org/10.1016/j.scienta.2017.06.056

Fabroni, S., Romeo, F.V., & Rapisarda, P. (2016). Nutritional composition of clementine (citrus x clementina) cultivars. In Nutritional Composition of

Fruit Cultivars, 1st ed.; Simmonds, M.S.J., Preedy, V.R., Eds.; Academic Press Elsevier: Amsterdam. The Netherlands, pp. 149–172. https://doi.org/10.1016/B978-0-12-408117-8.00007-6

Filho, F.D.A.A., Espinoza-Núñez, E., Stuchi, E.S., & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. *Scientia Horticulturae,* 114, 45–49. https://doi.org/10.1016/j.scienta.2007.05.007

Jimenez-Cuesta, M., Cuquerella, J., & Martínez-Jávega, J. (1981). Determination of a color index for citrus fruit degreening. *Proceedings of the International Society of Citriculture*, 2, 750–753.

Julhia, L., Belmin, R., Meynard, J.M., Pailly, O., & Casabianca, F. (2019). Acidity drop and coloration in clementine: Implications for fruit quality and harvesting practices. *Frontiers in plant science, 10,* 754. https://doi.org/10.3389/fpls.2019.00754

Khan, M.K., & Dangles, O. (2014). A comprehensive review on flavanones, the major citrus polyphenols. *Journal of Food Composition and Analysis*, 33(1), 85–104. https://doi.org/10.1016/j.jfca.2013.11.004

Ladanyia, M.S. (2008). Fruit Biochemistry. In Citrus Fruit: Biology, Technology and Evaluation, 1st ed.; Ladanyia, M.S., Ed.; Academic Press Elsevier: San Diego, CA, USA, pp. 125–190.

Legua, P., Forner, J.B., Hernández, F., & Forner-Giner, M.A. (2013). Physicochemical properties of orange juice from ten rootstocks using multivariate analysis. *Scientia Horticulturae*, 160, 268–273. https://doi.org/10.1016/j.scienta.2013.06.010

Legua, P., Forner, J.B., Hernández, F., & Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. *Scientia Horticulturae*, 174, 60–64.

https://doi.org/10.1016/j.scienta.2014.05.004

Legua, P., Martinez-Cuenca, M.R., Bellver, R., & Forner-Giner, M.A. (2018). Rootstock's and scion's impact on lemon quality in southeast. *International* Agrophysics, 32(3), 325–333. https://doi.org/10.1515/intag-2017-0018

Machado, F.L.D.C., Costa, J.D.P.D., Teixeira, A.D.S., & Costa, J.M.C.D. (2015). The influence of rootstock and time of harvest on the fruit quality during storage of in two grapefruit cultivars. *Acta Scientiarum. Agronomy*, *37(3),* 339–346. https://doi.org/10.4025/actasciagron.v37i3.16970

Magwaza, L.S., Mditshwa, A., Tesfay, S.Z., Opara, U.L. (2017). An overview of preharvest factors affecting vitamin C content of citrus fruit. *Scientia Horticulturae, 216,* 12–21. https://doi.org/10.1016/j.scienta.2016.12.021

Martínez-Jávega, J.M., Cuquerella, J., Salvador, A., Monterde, A., & Navarro, P. (2004). Adecuación de tratamientos postcosecha a parámetros de recolección de mandarinas y naranjas de España. *Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones*, 422-431.

Mazidi, M., Sadrnia, H., & Khojastehpour, M. (2016). Evaluation of orange mechanical damage during packaging by study of changes in firmness. *International Food Research Journal*, *23(2),* 899–903.

McCollum, T.G., Bowman, K.D., & Castle, W.S. (2002). Effects of rootstock on fruit quality and postharvest behavior of 'Marsh' grapefruit. *Proceedings of Florida State Horticultural Society*, 115, 44–46.

Pallottino, F., Menesatti, P., Lanza, M.C., Strano, M.C., Antonucci, F., & Moresi, M. (2012). Assessment of quality-assured Tarocco orange fruit sorting rules by combined physicochemical and sensory testing. *Journal of the Science of Food and Agriculture,* 93(5), 1176–1183. https://doi.org/10.1002/jsfa.5871

Saini, M.K., Capalash, N., Kaur, C., & Singh, S.P. (2019). Comprehensive metabolic profiling to decipher the influence of rootstocks on fruit juice metabolome of Kinnow (C. nobilis × C. deliciosa). *Scientia Horticulturae, 257*, 108673. https://doi.org/10.1016/j.scienta.2019.108673

Sdiri, S., Bermejo, A., Aleza, P., Navarro, P., & Salvador, A. (2012). Phenolic composition, organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid late-season mandarins. *Food Research International,* 49(1), 462–468. https://doi.org/10.1016/j.foodres.2012.07.040

Sdiri, S., Salvador, A., Farhat, I., Navarro, P., & Besada, C. (2014). Influence of postharvest handling on antioxidant compounds of Citrus fruits. In Citrus: Molecular Phylogeny, Antioxidant Properties and Medicinal Uses; Hayat, K., Ed.; Nova Publisher Inc.: New York, NY, USA, pp. 73–94.

Sharma, R.M., Dubey, A.K., Awasthi, O.P., & Kaur, C. (2016). Growth, yield, fruit quality and leaf nutrient status of grapefruit (Citrus paradisi macf.): Variation from rootstocks. *Scientia Horticulturae,* 210, 41–48. https://doi.org/10.1016/j.scienta.2016.07.013

Sumiasih, I.H., Poerwanto, R., Efendi, D., Agusta, A., & Yuliani, S. (2018). β-Cryptoxanthin and Zeaxanthin Pigments Accumulation to Induce Orange Color on Citrus Fruits. *IOP Conference Series: Materials Science and Engineering,* 299, 1–8.

Syvertsen, J.P., & Graham, J.H. (1985). Hydraulic conductivity of roots, mineral nutrition, and leaf gas exchange of citrus rootstocks. *Journal of the American Society for* Horticultural, 110, 865–869.

Tripoli, E., La Guardia, M., Giammanco, S., Di Majo, D., & Giammanco, M. (2007). Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry, 104(2),* 466–479. https://doi.org/10.1016/j.foodchem.2006.11.054

United Nations. UNECE Standard FFV-14: Citrus Fruit; United Nations: Geneva, Switzerland, 2018, p. 5.

Yang, X.Y., Xie, J.X., Wang, F.F., Zhong, J., Liu, Y.Z., Li, G.H., & Peng, S.A. Comparison of ascorbate metabolism in fruits of two citrus species with obvious difference in ascorbate content in pulp. *Journal of plant physiology*, *168(18),* 2196–2205. https://doi.org/10.1016/j.jplph.2011.07.015

Yıldırım, F., Yıldırım, A.N., San, B., & Ercişli, S. (2016). The relationship between growth vigour of rootstock and phenolic contents in apple (malus×domestica). *Erwerbs-Obstbau 58,* 25–29. https://doi.org/10.1007/s10341-015-0253-7

Yin, X.R., Xie, X.L., Xia, X.J., Yu, J.Q., Ferguson, I.B., Giovannoni, J.J., & Chen, K.S. (2016). Involvement of an ethylene response factor in chlorophyll degradation during citrus fruit degreening. *The Plant Journal,* 86(5), 403–412. https://doi.org/10.1111/tpj.13178

III.2. TANGO MANDARINS

CHAPTER II

Physico-Chemical, Sensorial and Nutritional Quality during the Harvest Season Of 'Tango' Mandarins Grafted Onto Carrizo Citrange and Forner-Alcaide No.5

Julia Morales [a], Alejandra Salvador [a], Cristina Besada [a], Pilar Navarro [a], Almudena Bermejo [b]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

Reference: *Food Chemistry*, 2021, 339: 127781.

Abstract

‘Tango’ mandarin is becoming one of the most demanded varieties in the Mediterranean Region. However, no information on the quality of ‘Tango’ fruit in this citrus area has been reported. In this study, the physico-chemical, nutritional and sensorial quality of ‘Tango’ mandarins grafted onto Carrizo Citrange and FA5 rootstocks from two locations (Sevilla and Huelva) was evaluated by harvest season. The fruit from Sevilla exhibited lower levels of acids and sugars than those from Huelva, which was associated with a higher sandy soil percentage in the Huelva orchard. In both orchards, the FA5 produced fruit had higher sugars and acids. Flavonoids were affected mainly by location, and the Huelva fruit exhibited the highest levels. The highest vitamin C was for the FA5 fruit. The decreased antioxidant capacity observed throughout the harvest season was related to reduce vitamin C. The sensorial evaluation corroborated changes in the quality parameters.

Keywords: Biocomponents, harvest, sugars, acidity, physico-chemical quality, sensorial quality

1. Introduction

‘Tango’ mandarin is a recent cultivar that was developed at the University of California, Riverside from an irradiated bud of the diploid mandarin cultivar ‘W. Murcott’ (USPP17863; Roose & Williams, 2007). It is characterized by its mid-late harvest period and having high-quality seedless fruits. Currently this cultivar is becoming one of the most demanded mandarin varieties in the world’s main citrus production areas.

In Spain, the ‘Tango’ cultivar is being introduced as an interesting alternative to be commercialized when the Clementine season ends. Nowadays, most ‘Tango’ plots are located in Andalucia (S. Spain), although there is interest in extending its production throughout the Mediterranean Region. The harvest period in this area roughly starts at the end of December and continues to the end of February. Presently, there is no information on the changes that ‘Tango’ fruit undergoes during this period, or if an optimal harvest time exists when this fruit exhibits optimal quality.

It has been reported that fruit quality can be influenced by biotic and abiotic factors (Iglesias et al., 2007). In citrus, one important aspect to consider is the rootstock onto which a specific cultivar is grafted because it may influence several tree growth and development aspects, including yield, fruit quality and tolerance to stress caused by biotic and abiotic factors (Filho, Espinoza-Núñez, Stuchi, & Ortega, 2007).

Likewise, the nutritional composition of citrus fruit can vastly vary depending on the growth conditions of groves, including rootstock, soil composition and elevation (Zhang, Breksa, Mishchuk, & Slupsky, 2011). Indeed, the nutritional quality of citrus fruit juice has been reported to be directly linked with rootstock effects on plant-water relations regulating sucrose transport (Barry, Castle, & Davies, 2004).

The most widely used rootstock in Spain is Carrizo Citrange (CC) [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.]. This rootstock is tolerant to CTV (citrus tristeza virus), and induces good productivity and fruit quality, but frequently presents iron chlorosis and salinity problems (Forner, Forner-Giner, & Alcaide, 2003). The search for new better performing citrus rootstocks than those normally used is one of the citrus industry's main challenges in many countries. In this context, an ambitious program for breeding citrus rootstocks is being undertaken at the Institute Valenciano de Investigaciones Agrarias (IVIA) (Valencia, Spain). As a result of this programme, different rootstocks have been selected and released. One of the most interesting obtained rootstock has been Forner-Alcaide no. 5 [*Citrus reshni* Hort. Ex Tan. × *Poncirus trifoliata* (L.) Raf.], (FA5). FA5 is a hybrid of the Cleopatra mandarin [*Citrus reshni* Hort. ex Tan) × *Poncirus trifoliata* (L.) Raf.] obtained by traditional hybridisation. This rootstock offers high productivity and good fruit quality of the scion cultivar. It is tolerant to CTV, salinity and lime-induced chlorosis, and is resistant to *Phytophthora* sp. and citrus nematode *Tylenchulus semipenetrans* Cobb (Forner et al., 2003; Forner-Giner, Primo-Millo, & Forner, 2009). For all these reasons, it would be very interesting to know the behaviour of the 'Tango' cultivar grafted onto FA5. In this context, it is important to know the influence of this rootstock on physico-chemical and nutritional fruit quality as it has been reported that rootstock can also affect internal quality, organoleptic and nutritional quality, and the concentration of some bioactive compounds (Legua, Forner, Hernández, & Forner-Giner, 2014).

The aim of the present work was to evaluate the changes in the physico-chemical, sensorial and nutritional quality during harvest of 'Tango' mandarins from trees grafted onto Carrizo Citrange or Forner-Alcaide no. 5 under Mediterranean conditions.

2. Materials and Methods

2.1. Plant material

Experiments were conducted with the 'Tango' mandarins taken from the trees grafted onto Carrizo Citrange (CC) [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], and also grafted onto Forner-Alcaide no. 5 (FA5) [*Citrus reshni* Hort. Ex Tan. × *Poncirus trifoliata* (L.) Raf.] in two commercial orchards located in different growing areas of Andalucia. One of the orchards is located inland in Sevilla and has loamy soil (45% sand; 36% lime; 19% clay). This orchard had $EC_{1-5}< 70$ µS cm^{-1} (20°C) and a pH of 6.19. The other orchard is found in Huelva on the Mediterranean coast with sandy loam soil (80% sand, 10% lime; 10% clay). This orchard had EC1-5<106 µS cm^{-1} (20°C) and a pH of 6.75. The fruit from six trees grafted onto each rootstock in both orchards were used for the physico-chemical evaluations. On seven harvest dates, 20 fruit were picked in both orchards from two trees grafted onto each rootstock to perform three replicates: 5 January (H1); 9 January (H2); 17 January (H3); 24 January (H4); 30 January (H5); 6 February (H6) and 13 February (H7). After each harvest, fruit were transported to the IVIA where the physico-chemical, nutritional and sensorial analyses were carried out.

2.2. Determination of the physico-chemical parameters

The external peel color of fruit was measured by a Minolta colorimeter (model CR-400, Minolta Co. Ltd, Osaka, Japan) over 15 fruit per lot by taking two measurements from the opposite equatorial sides of each fruit. The mean values for the 'L', 'a' and 'b' Hunter parameters were calculated with each fruit and expressed as the Citrus Colour Index (CCI) (CCI=1000a/Lb) (Sdiri, Navarro, Monterde, Benabda, and Salvador, 2012).

Firmness measurements were taken by a Universal Testing Machine (model 3343, Instron Limited, Buckinghamshire, England) using 15 fruit per lot. The results were expressed as the percentage of millimetres of fruit deformation that resulted from a 10 N force on the longitudinal axis at constant speed (Sdiri, Navarro, et al., 2012).

In each lot of fruit, three samples of five fruit each were squeezed by an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain).

Juice yield was measured and expressed as a percentage, calculated by dividing the volume of juice by the total fruit weight. Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution using phenolphthalein as the indicator, expressed as g citric acid/100 mL of juice. The total soluble solids content (TSS) in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd, Tokyo, Japan) and data were expressed as °Brix. The maturity index (MI) was calculated as the TSS/TA ratio.

The ethanol content from each juice was quantified by a headspace analysis in a gas chromatograph (model 1020, Perkin Elmer Corp., Norwalk, CT, USA) following the method described by Sdiri, Navarro, et al. (2012), expressed as mg ethanol/100 mL juice.

2.3. Biocomponents analysis

2.3.1. Extraction and analysis of sugars

The extraction method was the same as the procedure by Bermejo, Pardo, Morales, and Cano (2016). Carbohydrates were analysed by HPLC according to the method described by Bermejo, Pardo, and Cano (2011). The results were expressed as g/L.

2.3.2. Extraction and analysis of organic acids

Organic acids extraction was carried out according to the method described by Bermejo et al. (2016), and compounds were analysed by HPLC-DAD and HPLC-MS under electrospray ion negative conditions. An ICSep ICE-COREGEL 87H3 column (Transgenomic) was used with an isocratic mobile phase of 0.1% H_2SO_4 solution at a flow rate of 0.6 mL/min. The injection volume was 5 µL. The HPLC-MS analysis was carried out according to Bermejo et al. (2016). The standard compounds came from Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g/L.

2.3.3. Extraction and analysis of flavonoids

The main flavonoids were extracted following the procedure described by Bermejo, Pardo, and Cano (2012), with some modifications to adapt the method to a microlitre format (Bermejo et al., 2016). Narirutin, hesperidin and didymin were analysed by HPLC-DAD and HPLC-MS under electrospray ion positive conditions according to the method by Bermejo et al. (2011). The standards came from Extrasynthesis (Genay, France), Sigma (Sigma Co., Barcelona, Spain) and ChromaDex (Irvine, CA, USA). The results were expressed as mg/100 mL.

2.3.4. Extraction and analysis of vitamin C

Total vitamin C was extracted according to the method reported by Sdiri, Bermejo, Aleza, Navarro, and Salvador (2012). DL-dithiothreitol (DTT) was used as the reducing reagent of dehydroascorbic acid to ascorbic acid. Ascorbic acid quantification was performed by HPLC-DAD as described by Bermejo et al. (2012). L-ascorbic acid was obtained from Sigma (Sigma Co., Barcelona, Spain) and DTT from Fluka (Sigma Co., Barcelona, Spain). The results were expressed as mg/100 mL.

2.3.5. Instruments for the HPLC analysis

An HPLC analysis were performed in an Alliance liquid chromatographic system (Waters, Barcelona, Spain), equipped with a 2695 separation module, a 2996 photodiode array detector and a ZQ2000 mass detector. Samples were detected at 5°C, and the column temperature was set at 25°C or 35°C. The sugars analyses were carried out with a Waters 515 HPLC pump, a 2414 refractive index detector and a 20 µL loop Rheodyne injector. Data were acquired and processed by the Empower 2 software (Waters, Spain). Grade solvents and Milli-Q water were used in all the biocompound HPLC analyses.

2.3.6. Analysis of total antioxidant activity (AA)

The antioxidant activity (AA) of citrus juice was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method and the ferric reducing antioxidant power (FRAP) assay. The DPPH free radical-scavenging activity measurement was assessed according to the procedure described by Sdiri, Bermejo, et al. (2012). Briefly in glass 96-well reaction plates, 30 µL of the diluted samples in 100% methanol were combined with 270 µL of methanolic DPPH (0.025 g/L in 100% methanol). Following incubation at 20°C for 50 min, absorbance at 515 nm was read by a Multiscan Spectrum microplate reader (Thermo Electron Corporation, SA, Finland). The ability of scavenging the DPPH radical was calculated by the following equation:

$\% \text{ Inhibition}=[(A_0-A_x)/A_0]100$

Where A_0 is the absorbance of a DPPH blank and A_x is the absorbance of the juice solution. The FRAP method was assessed according to the method described by Sdiri, Bermejo, et al. (2012). Briefly, the FRAP reagent was freshly prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. The assay was carried out by placing 30 µL of appropriately diluted samples in a 96-well microplate and then adding 270 µL of FRAP reagent. Absorbance was read at 593 nm After 10 min of incubation at 37°C and shaking. The results were compared with a standard curve prepared with different ascorbic acid concentrations and were expressed as an mM ascorbic acid equivalent (AAe).

2.4. Sensory evaluation

Panellists were employees from the Postharvest Technology Centre, who were classified as semi-experts given their ample experience in serving on citrus sensory panels. The sensorial evaluation was made up of between 15 and 17 panellists. Sensory sessions were carried out in a specifically adapted room, where panellists sat in individual evaluation booths, and samples were presented through a small door in front of the booths. Three segments from three different mandarins were presented per sample to compensate for biological variability. Room temperature distilled water was provided to cleanse palates between samples. Samples were served in random order to panellists in 50-mL stainless steel soufflé cups, identified by a unique three-digit number. Each fruit was tasted by at least three panellists, who gave a hedonic score (liking) for each sample, which ranged from 1 to 9: 1 was “dislike very much” and 9 was “like very much”. Panellists were also asked about their purchase intention on a 5-point scale, which went from 1 “definitely would not buy” to 5 “definitely would buy”. Sweetness and acidity levels were also evaluated using 5-point scales that went from 1 “very low” to 5 “very high”. Finally, the off-flavour level was evaluated from 1 “absence” to 5 “a very high off-flavour”.

2.5. Statistical analysis

Statistical procedures were performed using a statistical software (Statgraphics plus 5.1. Manugistics, Inc., Rockville, MD, USA). All the data were subjected to an analysis of variance, and means were com-pared by an LSD test at P ≤ 0.05.

3. Results and discussion

3.1. Physico-Chemical quality

'Tango' fruit is characterised by having an intense reddish external color (USPP17863). In the present study, the fruit from the Sevilla orchard were harvested with high values close to 20 at the beginning of January (H1). Slight changes for the following harvests were observed. The fruit from the trees grated onto FA5 showed slightly higher values than those grafted onto CC (Fig. 1). Nevertheless, in the fruit from the Huelva orchard, the differences between the two rootstocks were more marked. While the CCI values of the CC fruit were similar to those observed in the fruit from Sevilla, the fruit from FA5 had lower values during the first harvest (CCI=17.4). Although a gradual increase was observed during the following harvests, the maximum value came close to 19.

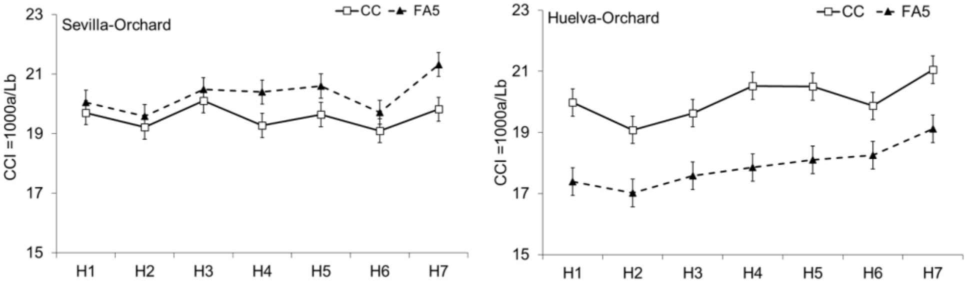


Figure 1. External color of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC and FA5) during harvest seasons from early January to mid-February. The vertical bars represent the interaction rootstock-harvest in each location (least significant difference (LSD) intervals (P ≤ 0.05)).

The ‘Tango’ mandarins from both orchards had high firmness values, with a percentage of deformation values close to 4.5–5% throughout the harvest period (data not shown). No influence of root-stock was observed on this parameter in any case. The juice yield of the fruit in all cases was higher than 33%, which is the minimum to commercialise hybrid mandarins according to EU Quality Citrus Standards (EU Regulation No. 543/2011). The juice yield content was higher in the fruit from the Huelva orchard (45–50%) than those from Sevilla (43–46%) throughout the study, with no differences among harvests (data not shown). Rootstock did not influence the juice yield content in any case.

Regarding total soluble solids content (TSS), at first harvest, the TSS values of the Sevilla orchard fruit were lower (between 10.3 °Brix and 11.2 °Brix) than those for the fruit from Huelva (between 12.3 °Brix and 13.0 °Brix; Fig. 2A). In both orchards, the highest TSS values were shown in the fruit from the trees grafted onto FA5. In all cases, TSS remained practically constant until 24 January (H4), after which time a slight gradual increase in later harvests took place. This increase was more marked in the fruit from Sevilla, with values close to 11 °Brix and 12 °Brix in the fruit from the trees on CC and FA5, respectively. A rise in total sugars during fruit ripening has been previously described in the pulp of other citrus fruit (Emmanouilidou & Kyriacou, 2017; Ladanyia, 2008). Our TSS values coincided with the value set out in the patent of the variety (USPP17863), and fell within the range of other mandarin varieties (Goldenberg et al., 2014).

Similarly to that observed for TSS, at each harvest fruit acidity (TA) was higher in the fruit from Huelva than from Sevilla. Although the fruit from FA5 in both orchards also obtained higher values, the differences between rootstocks were more marked in the Huelva orchard. The fruit from both rootstocks from Sevilla had stable acidity values as no changes were observed throughout the study season, and only a slight decrease was noted for the last harvest. In Huelva, a gradual decrease in fruit acidity took place throughout the harvest with values coming close to 1.1 g citric acid/100 mL juice in the fruit from FA5, and close to 0.9 g citric acid/100 mL juice in CC-fruit at the end of the harvest season. According to the patent text of ‘Tango’ mandarin (USPP17863), fruit acidity values between 0.54 and 1.19 g citric acid/ 100 mL juice are reported, which are slightly lower than those obtained for our assayed conditions.

The Maturity Index (MI), calculated as the TSS/TA ratio, is the parameter included in most quality standards for citrus fruit. In our case, the high TA of the fruit values led to lower MI values than we expected when considering the high TSS value. In all cases, a slight increase was observed in the first four harvests, which then became more marked.

In both orchards, the fruit from trees grafted onto CC, with the lowest TSS and TA values, showed the highest MI throughout the harvest period. The fruit from Sevilla, with lower TSS and TA values, obtained higher values than those from Huelva.

It is worth mentioning that in all cases the MI of fruit exceeded the minimum value for mandarins and hybrids (7.5), established by The European Regulation for the commercialisation of citrus fruits (EU Regulation No. 543/2011).

The higher TSS and TA levels in the ‘Tango’ mandarins from Huelva could be attributed to texture soil composition. The Huelva orchard had a higher sandy soil percentage, and sandy soils have been reported to lead to higher radicular development, mainly fibrous roots (Li, Xu, & Cohen, 2005). An increase in fibrous roots density improves the plant water status due to more effective water uptake and greater hydraulic conductivity, which induce higher TSS and TA levels (Barry et al., 2004). Rootstock also influences water uptake, nutrients transport and scion budded vigour (Castle, 1995). In the specific case of FA5, the highest TSS and TA values could be explained by their higher photosynthetic rate compared to CC, as reported by González-Mas, Llosa, Quijano, & Forner-Giner (2009). Higher net photosynthetic flux higher photoassimilate compounds are transported from leaves to fruits. Sucrose is the main sugar transported through the phloem in citrus (Jover et al., 2012).

Regarding ethanol content, it is must be noted that it is presented in the aroma of citrus fruits and has been reported to be a natural precursor of aromatic compounds (Sdiri, Rambla, Besada, Granell, & Salvador, 2017). In the present study, very low values with no relevant changes throughout the studied harvest period were found in all cases (data not shown). Notwithstanding, the fruit from Huelva exhibited higher values (between 14.3 and 20.14 mg ethanol/100 mL juice) than those from Sevilla (between 3.17 and 11.88 mg ethanol/100 mL juice).

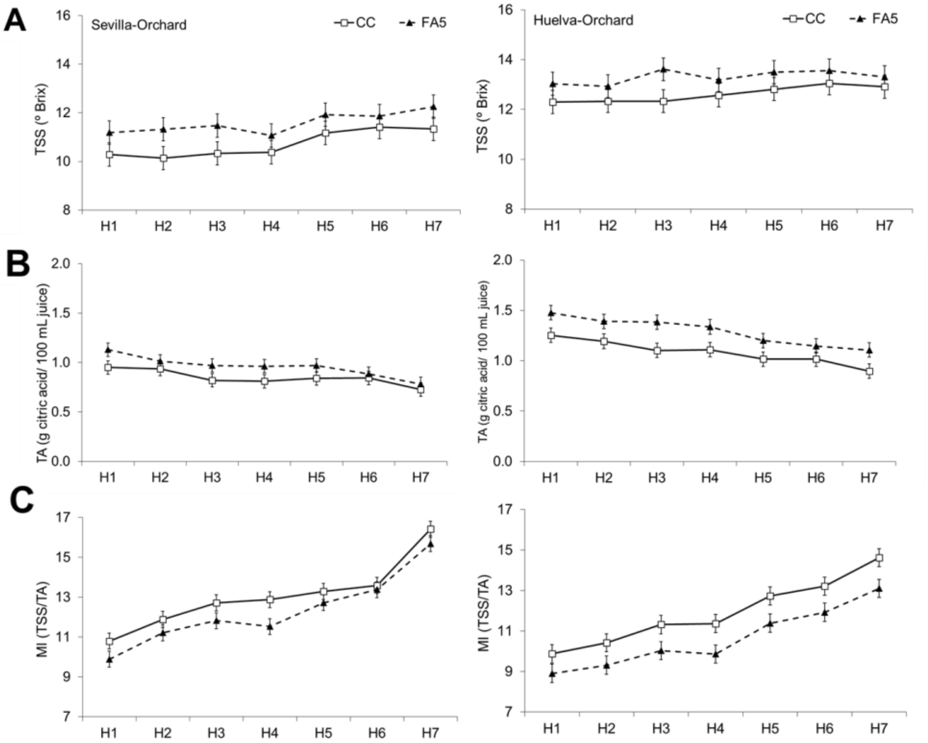


Figure 2. Total soluble solids (TSS) (A), Titratable acidity (TA) (B) and Maturity Index (C) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC and FA5) during harvest seasons from early January to mid-February. The vertical bars represent the interaction rootstock-harvest in each location (least significant difference (LSD) intervals (P≤0.05)).

3.2. Nutritional quality

3.2.1. Sugars and organic acids contents

The main detected sugar was sucrose, followed by fructose and glucose (Table 1). The sucrose-fructose-glucose ratio (2:1:1) was similar for all the 'Tango' mandarins. As previously shown in TSS, the concentration of total sugars in the fruit from Huelva was higher than for the fruit from Sevilla. The values of the three detected sugars agree with those reported in the literature for juices from different mandarins (Bermejo et al., 2016), but are slightly higher than those reported for the parental 'Murcortt' (Sdiri et al., 2012).

No differences in the total sugars concentration were found between H1 and H4. An increment was observed at H7, except in the CC fruit from Sevilla, for which a similar total sugar content was observed in the three harvests. A significant rootstock effect on sugars accumulation was found in the Huelva orchard, where the FA5 fruit obtained higher values for the three individual sugars. In the Sevilla orchard, the rootstock influence was only significant on the sucrose concentration.

The measurements taken of the main organic acids revealed that the major acid was citric acid, followed by malic and succinic acids, as previously reported (Kelebek & Selli, 2011). The citric: malic: succinic ratio (2:1:1) was similar for all the mandarins under study. The citric acid levels found in this study fell within the previously reported range for other mandarins in different ripening stages (Bermejo & Cano, 2012).

Table 1. Content of individual sugars (sucrose, glucose, fructose and total sugars) and organic acids (citric acid, malic acid, succinic acid and total organic acids) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P ≤ 0.05).

		Sevilla-Orchard		**Huelva-Orchard**	
	Harvest	**CC**	**FA5**	**CC**	**FA5**
Sucrose (g/L)	H1	38,16 a	40,83 ab	46,40 a	49,20 ab
	H4	37,76 a	40,44 ab	47,96 a	50,86 b
	H7	41,82 ab	47,81 b	53,08 b	52,33 b
Glucose (g/L)	H1	13,68 b	13,47 b	13,95 a	16,23 bc
	H4	13,67 b	12,67 a	15,33 ab	15,83 b
	H7	13,90 b	16,58 c	19,06 c	18,27 c
Fructose (g/L)	H1	15,58 ab	15,32 ab	16,47 a	17,75 ab
	H4	15,47 ab	14,23 a	16,90 a	17,51 ab
	H7	16,29 ab	17,97 b	19,52 b	18,52 b
Total Sugars (g/L)	H1	67,42 a	69,62 a	76,83 a	83,18 b
	H4	66,90 a	67,33 a	80,20 ab	84,19 b
	H7	72,01 ab	82,36 b	91,66 c	89,12 c
Citric Acid (g/L)	H1	9,17 b	9,38 b	10,87 ab	12,10 c
	H4	7,14 a	9,38 b	9,22 a	11,46 b
	H7	7,30 a	8,11 ab	8,40 a	10,48 ab
Malic Acid (g/L)	H1	2,10 ab	1,97 a	1,93 b	1,69 a
	H4	1,93 a	2,25 b	1,84 b	1,67 a
	H7	1,92 a	2,17 b	1,70 ab	1,73 ab
Succinic Acid (g/L)	H1	1,97 a	1,76 a	1,92 c	1,64 ab
	H4	1,82 a	1,90 a	1,70 b	1,46 a
	H7	1,82 a	1,79 a	1,55 ab	1,49 a
Total Organic Acids (g/L)	H1	13,24 b	13,10 b	14,72 ab	15,43 b
	H4	10,89 a	13,52 b	12,76 ab	14,60 ab
	H7	11,04 a	12,07 ab	11,65 a	13,69 ab

P-Value	Sucrose	Glucose	Fructose	Total Sugars	Citric	Malic	Succinic	Total Acids
Sevilla-Orchard								
A: Rootstock	0,033 *	0,439	0,667	0,239	0,025 *	0,014 *	0,823	0,087
B: Harvest	0,045 *	0,010 *	0,061	0,089	0,026 *	0,648	0,547	0,264
AB	0,702	0,010 *	0,265	0,231	0,188	0,006	0,174	0,135
Huelva-Orchard								
A: Rootstock	0,017 *	0,019 *	0,040 *	0,001 *	0,025 *	0,012 *	0,006 *	0,063
B: Harvest	0,005 *	0,000 *	0,016 *	0,000 *	0,109	0,188	0,008 *	0,073
AB	0,035 *	0,001 *	0,031 *	0,002 *	0,829	0,072	0,321	0,754

3.2.2. Flavonoids composition

Flavonoids are widely distributed in fruits and each species is characterised by a particular flavanone glycoside pattern. Flavonoids generally contribute to fruit and juice quality in many ways, which influences the appearance, taste and nutritional value of fruits (Bermejo et al., 2016).

Flavanones are the major flavonoids found in mandarin fruits (Bermejo et al., 2016). It was observed that the ‘Tango’ mandarins under all the study conditions did not differ in terms of individual flavonoid chromatographic profiles. As in other mandarin varieties, the most abundant flavanone glycoside was hesperidin, followed by narirutin and didymin (Sdiri, Bermejo, et al., 2012) (Table 2). The contents of hesperidin (7.67–10.69 mg/100 mL juice), narirutin (3.15–4.85 mg/ 100 mL juice) and didymin (1.51–2.15 mg/100 mL juice) were slightly below those reported for other irradiated ‘Murcott’ mandarin clones, an overall narirutin compound (Bermejo et al., 2012).

The values of the three identified flavanones were higher in the fruit from Huelva than in those from Sevilla. Harvest time did not affect the content of these compounds, except in fruit from Huelva whose hesperidin content lowered while harvest advanced. Rootstock did not influence the hesperidin content in both orchards. Nevertheless, higher levels of narirutin and didymin were observed in the FA5-fruit, but this rootstock effect was exhibited only on the fruit from Huelva. Although previous studies have observed a rootstock effect on flavonoids com-position, this effect can be affected by other preharvest factors (Gil- Izquierdo, Riquelme, Porras, & Ferreres, 2004).

Table 2 Content of the main flavonoids (herperidin, narirutin and dydimin), vitamin C (ascorbic acid) and antioxidant capacity (by the DPPH and FRAP methods) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P ≤ 0.05)

		Sevilla-Orchard		**Huelva-Orchard**	
	Harvest	**CC**	**FA5**	**CC**	**FA5**
Hesperidin (mg/100 mL Juice)	H1	7,77 a	7,81 a	10,33 b	10,69 b
	H4	7,95 a	7,19 a	9,70 ab	9,04 a
	H7	7,67 a	7,89 a	9,02 a	9,36 a
Narirutin (mg/100 mL Juice)	H1	3,15 a	3,31 a	3,95 ab	4,63 c
	H4	3,36 a	3,33 a	4,04 b	4,73 c
	H7	3,41 a	3,35 a	3,45 a	4,85 c
Didymin (mg/100 mL Juice)	H1	1,51 a	1,67 a	1,81 ab	2,13 b
	H4	1,62 a	1,62 a	1,91 ab	2,15 b
	H7	1,58 a	1,69 a	1,60 a	2,08 b
Ascorbic Acid (mg/100 mL Juice)	H1	25,12 b	27,25 c	24,74 bc	28,11 d
	H4	25,06 b	25,58 b	23,38 b	25,12 c
	H7	22,50 a	24,71 b	20,93 a	22,53 b
Inhibition DPPH (%)	H1	24,39 a	23,40 a	23,14 b	23,38 b
	H4	24,23 a	22,57 a	21,45 ab	22,66 ab
	H7	22,97 a	22,42 a	20,75 a	22,81 ab
FRAP (mM Aae)	H1	5,87 b	5,93 b	5,87 b	5,73 b
	H4	5,43 b	5,33 b	5,03 b	5,03 b
	H7	4,33 a	4,13 a	4,07 a	4,13 a

P-Value	Hesperidin	Narirutin	Didymin	Ascorbic Acid	Inh. DPPH	FRAP
Sevilla-Orchard						
A: Rootstock	0,983	0,461	0,239	0,004 *	0,768	0,663
B: Harvest	0,782	0,795	0,899	0,001 *	0,279	0,000 *
AB	0,247	0,771	0,660	0,284	0,816	0,822
Huelva-Orchard						
A: Rootstock	0,971	0,000 *	0,001 *	0,000 *	0,309	0,921
B: Harvest	0,023 *	0,365	0,199	0,000 *	0,004 *	0,000 *
AB	0,444	0,102	0,488	0,062	0,704	0,931

3.2.3. Vitamin C content

Vitamin C is the major antioxidant compound in citrus juice (Gardner, White, McPhail, & Duthie, 2000). Vitamin C content can be influenced by several factors, such as genotypic differences, preharvest factors, climate conditions, cultural practices, environmental stress during fruit development and postharvest treatment (Yang et al., 2011).

In the present study at the beginning of January (H1), 'Tango' mandarins had high ascorbic acid values that range from 25.12 to 27.25 mg/100 mL in the fruit from Sevilla, and from 24.74 to 28.11 mg/100 mL in the fruit from Huelva (Table 2). These vitamin concentration values detected in 'Tango' mandarins fell within the range reported for other mandarins and were slightly higher than those found in its parental 'Murcott' (Bermejo et al., 2012). A reduced vitamin C content in fruit as season advanced was observed. This finding agrees with the results reported in the literature, where less mature fruits have been found to contain the highest vitamin C concentration (Ladanyia, 2008). In this study, rootstock strongly influenced the total amount of vitamin C. Therefore, in both the studied orchards, the fruit grafted onto FA5 had the highest vitamin C content. Different studies have shown that rootstock influences vitamin C levels (Bassal, 2009; Bermejo & Cano, 2012), and this effect depends on the interaction between each cultivar and each rootstock. This interaction is a thought-provoking approach to adapt citrus cultivars to diverse climatic conditions (Legua et al., 2014).

3.2.4. Antioxidant activity

Antioxidant activity (AA) denotes the ability of a bioactive com-pound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage (Bravo, 1998). Vitamin C is considered one of the most important nutrients in citrus fruit, and it is an important water-soluble antioxidant that plays a crucial role in suppressing su-peroxide radicals (Kaur & Kapoor, 2001). Similarly, flavonoids play a direct role in scavenging reactive oxygen species (ROS), which can counteract lipid oxidation in vitro and improve the body's antioxidant enzyme activity (Nakao et al., 2011). Rekha et al. (2012) also found that the antioxidant capacity of fruit juices to be directly related to total phenolics and vitamin C content. As a synergistic effect may exist among different antioxidants in juice, the use of more than one assay is highly recommended to evaluate the AA of citrus juice by different assays (Sdiri, Bermejo, et al., 2012). The DPPH radical scavenging test is simple rapid method used to evaluate the AA of a sample by changing the absorbance of the solution after combining antioxidant compositions and DPPH radicals (Alam, Bristi, & Rafiquzzaman, 2013). The FRAP assay measures AA by evaluating the ability of a sample to reduce Fe^{3+} to Fe^{2+} in the medium (Benzie & Strain, 1996). Both methods are based on the electron transfer reaction. However, the DPPH assay is less sensitive than FRAP because the interaction of antioxidant compounds and DPPH radical depends on its structural conformation (Barros, de Castro Ferreira, & Genovese, 2012).

In ‘Tango’ mandarins, AA slightly decreased with harvest date, and changes became more evident when the FRAP method was used (Table 2). AA detriment could be due mainly to reductions in vitamin C as only a few changes in the main flavonoids were observed. It has been reported that ascorbic acid in citrus juice contributes more than 50% total antioxidant capacity (Arena, Fallico, & Maccarone, 2001; Del Caro, Piga, Vacca, & Agabbio, 2004; Xu et al., 2008). Regarding rootstock, the effect on AA, although FA5 gave higher vitamin C values, no rootstock effect was found on antioxidant capacity when evaluated by the DPPH radical scavenging method and the FRAP assay.

3.3. Sensorial analysis

Fruit taste, one of the most important traits of citrus fruit quality, is principally governed by the levels and ratios of sugars and organic acids in juice sacs (Obenland et al., 2009). In addition to sugars and acids, mandarins possess a typical uniquely rich flavour, attributed to the presence of a mixture of aroma volatiles in pulp (Miyazaki, Plotto, Baldwin, Reyes-De-Corcuera, & Gmitter, 2012).

The sensory evaluation revealed that panellists detected higher acidity levels in the fruit from Huelva than in those from Sevilla (Table 3). In the fruit from the Sevilla orchard, the highest values were reported for FA5-fruit. No rootstock effect was found in the fruit from Huelva. In all cases, the fruit acidity scores slightly dropped for the first four harvests, although a more marked acidity loss took place after H4. The acidity scores given by panellists well relate to the above-men-tioned TA values.

The sweetness scores were always between 2.3 and 3, which came close to optimal values. Panellists established that the fruit from Huelva were slightly sweeter than those from Sevilla. No effect of rootstock or harvest was perceived by panellists for sweetness.

The liking scores were high in all cases, over 6, with slight changes noted throughout the harvest period. Although these changes are not significant, it is worth noting that while the scores increased as season advanced for the fruit from both rootstocks in Huelva and in the FA5-fruit from Seville, the CC-fruit scores lowered in the Seville orchard. This fact could be related to the changes in the acidity level rather than to sweetness. Thus upon the first harvest in Sevilla, the highest liking scores were obtained for the CC-fruit at H1 (7.13), along with the lowest acidity scores, which were close to 3. At the seventh harvest (H7), this fruit had the lowest liking values when acidity scores fell to 2.4. Conversely, all the other fruit obtained the lowest liking scores at the first harvest, with acidity levels coming close to 4. In all cases, the highest liking scores were obtained when acidity dropped to values close to 3, which happened after the fourth harvest. Purchase intention followed the same pattern as panellists' likings, with maximum scores close to 4 obtained when liking values exceeded 7.

Table 3. Sensorial evaluation (acidity, sweetness, likely and purchase intention) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva- Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P ≤ 0.05).

		Sevilla-Orchard		**Huelva-Orchard**	
	Harvest	**CC**	**FA5**	**CC**	**FA5**
Acidity (1-5)	H1	3,38 b	4,13 c	4,00 b	4,25 b
	H2	2,75 ab	3,63 c	3,38 ab	4,25 b
	H3	2,73 ab	3,70 bc	3,50 ab	4,11 b
	H4	2,90 ab	3,40 bc	3,50 ab	3,80 b
	H5	2,88 ab	3,00 ab	3,36 ab	3,25 ab
	H6	2,40 a	2,70 ab	3,10 a	3,20 a
	H7	2,44 a	2,63 a	2,78 a	3,20 a
Sweetness (1-5)	H1	2,63 a	2,50 a	2,88 a	2,63 a
	H2	2,63 a	2,50 a	2,75 a	2,63 a
	H3	2,09 a	2,40 a	2,70 a	2,78 a
	H4	2,30 a	2,60 a	2,70 a	2,90 a
	H5	2,25 a	2,88 ab	2,75 a	2,88 a
	H6	2,40 a	2,80 ab	2,90 a	2,80 a
	H7	2,44 a	3,13 b	3,11 a	3,00 a
Likely (1-9)	H1	7,13 a	6,63 a	6,88 ab	6,38 a
	H2	7,00 a	7,00 a	7,50 b	6,25 a
	H3	6,27 a	6,70 a	6,90 ab	6,44 a
	H4	6,80 a	6,70 a	7,20 ab	6,70 ab
	H5	6,25 a	7,13 a	7,50 b	7,38 ab
	H6	6,30 a	7,50 a	7,40 ab	7,40 ab
	H7	6,67 a	7,00 a	7,22 ab	7,60 b
Purchasing (1-5)	H1	4,00 a	3,63 a	3,50 a	3,63 ab
	H2	3,75 a	4,00 a	3,75 ab	3,35 a
	H3	3,55 a	3,60 a	3,80 ab	3,22 a
	H4	4,10 a	3,90 a	3,90 ab	3,80 ab
	H5	3,38 a	4,38 a	4,63 b	4,63 b
	H6	3,60 a	4,20 a	4,40 b	4,30 b
	H7	3,78 a	4,38 a	4,22 b	4,50 b

P-Value	Acidity	Sweetness	Likely	Purchasing
Sevilla-Orchard				
A: Rootstock	0,001 *	0,067	0,506	0,661
B: Harvest	0,000 *	0,255	0,427	0,376
AB	0,162	0,456	0,232	0,227
Huelva-Orchard				
A: Rootstock	0,507	0,977	0,365	0,605
B: Harvest	0,002 *	0,708	0,039 *	0,011 *
AB	0,145	0,465	0,694	0,557

4. Conclusion

In this study, the physico-chemical, nutritional and sensorial quality of 'Tango' mandarins grafted onto two rootstocks during harvest seasons was evaluated in two different Spanish orchards.

The results herein obtained revealed that 'Tango' mandarin displays an attractive external color and commercial sugars and acidity levels from early January to mid-February for all the studied conditions. Nevertheless, we must take into account that orchard location influenced the main quality parameters as the fruit from Sevilla orchard had lower levels of acids and sugars than those from Huelva. This location influence could be associated with the higher sandy soil percentage in the Huelva orchard. In all cases, the fruit from the trees grafted onto FA5 displayed higher contents of sugars and acids, which is related to the good photosynthetic capacity reported for this rootstock.

The sensorial analysis results were clearly related to the physico-chemical parameters and allowed an optimum harvest time to be established for the different studied conditions. Indeed, the fruit from CC in the Sevilla orchard exhibited the best quality at the first harvest (H1, early January), while the fruit from FA5 did so at the end of January (H5). In the Huelva orchard, the fruit from both rootstocks achieved maximum organoleptic quality in the second half of February.

Regarding the main flavonoids contents, the Huelva fruit had higher levels of hesperidin, narirutin and didymin. Although the rootstocks in both orchards not affect hesperidin content, the FA5 fruit from Huelva had higher narirutin and didymin than the CC-fruit. The vitamin C content in both evaluated orchards was higher in the fruit from the trees grafted onto FA5. The slightly lowering antioxidant capacity values, as reflected mainly by the FRAP method, were related to the drop in the vitamin C values.

Acknowledgements

The authors thank Frutaria Agricultura, S.L for supplying the fruit used herein and for its technical support. This study has been co-financed by European Social Funds.

References

Alam, M.N., Bristi, N.J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi *Pharmaceutical Journal, 21,* 143-152.
https://doi.org/10.1016/j.jsps.2012.05.002

Arena, E., Fallico, B., & Maccarone, E. (2001). Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. *Food Chemistry, 74(4),* 423-427.
https://doi.org/10.1016/S0308-8146(01)00125-X

Bassal, M.A. (2009). Growth, yield and fruit quality of 'Marisol' clementine grown on four rootstocks in Egypt. *Scientia Horticulturae, 119(2),* 132-137.
https://doi.org/10.1016/j.scienta.2008.07.020

Barry, G.H., Castle, W.S. & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. *Journal of the American Society for Horticultural Science, 129(6),* 881-889.
https://doi.org/10.21273/JASHS.129.6.0881

Barros, H.R.dM, de Castro Ferreira, T.A.P.dCF., & Genovese, M.I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chemistry, 134(4),* 1892-1898.
https://doi.org/10.1016/j.foodchem.2012.03.090

Benzie, I.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry, 239(1),* 70-76. https://doi.org/10.1006/abio.1996.0292

Bermejo, A. Pardo, J. & Cano, A. (2011). Influence of gamma irradiation on seedless citrus production: pollen germination and fruit quality. *Food and Nutrition Sciences, 2(3),* 169-180. https://doi.org/10.4236/fns.2011.23024

Bermejo, A. & Cano, A. (2012). Analysis of nutritional constituents in twenty citrus cultivars from the Mediterranean area at different stages of ripening. Food and Nutrition Sciences, 3(5), 639-650. https://doi.org/10.4236/fns.2012.35088

Bermejo, A., Pardo, J., & Cano, A. (2012). Murcott seedless: influence of gamma irradiation on citrus production and fruit quality. Spanish Journal of Agricultural Research, 10(3), 768-777. http://dx.doi.org/10.5424/sjar/2012103-460-11

Bermejo, A., Pardo, J.L., Morales, J., & Cano, A. (2016). Comparative study of bioactive components and quality from juices of different mandarins: discriminant multivariate analysis of their primary and secondary metabolites. Agricultural Sciences, 7(6), 341-351. https://doi.org/10.4236/as.2016.76035

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews, 56(11), 317-333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x

Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. New Zealand Journal of Crop and Horticultural Science, 23(4), 383-394. https://doi.org/10.1080/01140671.1995.9513914

Del Caro, A., Piga, A., Vacca, V., & Agabbio, M. (2004). Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chemistry, 84(1), 99-105. https://doi.org/10.1016/S0308-8146(03)00180-8

Emmanouilidou, M.G., & Kyriacou, M.C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of 'Lane Late' and 'Delta' sweet orange. Scientia Horticulturae, 225, 112-121. https://doi.org/10.1016/j.scienta.2017.06.056

Eienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D., & Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. Journal of Experimental Botany, 64(6), 1451-1469. https://doi.org/10.1093/jxb/ert035

Filho, F.A.A., Espinoza-Núñez, E., Stuchi, E.S. & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. Scientia Horticulturae, 114(1), 45-49. https://doi.org/10.1016/j.scienta.2007.05.007

Forner, J.B., Forner-Giner, M.A., & Alcaide, A. (2003). Forner-Alcaide 5 and Forner-Alcaide 13: two new citrus rootstocks released in Spain. HortScience, 38(4), 629-630.

Forner-Giner, M.A., Primo-Millo, E., & Forner, J.B. (2009). Performance of Forner-Alcaide 5 and Forner-Alcaide 13, hybrids of Cleopatra mandarin x Poncirus trifoliate, as salinity-tolerant citrus rootstocks. Journal of the American Pomological Society, 63(2), 72-80.

Gardner, P.T., White, T.A., McPhail, D.B., & Duthie, G.G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food chemistry, 68(4), 471-474. https://doi.org/10.1016/S0308-8146(99)00225-3

Gil-Izquierdo, A., Riquelme, M.T., Porras, I. & Ferreres, F. (2004). Effect of the rootstock and interstock grafted in lemon tree (Citrus limon (L.) Burm.) on the flavonoid content of lemon juice. Journal of Agricultural and Food Chemistry, 52(2), 324-331. https://doi.org/10.1021/jf0304775

Goldenberg, L., Yaniv, Y., Kaplunov, T., Doron-Faigenboim, A., Porat, R. & Carmi, N. (2014). Genetic diversity among mandarins in fruit-quality traits. Journal of Agricultural and Food Chemistry, 62(21), 4938-4946. https://doi.org/10.1021/jf5002414

González-Mas, M.C., Llosa, M. J., Quijano, A., & Forner-Giner, M.A. (2009). Rootstock effects on leaf photosynthesis in 'Navelina' trees grown in calcareous soil. HortScience, 44(2), 280-283. https://doi.org/10.21273/HORTSCI.44.2.280

Iglesias, D.J., Cercós, M., Colmenero-Flores, J.M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-Rivero, O., Lliso, I., Morillon, R., Tadeo, F.R., & Talón, M. (2007). Physiology of citrus fruiting. Brazilian Journal of Plant Physiology 19, 333–362. http://dx.doi.org/10.1590/S1677-04202007000400006

Jover, S., Martínez-Alcántara, B., Rodríguez-Gamir, J., Legaz, F., Primo-Millo, E., Forner, J., & Forner-Giner, M. (2012). Influence of rootstocks on photosynthesis in Navel orange leaves: effects on growth, yield, and carbohydrate distribution. Crop Science, 52(2), 836-848. https://doi.org/10.2135/cropsci2011.02.0100

Kaur, C., & Kapoor, H.C. (2001). Antioxidants in fruits and vegetables–the millennium's health. International *Journal of Food Science & Technology, 36(7),* 703-725. https://doi.org/10.1111/j.1365-2621.2001.00513.x

Kelebek, H., & Selli, S. (2011). Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (Citrus sinensis L. Osbeck) orange juice. *Journal of the Science of Food and Agriculture, 91(10)*, 1855-1862. https://doi.org/10.1002/jsfa.4396

Ladanyia, M. S. (2008). Citrus fruit: biology, technology and evaluation. San Diego, CA. Academic press (Elsevier).

Legua, P., Forner, J.B., Hernández, F. & Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. *Scientia Horticulturae, 174,* 60-64. https://doi.org/10.1016/j.scienta.2014.05.004

Li, Y.A.N., Xu, H.A.O. & Cohen, S. (2005). Long-term hydraulic acclimation to soil texture and radiation load in cotton. *Plant, Cell and Environment, 28(4),* 492-499. https://doi.org/10.1111/j.1365-3040.2005.01291.x

Miyazaki, T., Plotto, A., Baldwin, E.A., Reyes-De-Corcuera, J.I., & Gmitter Jr, F.G. (2012). Aroma characterization of tangerine hybrids by gas-chromatography–olfactometry and sensory evaluation. *Journal of the Science of Food and Agriculture, 92(4),* 727-735. https://doi.org/10.1002/jsfa.4663

Nakao, K., Murata, K., Itoh, K., Hanamoto, Y., Masuda, M., Moriyama, K., & Matsuda, H. (2011). Anti-hyperuricemia effects of extracts of immature Citrus unshiu fruit. *Journal of Traditional Medicines, 28(1),* 10-15. https://doi.org/10.11339/jtm.28.10

Obenland, D., Collin, S., Mackey, B., Sievert, J., Fjeld, K., & Arpaia, M. L. (2009). Determinants of flavor acceptability during the maturation of navel oranges. *Postharvest Biology and Technology, 52(2),* 156-163. https://doi.org/10.1016/j.postharvbio.2009.01.005

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Devi, J.P., Kumar, V.H.T. & Kekuda, T.R.P. (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transactions, 1(2*), 303-310. https://doi.org/10.7598/cst2012.182

Roose, M. & Williams, T. (2007). U.S. Patent Application No. 11/220,875 (USPP17863).

Sdiri, S., Navarro, P., Monterde, A., Benabda, J., & Salvador, A. (2012a). Effect of postharvest degreening followed by a cold-quarantine treatment on vitamin C, phenolic compounds and antioxidant activity of early-season citrus fruit. *Postharvest Biology and Technology, 65,* 13-21. https://doi.org/10.1016/j.postharvbio.2011.10.010

Sdiri, S., Bermejo, A., Aleza, P., Navarro, P. & Salvador, A. (2012b). Phenolic composition, organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid late-season mandarins. *Food Research International, 49(1),* 462-468. https://doi.org/10.1016/j.foodres.2012.07.040

Sdiri, S., Rambla, J. L., Besada, C., Granell, A. & Salvador, A. (2017). Changes in the volatile profile of citrus fruit submitted to postharvest degreening treatment. *Postharvest Biology and Technology, 133,* 48-56. https://doi.org/10.1016/j.postharvbio.2017.07.001

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y. & Shi, J. (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food chemistry, 106(2),* 545-551. https://doi.org/10.1016/j.foodchem.2007.06.046

Yang, X.Y., Xie, J.X., Wang, F.F., Zhong, J., Liu, Y.Z., Li, G.H. & Peng, S.A. (2011). Comparison of ascorbate metabolism in fruits of two citrus species with obvious difference in ascorbate content in pulp. *Journal of Plant Physiology, 168(18),* 2196-2205. https://doi.org/10.1016/j.jplph.2011.07.015

Zhang, X., Breksa III, A.P., Mishchuk, D.O. & Slupsky, C.M. (2011). Elevation, rootstock, and soil depth affect the nutritional quality of mandarin oranges. *Journal of Agricultural and Food Chemistry, 59(6),* 2672-2679. https://doi.org/10.1021/jf104335z

CHAPTER III

Physicochemical Changes and Chilling Injury Disorders in ‘Tango’ Mandarins Stored at Low Temperatures

Julia Morales [a], Almudena Bermejo [b], Cristina Besada [a], Pilar Navarro [a], Rebeca Gil [a], Isabel Hernando [c] and Alejandra Salvador [a]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[c]Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Valencia, Spain

Reference: *Journal of Science of Food and Agriculture*, 2020, 100, 2750-2760.

Abstract

BACKGROUND: The susceptibility to chilling injury and quality changes of 'Tango' mandarins stored at different temperatures was evaluated in fruit grown at two locations in Andalusia (Spain) and grafted on Carrizo Citrange or FA5 rootstock. The peel disorders were also characterized by a microstructural study.

RESULTS: Fruit developed chilling injuries, manifested as pitting lesions affecting the equatorial area of the fruit stored at 1 °C or 5 °C; fruit growing on FA5 rootstock showed a slightly lower incidence. The microstructural study revealed that only the upper layers of flavedo were affected in the damaged fruit, the epidermal and hypodermal tissues being dramatically collapsed. Although the fruit was prone to accumulate ethanol, especially after the shelf life that followed the different periods of cold storage, the ethanol did not compromise the overall flavor.

CONCLUSIONS: Storage of 'Tango' fruit was limited by chilling injuries when stored at 1 °C or 5 °C for more than 20 days. Moreover, at these temperatures, the fruit was prone to accumulate ethanol and develop off flavors. At 9 °C, the fruit could be stored for 30 days without compromising external or internal quality. Growing location and rootstock influenced some quality attributes at harvest but not during storage.

Keywords: rootstock; growing location; storage temperature; sensorial quality; chilling injury

1. Introduction

Rootstocks are frequently used in citriculture to improve crop adaptation to different biotic and abiotic conditions by regulating the uptake and movement of water and nutrients among plant organs (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007; Sharma, Dubey, Awasthi & Kaur, 2016; Yıldırım, Yıldırım, San & Ercişli, 2016). The rootstock can also influence fruit quality parameters (Castle, 1996) such as color (Machado, Costa, Teixeira & Costa, 2015; Legua, Martinez-Cuenca, Bellver, Forner-Giner, 2018) or maturity index (McCollum, Bowman & Castle, 2002; Bassal, 2009) and nutritional juice parameters, such as individual sugars and organic acids (Legua, Forner, Hernández & Forner-Giner, 2013; Saini, Capalash, Kaur & Singh, 2019), phenolic compounds (Legua, Forner, Hernández & Forner-Giner , 2014; Sharma et al., 2016) or vitamin C (Magwaza, Mditshwa, Tesfay & Opara, 2017). The effects of rootstock strongly depend on the rootstock-scion interaction.

Spain is the fifth largest producer of citrus in the world and the world's leading exporter of mandarins (FAO, 2016). Citrus fruit production in Spain is destined mainly for fresh fruit markets. Mandarin cultivars include satsumas (*Citrus unshiu* (Mak.) Marc.), clementines (*Citrus clementina* Hort. ex Tanaka) and mandarin hybrids. Satsumas and clementines are traditionally harvested from the beginning of September to mid-January. Nevertheless, international markets demand high-quality mandarins throughout the season. In recent years, therefore, one of the objectives in Mediterranean citriculture has been to introduce new, high-quality, mid and late-season mandarin varieties with seedless fruits. In this context, the 'Tango' mandarin is becoming one of the most popular mid-late season maturing varieties by Spanish citrus producers. This mandarin is a recent cultivar, developed at the University of California Riverside from an irradiated bud of the diploid mandarin cultivar 'W. Murcott' (Roose & Williams, 2007). This mid-late season maturing variety is distinguished because it is very low seeded in all cross-pollination situations, and has fruit of a high organoleptic quality. In Spain, 'Tango' production is expected to grow exponentially in the coming years.

The introduction of a variety into a new cultivation area requires studying the postharvest behavior of fruit. In citrus fruit, susceptibility to low temperature is a key aspect as citrus fruit is prone to develop chilling injury (Lafuente & Zacarias, 2006; Salvador, Carvalho, Monterde & Martínez-Jávega, 2006). Maintaining refrigerated conditions during transport and storage is required to prolong postharvest life, especially during shipping to international markets. The patent text of ‘Tango’ states that fruit can be stored at 5–6 °C for 60 days to maintain their optimal characteristics (Roose & Williams, 2007). These data come from studies that were conducted under specific conditions, and it is known that postharvest citrus fruit quality is strongly influenced by preharvest biotic and abiotic factors, such as location and rootstock (Arpaia, 1992; Treeby, Henriod, Bevington, Milne & Storey, 2007).

In Spain, the production of ‘Tango’ mandarins is located mainly in Andalusia. Although Carrizo Citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.) (CC) is one of the most important rootstocks in this area, Forner-Alcaide 5 (*Citrus reshni* Hort. Ex Tan. × *Poncirus trifoliata* (L.) Raf.) (FA5), a new citrus rootstock obtained from the breeding program carried out at the Valencian Institute for Agricultural Research (IVIA), is now becoming important for its better tolerance to *tristeza virus, Phytophthora* sp. salinity and flooding (Forner, Forner-Giner & Alcaide, 2003; Martínez-Cuenca, Primo-Capella A & Forner-Giner, 2017).

This study evaluated the effect of cold storage at different temperatures on the physico-chemical and sensory quality, and on the chilling injury sensitivity of ‘Tango’ mandarins grafted onto two different rootstocks.

2. Materials and Methods

2.1. Plant material and treatments

Five-year-old ‘Tango’ mandarin trees grafted onto CC and FA5, in two commercial orchards located in different growing areas of Andalusia, were used in this experiment. One of the orchards is located inland in Seville (37° 40’ 38.80’’ N, 5° 49’ 50.80’’ W) with loamy soil (45% sand; 36% lime; 19% clay; Orchard A). The soil has an organic matter content of 0.54% (dry soil), EC_{1-5} (electric conductivity) of 1.24 dS m^{-1} (20 °C), and a pH of 6.2. The other orchard is located in Huelva (37° 24’ 12.12’’ N, 7° 9’ 22.91’’ W) on the Mediterranean coast, with sandy loam soil (80% sand, 10% lime; 10% clay; Orchard B). In this orchard the soil has an organic matter content of 0.72%, $EC_{1–5}$ of 0.82 dS m^{-1} (20° C), and a pH of 7.8. In both orchards the annual precipitation was similar, ranging from 244 to 300 mm in 2017 and from to 543 to 594 mm in 2018. The relative humidity was above 72–77%.

Fruit taken from six trees grafted onto each rootstock in both orchards were harvested on three harvest dates: January 24, February 6, and February 18. After each harvest date, fruit samples were sent to the Postharvest Technology Center at IVIA, where cold-storage assays were carried out. At IVIA, fruit were selected, sorted on the basis of uniform size and absence of visible defects, washed in a foam curtain with biodegradable detergent (Fruit-Cleaner®, Fomesa Fruitech SLU, Valencia, Spain), and waxed with a shellac-based commercial coating of 14% total solids with 2000 ppm of Imazalil as a fungicide (Waterwax TTT-2I®, Fomesa Fruitech SLU). Afterwards, six lots of 60 fruit from each location and rootstock were exposed to one of three different storage temperatures (1 °C, 5 °C, and 9 °C) for two periods (20 and 30 days). After each storage period, fruit were transferred to 20 °C for 6 days to simulate shelf-life conditions.

The following physico-chemical parameters were evaluated at harvest, after the cold storage and the subsequent shelf-life period: peel color index, fruit firmness, juice total soluble solids content (TSS), titratable acidity (TA), TSS:TA ratio, and juice ethanol content. The sensory quality of fruit was evaluated by a semi-trained panel. The incidence of external peel disorder was also evaluated and microstructural studies were carried out to describe them.

2.2. Determination of physico-chemical parameters

The external peel color of fruit was measured by a Minolta colorimeter (model CR-400, Minolta Co. Ltd, Osaka, Japan) on 15 fruit per lot by taking two measurements from the opposite equatorial sides of each fruit. The mean values for the 'L', 'a', and 'b' Hunter parameters were calculated and expressed using the Citrus Color Index ($CCI = 1000a\ Lb{-}^{1}$) (Jimenez-Cuesta, Cuquerella & Martínez-Jávega, 1981).

Fruit firmness was measured on 15 fruit per lot using a Universal Testing Machine (model 3343, Instron Ltd, High Wycombe, England) with a 35 mm probe with a compression speed of 5 mm min^{-1}. The results were expressed as the percentage of millimetres of fruit deformation resulting from a 10 N force on the longitudinal axis.

In each lot of fruit, three samples of five fruit each were squeezed by an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). The juice yield was measured and expressed as a percentage, calculated by dividing the volume of juice by the total fruit weight. The TA was determined by titration with 0.1N NaOH solution, using phenolphthalein as the indicator, expressed as g citric acid L^{-1} of juice. The TSS in the juice was measured by a digital refractometer (Atago PR-1, Atago Co. Ltd, Tokyo, Japan) and data were expressed as °Brix.

For the ethanol and acetaldehyde content, three samples of juice were analyzed from each lot of fruit. Five millilitres of the juice were transferred to 10 mL vials with crimp-top caps, with TFE/silicone septa seals, and then frozen at −20°C until analysis. The ethanol and acetaldehyde content was quantified by a headspace analysis in a gas chromatograph (model 1020, Perkin Elmer Corp., Norwalk, CT, USA) according to the method described by Sdiri, Navarro, Monterde, Benabda & Salvador (2012) and was expressed as mg 100 mL^{-1} of juice.

2.3. Sensory evaluation

Panelists were employees from the Postharvest Technology Center. They were classified as a trained panel given the selection process that they had passed, the amount of training sessions they had undertaken, and their wide experience as members of citrus sensory panels. At least 13 panelists among the 17 that make up the panel were present per test. The day before starting the experiment, the panel participated in a 2 h session to refresh the use of scales selected for the evaluation of the different sensory attributes. Sensory sessions were carried out in a specifically adapted room. Three segments from three different mandarins were presented per sample to compensate for biological variability. Room-temperature distilled water was provided to cleanse palates between samples. Samples were served in random order to the panelists in 50 mL stainless steel soufflé cups, identified by a unique three-digit number. Each fruit was tasted by at least three panelists. Panelists were asked to rate the level of sweetness and acidity using a structured five-point scale that was from 0 (very low) to 4 (very high). Finally, the off-flavor level was evaluated from 0 (absent) to 4 (a very high off flavor).

2.4. Evaluation of physiological disorders and structural studies

Physiological disorders were visually evaluated on all fruit within each lot. The results were expressed as a percentage of damaged fruit. According to the affected rind surface, the scale of intensity used was: 0-no disorders (0%); 1-light (<25%); 2-moderate (25%–50%); 3-severe (>50%). The intensity of damage was expressed by an Index of Peel Disorders using the weighted average of the data obtained.

Structural studies were conducted to characterize the rind disorders in the ‘Tango’ mandarins. The surface and cross-section of the healthy and the affected rind were examined under an optical microscope (stereoscopic zoom microscope model, SMZ-645, Nikon, Tokyo, Japan). A small section (2 mm^2) was cut from the equatorial area, where peel injury was more frequent, for cryo scanning electron microscopy (Cryo-SEM) analyses. The rind section was immersed in slush nitrogen (-210°C) and was transferred to a cryo-trans (CT 15000C, Oxford Instruments, Oxford, England) linked to a scanning electron microscope (model JSM-5410, JEOL, Tokyo, Japan), which ran at a temperature below -130°C. Some samples were cryo-fractured at -180°C and etched at -90 °C. Microscopic observations were made at 15 kV and at a working distance of 15 mm.

2.5. Statistical analysis

To verify sample differences in each studied parameter based on the two orchards, two rootstocks, storage temperature, and storage duration at each harvest moment, an analysis of variance test (ANOVA) was carried out. When ANOVA indicated significant differences, they were tested by a multiple mean comparison test (LSD) at a significance level of 5%. Statistical procedures were performed using statistical software (Statgraphics plus 5.1. Manugistics, Inc., Rockville, MD, USA).

3. Results

3.1. Physico-chemical and sensorial changes

On the three harvest dates, all fruit presented a very intense orange color, close to 20, which was typical of the variety (Roose & Williams, 2007) (Fig. 1A). However, significant differences among the fruit from different locations and rootstocks were observed. The fruit budded on rootstock FA5 in Orchard A (A-FA5) and those from CC in Orchard B (B-CC) exhibited the highest citrus color index (CCI). Throughout cold storage, the fruit did not undergo any evident increase in external coloration and the fruit with the highest CCI values at harvest maintained the highest color values.

Fruit firmness was similar on all three harvest dates, with no differences between rootstocks or location, and the percentage of deformation ranged from 4% to 5% (data not shown). After cold storage, all the fruit suffered evident firmness loss as values of 7.5% and 8.5% were detected after 20 or 30 storage days, respectively. No effect of storage temperature was found. The juice yield of the fruit in all cases was higher than 33%, which is the minimum to commercialize hybrid mandarins according to EU Quality Citrus Standards (EU Regulation No. 543/2011, 2011). The juice yield was not affected by either storage temperatures or rootstock (Fig. 1B). Nevertheless, differences between the fruit from both locations were found; the fruit from Orchard B obtained higher values (50%–52%) than those from Orchard A (43.5%–49.8%).

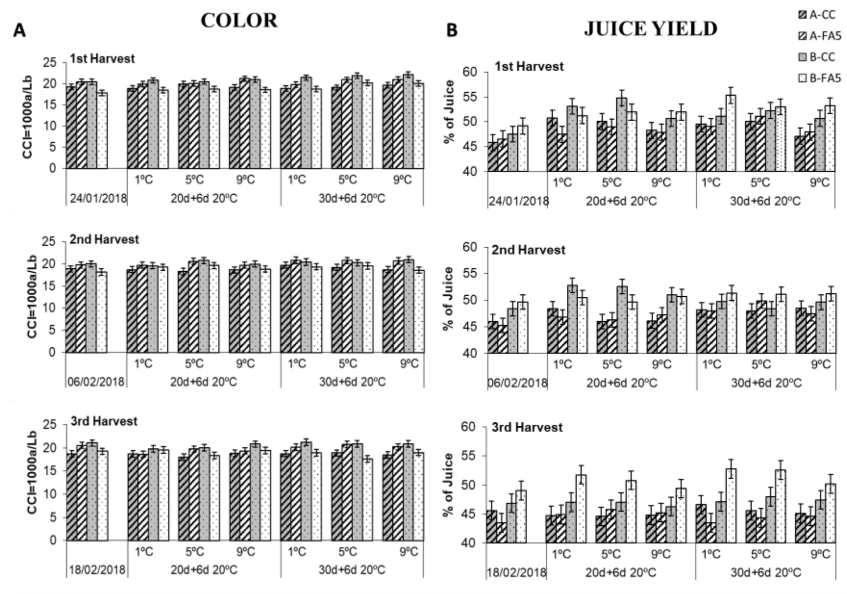


Figure 1. Effect of cold storage at 1°C, 5°C or 9°C on the color (ICC = 1000a/Lb) (A) and on juice yield (% of juice) (B) of 'Tango' mandarins from two growth locations (Sevilla – Orchard A or Huelva – Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 days shelf- life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

Regarding TSS, on all three harvest dates the Orchard B fruit had higher TSS values (12.5–13.5 °Brix) than those from Orchard A with values of 10–12 °Brix (Fig. 2(A)). Only in the second and third harvests in Orchard A did FA5 fruit show a higher TSS content than CC fruit.

After 20 and 30 days of storage at three temperatures, the TSS levels remained practically constant at the three storage temperatures. The differences among orchards and rootstocks observed at harvest continued throughout storage.

Like the observation for the TSS content at harvest, the Orchard B fruit also exhibited higher TA values than those grown in Orchard A (Fig. 2B). The FA5 fruit exhibited the highest acidity level, but the differences between rootstocks were only significant at the first harvest. Cold storage led to a slight drop in acidity in all cases, which was more marked in the fruit stored at 9 °C, mostly in the first harvest. The acidity drop was more evident in the first harvest. In general, fruit from Orchard A, with lower values of TA and TSS, showed the highest values of TSS:TA ratio (Fig. 3). No relevant differences between rootstocks were found throughout the storage period.

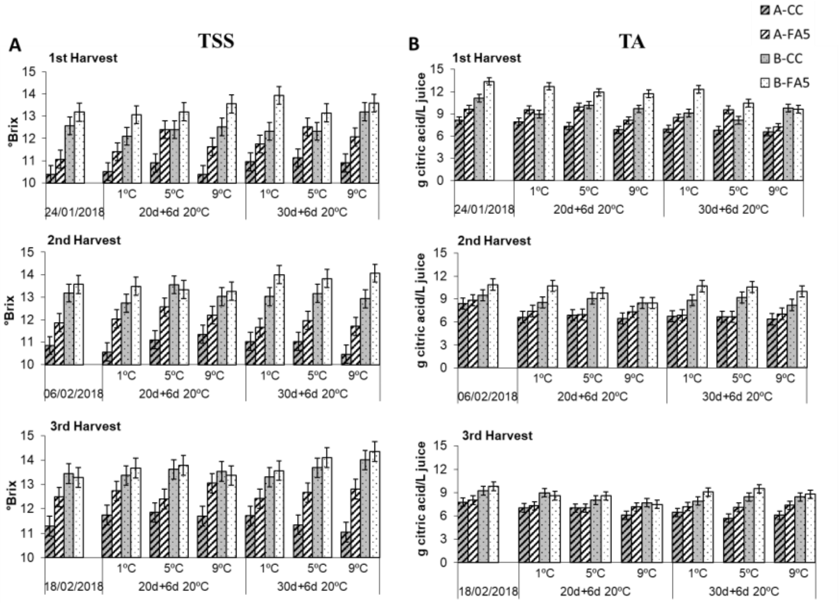


Figure 2. Effect of cold storage at 1°C, 5°C or 9°C on the soluble solid content (TSS) (A) and on acidity (TA) (B) of 'Tango' mandarins from two growth locations (Sevilla-Orchard A or Huelva-Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 day shelf-life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

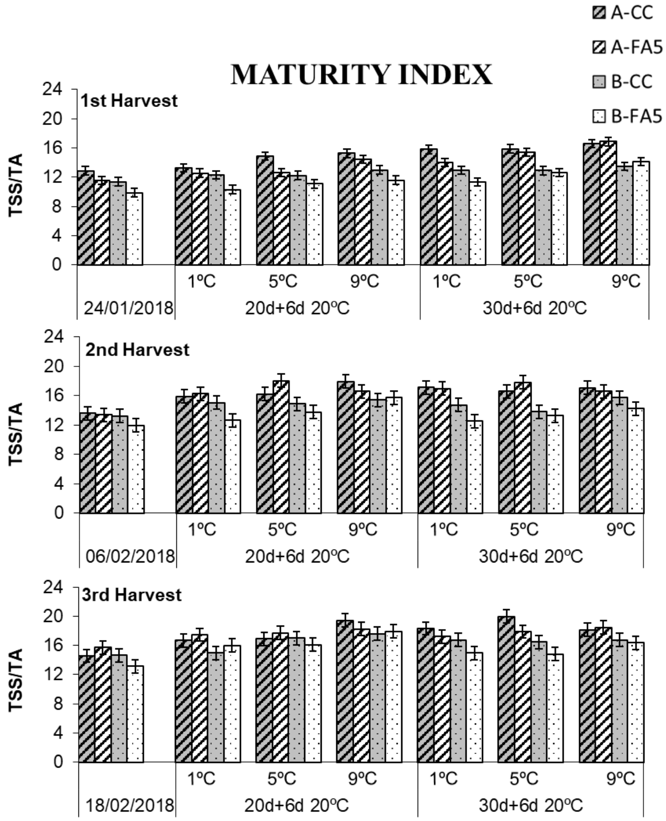


Figure 3. Effect of cold storage at 1°C, 5°C, or 9°C on the maturity index (ratio TSS:TA) of 'Tango' mandarins from two growth locations (Sevilla- Orchard A or Huelva-Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 days' shelf-life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

The ethanol content at harvest was very low in all cases; the Orchard A fruit obtained a range of values between 6.8 and 12 mg 100 mL^{-1}, whereas those from Orchard B tended to present higher values, from 14 to 29.4 mg 100 mL^{-1} (Fig. 4). Throughout cold storage, all the fruit exhibited a significantly increased ethanol content, which was more marked after transferring fruit to the shelf-life conditions. Storage time affected ethanol levels. Thus, in the first and overall in the second harvest, higher ethanol concentrations were obtained after 30 days than after 20 days.

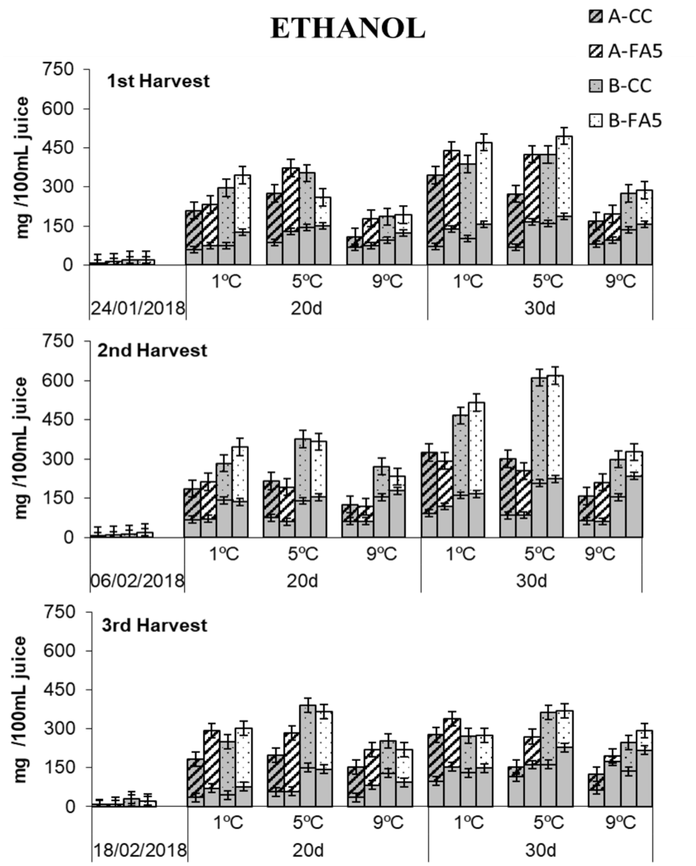


Figure 4. Effect of cold storage at 1°C, 5°C, or 9°C on the ethanol content of 'Tango' mandarins from two growth locations (Sevilla-Orchard A or Huelva-Orchard B) grafted onto two different rootstocks (CC or FA5). The data after cold storage periods are shown in bars without texture and the data after 6 day shelf-life periods at 20°C following different cold storage periods are shown in bars with texture. Vertical bars represent the LSD test (P ≤ 0.05).

While fruit remained in cold storage, no main differences in ethanol values were found for the different storage temperatures. However, when they were transferred to 20°C, the increase in ethanol was much more marked in the fruit stored at 1°C or 5°C than in those stored at 9°C. No differences were observed between the fruit stored at 1°C or 5°C; only in some cases, the fruit stored at 5°C displayed higher ethanol values. The increased ethanol content during storage was also affected by orchard location because the fruit harvested in Huelva (Orchard B), with higher ethanol values at harvest, in general presented more increases throughout storage. This was evidenced at the second harvest, when the fruit stored at 1°C or 5°C had the highest values after 30 days' plus shelf-life, which came close to 500 and 600 mg ethanol 100 mL^{-1} of juice, respectively, while the rootstock did not strongly influence the increase in ethanol caused by storage.

Regarding acetaldehyde, the values at the three harvests were very low, ranging between 0.4 to 0.6 mg 100 mL^{-1} (data not shown). Although increased acetaldehyde was observed throughout cold storage, the content did not exceed 2 mg 100 mL^{-1}. No clear differences were shown among orchard or rootstock in any storage temperature.

Trained sensory panelists, at harvest, found no significant differences in fruit acidity or sweetness between growing locations or rootstocks. Throughout the storage, the fruit sweetness (Fig. 5 (A)) remained stable and a gradual loss of acidity took place (Fig. 5(B)). No significant effect of storage temperature was observed. In general, loss of acidity was more marked in the fruit grown in Orchard A than in Orchard B. The off-flavor perception became more pronounced when fruit were transferred from refrigeration (data not shown) to the shelf-life conditions (Fig. 6). A clear effect of storage temperature was observed as off-flavors were higher in the fruit stored at 1 °C and 5 °C than in those at 9 °C, except in the third harvest, when all the values were relatively low. Even so, the maximum values achieved in fruit at 5 °C in the second harvest were close to two (the off-flavor is noticeable). In fruit stored at 9 °C, in any case, the values exceed 0.5.

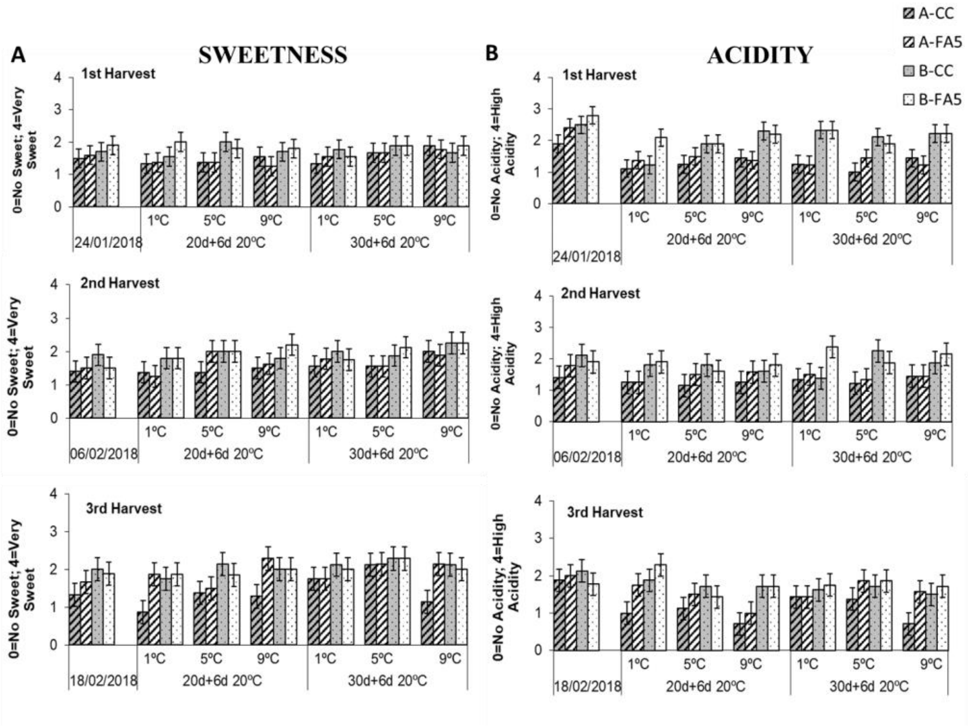


Figure 5. Effect of cold storage at 1°C, 5°C, or 9°C on the sensorial evaluation (sweetness (A) and acidity (B)) of ‘Tango’ mandarins from two growth locations (Sevilla-Orchard A or Huelva-Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 day shelf-life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

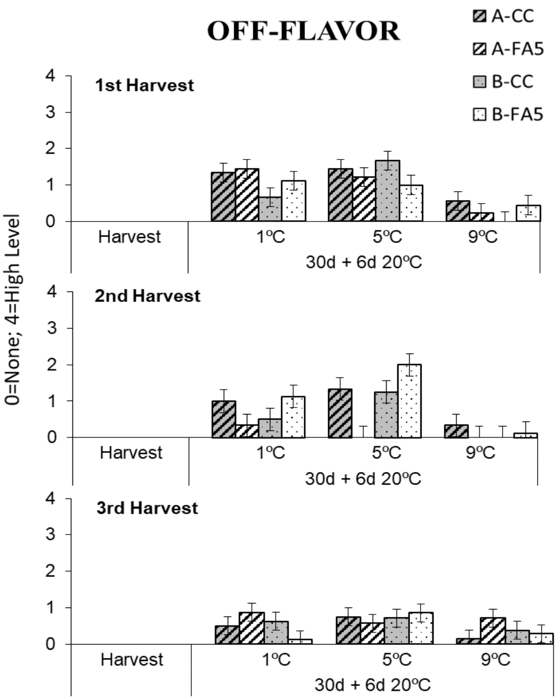


Figure 6. Effect of cold storage at 1°C, 5°C, or 9°C on off-flavor development of 'Tango' mandarins from two growth locations (Sevilla – Orchard A or Huelva – Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 day shelf-life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

3.2. Chilling injury incidence

Fruit at harvest did not show rind damage. Throughout the cold storage fruit manifested chilling injury, which was more evident after the shelf-life period (Fig. 7, only the data after shelf-life periods are shown). In the first and second harvest, the skin damage was higher in the fruit stored at 1°C and 5°C than in the fruit maintained at 9°C. This effect was more evident after 30 days of cold storage plus shelf-life period when the fruit stored at 1°C exhibited the highest disorders values. In the third harvest, in which all the fruit exhibited more chilling injury incidence, the differences among temperatures were less marked. Even so, the fruit stored at 9°C presented the lowest values. Fruit grown on FA5 rootstock and stored at 1°C or 5°C had lower incidence of peel disorders than CC fruit, especially in fruit from Orchard A.

The rind disorders observed in Tango were characterized by sunken brown spots that affected mainly the equatorial area of each fruit (Fig. 8(A),(B)). Structural studies of fruit rind were carried out. The optical stereo microscope observations of the cross sections of healthy and damaged rinds revealed that only the upper layers of flavedo were affected. A thin outer layer of flavedo showed clear signs of necrosis (Fig. 8(C),(D)). The inner cell layers of flavedo and albedo in the affected rind remained intact, with no differences from healthy rind found. Albedo presented the typical spongy morphology, with large intercellular spaces between cells. Oil glands were not affected, and the gland wall remained intact with no sign of rupture and no indication of oil being released, although some oil glands appeared squashed and deformed. Similarly, the interglandular zone presented no symptoms of alteration.

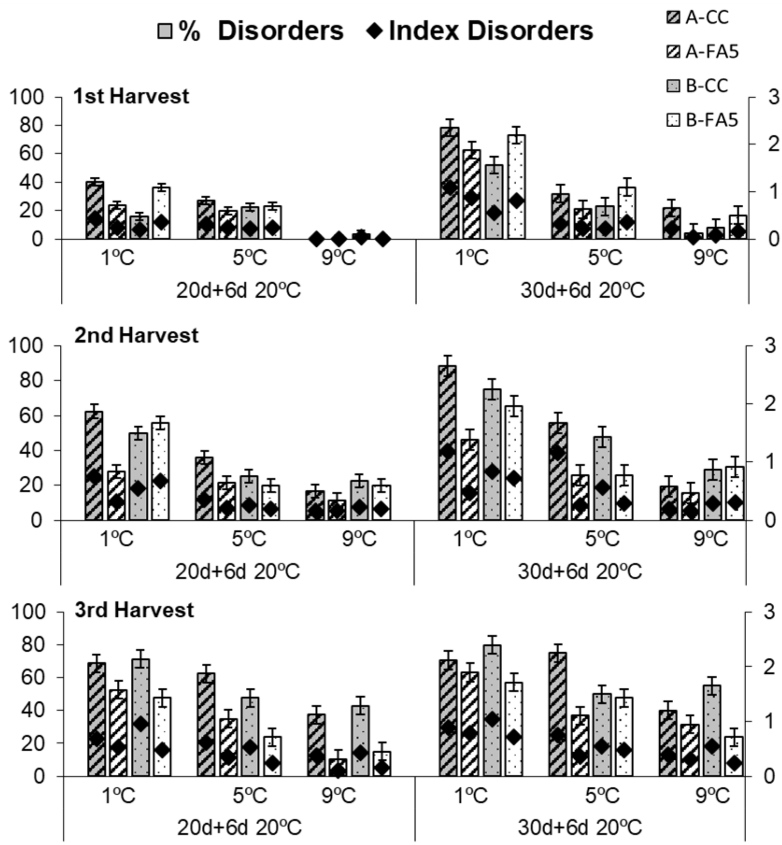


Figure 7. Effect of cold storage at 1°C, 5°C or 9°C on peel disorders of 'Tango' mandarins from two growth locations (Sevilla-Orchard A or Huelva-Orchard B) grafted onto two different rootstocks (CC or FA5). The data after the 6 day shelf-life periods at 20°C following different cold storage periods are shown. Vertical bars represent the LSD test (P ≤ 0.05).

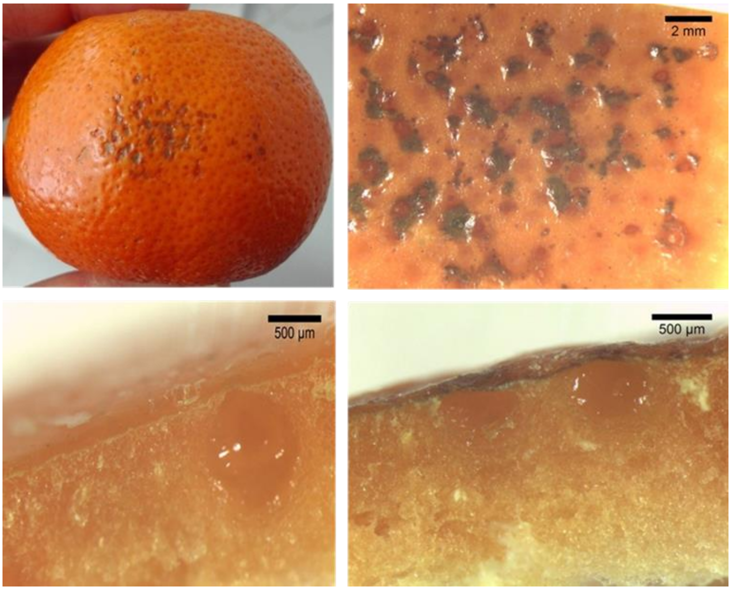


Figure 8. Peel disorders in 'Tango' mandarins stored at low temperature (A and B). Cross-section of the healthy (C) and the affected rind (D) examined under optical stereo microscope.

The Cryo-SEM evaluation made of the fruit rind surface revealed notable differences between healthy and damaged rinds (Fig. 9 (A),(B)). The surface of the affected rind was rough, with very pronounced sunken areas and some cuticle cracks compared to the flat surface with no wrinkling signs on healthy rinds. When Cryo-SEM was used to observe a cross-section of fruit rind, it showed that this disorder affected the cell layers from the epidermis downwardly. While healthy skin was perfectly structured (Fig. 9 (D)), the epidermal and hypodermal tissues in the altered rind had dramatically collapsed and displayed a compacted morphology of squashed cell layers (Fig9. 9(C)). Major differences were observed when a cross-section of healthy and damaged rinds were cryo-fractured (Fig. 9(E),(F)). In the damaged rind, the typical cell structure had disappeared with totally degraded cell walls and membranes, and intercellular spaces full of soluble, and even insoluble, material (Fig. 9(E)). A mass of collapsed material was located in the outer flavedo part, compared with the well-structured tissue that healthy rind tends to show.

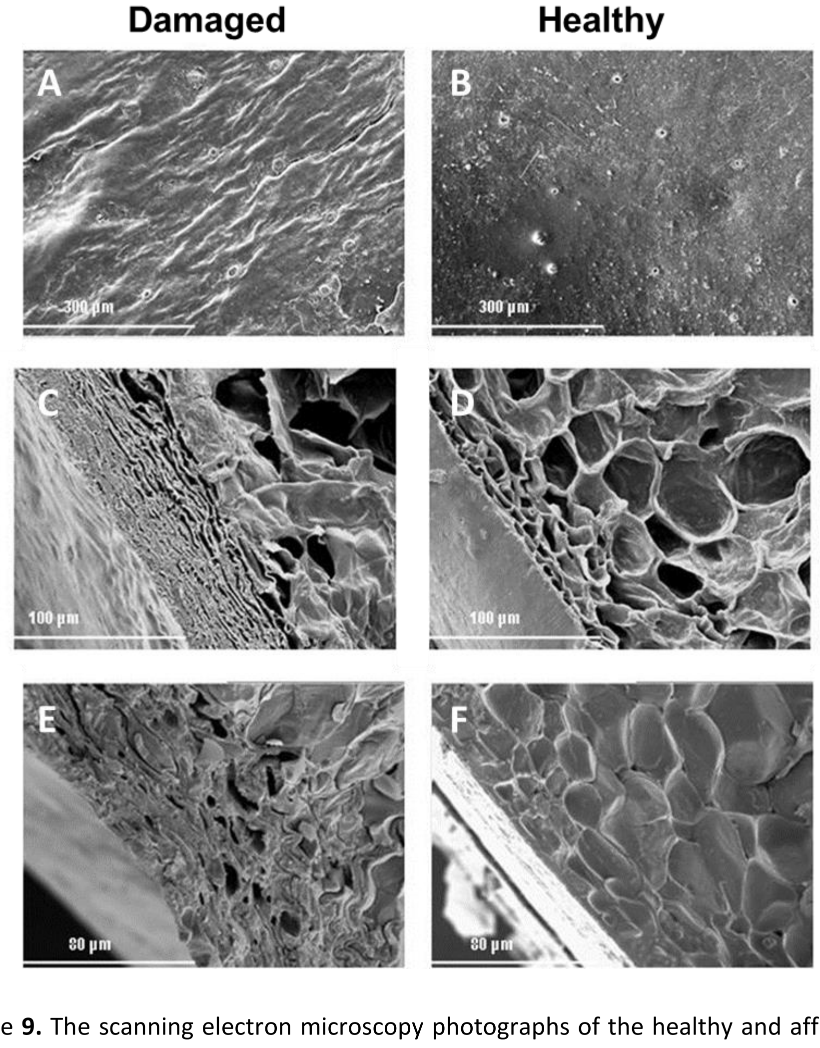


Figure **9.** The scanning electron microscopy photographs of the healthy and affected rinds of 'Tango' mandarins. Surface (A and B); flavedo and albedo cross-section (C and D); flavedo and albedo cryo-fractured cross-section (E and F)

4. Discussion and Conclusions

This work addressed the study of the postharvest behavior of ‘Tango’ fruit growth in two orchards in Andalusia (Spain) with different characteristics. One orchard with sandy soil was located in Huelva near the sea, whereas the other orchard was located inland in Seville and has loamy soil. The study was carried out with fruit from the trees grafted onto one of the most commonly used rootstocks, CC, and onto the new rootstock, FA5.

In this study, both growth location and rootstock influenced fruit quality at harvest. It has been reported that fruit quality is related to the inherent differences affecting plant water relations, including root distribution and water-uptake ability (Romero et al., 2006; Rodríguez-Gamir, Primo-Millo, Forner & Forner-Giner, 2010). The differences in the soil texture between both orchards evaluated could explain the higher TSS and acidity levels of the fruit from Huelva with a higher percentage of sandy soil. It has been established that the fibrous roots are higher in sandy soils than in loamy soils (Martínez, Bañuls, Quiñones, Martín, Primo-Millo & Legaz, 2002). The higher the fibrous root density, the higher the plant water status due to more effective water uptake and greater hydraulic conductivity, inducing higher TSS and TA ratios (Barry, Castle & Davies, 2004).

On the other hand, the rootstock's influence on fruit quality has also been related to the differences that it induces on the leaf photosynthetic rates of scion and photosynthetic product distribution (Morinaga & Ikeda, 1990). The better the photosynthetic capacity of the scion-rootstock, the more carbohydrate compounds are transported from leaves to fruits (Jover et al., 2012). Previous studies have reported that the leaf shoots grafted onto FA5 had a higher net photosynthetic flux than the leaves grafted onto CC (González-Mas, Llosa, Quijano & Forner-Giner, 2009), which would explain the higher TSS and acidity found in the fruit from the trees that budded on FA5. It must be taken into account that the bulk of the organic acids present in fruit flesh is not imported, but rather synthesized, in flesh from imported sugars (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié & Bugaud, 2013).

An aspect to bear in mind when preserving some citrus-fruit tastes during the postharvest life is reduced juicy acidity (Tietel, Plotto, Fallik, Lewinsohn &

Porat, 2011a). In this case, while cold storage caused slight fruit acidity loss, soluble solid content remained practically similar to the harvest values in all cases.

One of the problems of maintaining the quality of mandarins after harvest is the detriment of flavor, caused mainly by off-flavor volatiles accumulating (Tietel, Bar, Lewinsohn, Feldmesser, Fallik & Porat, 2010; Obenland, Collin, Mackey, Sievert & Arpaia, 2011). The off flavor of citrus fruit has been mainly related to the accumulation of ethanol and acetaldehyde as a result of the ethanol fermentation metabolism (Shi, Goldschmidt, Goren & Porat, 2007; Tietel, Lewinsohn, Fallik & Porat, 2011b). In this study, 'Tango' fruit increased ethanol content during cold storage. Storage temperature did not affect the ethanol level achieved immediately after cold storage but after shelf-life ethanol accumulation was greater at 1°C or 5°C than at 9°C. 'W. Murcott' mandarins and 'Barberina' oranges have also been reported to develop higher levels of ethanol when stored at 4 °C or 0 °C than at 8 °C (Obenland et al., 2011; Navarro, Gil, Guardado & Salvador, 2013). No significant influence of rootstock was exhibited on the ethanol accumulation caused by storage, but orchard location had a strong effect on the content of this volatile. Thus, the fruit from Huelva accumulated more ethanol during storage in the second harvest.

The off-flavor levels detected by the panelists were closely related to the ethanol concentration in juice. Hence the best off-flavor perception was obtained by the fruit stored at 1°C or 5°C, with more ethanol content accumulating. The threshold values for ethanol levels for off-flavor detection are not established. It has been reported that this depends not only on the cultivar but also on other factors like TSS and acidity levels or changes in other aroma-active compounds (Marcilla, Zarzo & Del Río, 2006; Tietel et al., 2010; Sdiri et al., 2012). In this sense, values of 150 mg 100 mL^{-1} in mandarins (Hagenmaier, 2002) or 500 mg 100 mL^{-1} in oranges (Hagenmaier, 2000) have been reported as limits for off flavors. In the present study, the ethanol accumulation values were generally elevated, but the off-flavor perception scores were much lower than expected, which could be related to the high soluble solid content and acidity of 'Tango' fruit.

Regarding sensitivity to low temperature, 'Tango' mandarins exhibited chilling injury incidence during storage. The critical temperature at which chilling injury can be manifested in different types of physiological disorders largely depends on variety and can be strongly influenced by preharvest biotic and abiotic factors (Lafuente, Zacarías, Martínez-Téllez, Sánchez-Ballesta & Granell, 2003; Martínez-Jávega, 2004). In our case, the fruit from both studied cultivation areas exhibited a similar sensitivity to the development of chilling injury, manifested as pitting lesions that generally affected the equatorial area of the fruit stored at 1°C or 5°C. The FA5 fruit exhibited a lower incidence than CC fruit, this being more noticeable in Orchard A. The effect of rootstock on postharvest disorders has been previously reported (Cronjé, 2013; Kullaj, 2018). The effect of rootstock on postharvest stem-end rind breakdown and the decay susceptibility of fresh citrus have also been described (Ritenour, Stover, Boman, Dou, Bowman & Castle, 2004). Rootstock has been found to play a role in fruit sensitivity to citrus peel disorders given its influence on water balance, mineral nutrition, plant growth regulators and fruit growth rates and, thus, affects the water and osmotic potentials in fruit rind (Alferez, Alquezar, Jacqueline & Zacarias, 2010; Cronjé, 2013; Magwaza, Opara, Cronjé, Landahl, Terry & Nicola, 2013).

The structural study revealed that only the upper flavedo layers were affected in the altered fruit. The epidermal and hypodermal tissues in the altered rind had dramatically collapsed and presented a compacted morphology of squashed cell layers. The drastic degeneration in cellular structure would lead to vacuolar content being released, which is rich in poliphenols and, consequently, oxidation processes would lead to brown spots, as described in other citrus peel alterations (Agustí, Almela, Juan, Alferez, Tadeo & Zacarías, 2001). The oil gland wall remained intact, with no oil apparently being released to the surface, unlike the case in oleocellosis, a well-known citrus rind disorder (Knight, Klieber & Sedgley, 2002). In some areas, glands looked as if they had been deformed through the action of the strongly collapsed tissue above them, but without oil being released. In the damaged rind, the inner cell flavedo and albedo layers remained intact, and no differences from healthy rind were observed. Peel pitting of citrus fruit has been related to the morphology and structure of the epicuticular wax layer (Vercher, Tadeo, Almela, Zaragoza, Primo-Millo & Agustí; Sala, 2000). Flavedo is covered by a thin waxy layer called the cuticle, which protects citrus fruit from dehydration and other external agents during growth (Garcia-Perez, Ortuño, Puig, Carcel & Perez-Munuera, 2012). When the epicuticular wax layer is damaged, flavedo water permeability increases and the water potential diminishes. It has been suggested that damage to the epicuticular wax structure may be a factor that influences the typical rind staining and peel pitting of mandarins, but it is not a determining factor (Vercher et al., 1994; Sala, 2000). In our case, damaged fruit showed considerable wrinkling and cracks on the surface, which would consequently affect epicuticular wax. Similarly, in Fortune mandarin affected by peel pitting, it has also been reported that the rind surface exhibited an undulating morphology (Vercher et al., 1994).

To summarize, the ‘Tango’ cultivar exhibited a high commercial quality level. Growth location and rootstock can affect solid soluble content, acidity, and ethanol accumulation. One point to highlight is that chilling sensitivity at 1°C and 5°C can limit ‘Tango’ storage, which must be taken into account when ‘Tango’ fruit is shipped at low temperature, especially if quarantine treatment is required. Fruit stored at 9°C for 20 or 30 days did not lose either external or internal quality.

Acknowledgements

The authors thank Frutaria Agricultura, S.L for supplying the fruit used and for its technical support. This study has been co-financed by the European Social Fund.

References

Albrigo, L.G., & Carter, R.D. (1977). Carbohydrates: Composition, distribution, significance. In Citrus Science and Technology, 1st ed.; Nagy, S., Shaw, P.E., Matthew, K., Veldhuis, M.K., Eds.; Avi Publishing Company: Westport, CT, USA; California University: Oakland, CA, USA, Volume 2, pp. 74–93.

Bassal, M.A. (2009). Growth, yield and fruit quality of 'Marisol' clementine grown on four rootstocks in Egypt. Scientia Horticulturae, 119, 132–137. https://doi.org/10.1016/j.scienta.2008.07.020

Agustí, M., Almela, V., Juan, M., Alferez, F., Tadeo, F.R., & Zacarías, F. (2001). Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Annals of Botany. 88 (3), 415–422. https://doi.org/10.1006/anbo.2001.1482

Alferez, F., Alquezar, B., Jacqueline, B.K., & Zacarias, L. (2010). Variation in water, osmotic and turgor potential in peel of Marsh grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology, 56(1), 44–49. https://doi.org/10.1016/j.postharvbio.2009.12.007

Arpaia, M.L. (1992). Preharvest factors influencing postharvest quality of tropical and subtropical fruit. HortScience 27(6), 982-985. https://doi.org/10.21273/HORTSCI.27.6.568c

Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and Plant Water Relations Affect Sugar Accumulation of Citrus Fruit via Osmotic Adjustment. Journal of the American Society of Horticultural Science, 129(6), 881–889. https://doi.org/10.21273/JASHS.129.6.0881

Cronjé, P.J.R. (2013). Postharvest rind disorders of "Nadorcott" mandarin are affected by rootstock in addition to postharvest treatments. Acta Horticulturae, 1007, 111–117. https://doi.org/10.17660/ActaHortic.2013.1007.9

Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D., & Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. Journal of Experimental Botany, 64(6), 1451-1469. https://doi.org/10.1093/jxb/ert035

European Union (2011). Execution Regulation (UE) No. 543/2011. Official Journal of the European Union; 2011:L157:71-75.

Food and Agriculture Organization of the United Nations (2016). Citrus fruit fresh and processed. Statistical Bulletin.

Forner, J.B., Forner-Giner, M.A., & Alcaide, A. Forner-Alcaide 5 and Forner-Alcaide 13: Two New Citrus Rootstocks Released in Spain. HortScience, 38(4), 629–630.

Garcia-Perez, J.V., Ortuño, C., Puig, A., Carcel, J.A., & Perez-Munuera, I. (2012). Enhancement of Water Transport and Microstructural Changes Induced by High-Intensity Ultrasound Application on Orange Peel Drying. Food and Bioprocess Technology, 5(6), 2256-2265. https://doi.org/10.1007/s11947-011-0645-0

González-Mas, M.C., Llosa, M.J., Quijano, A., & Forner-Giner, M.A. (2009). Rootstock Effects on Leaf Photosynthesis in 'Navelina' Trees Grown in Calcareous Soil. HortScience, 44(2), 280–283. https://doi.org/10.21273/HORTSCI.44.2.280

Hagenmaier, R.D. (2000). Evaluation of a polyethylene-candelilla coating for 'Valencia' oranges. Postharvest Biology and Technology, 19(2), 147-154. https://doi.org/10.1016/S0925-5214(00)00087-9

Hagenmaier, R.D. (2002). The flavor of mandarin hybrids with different coatings. Postharvest Biology and Technology, 24 (1), 79–87. https://doi.org/10.1016/S0925-5214(01)00121-1

Jimenez-Cuesta, M., Cuquerella, J., & Martínez-Jávega, J. (1981). Determination of a color index for citrus fruit degreening. Proceedings of the International Society of Citriculture, 2,750-753.

Jover, S., Martínez-Alcántara, B., Rodríguez-Gamir, J., Legaz, F., Primo-Millo, E., Forner, J., & Forner-Giner, M.A. (2012). Influence of Rootstocks on

Photosynthesis in Navel Orange Leaves: Effects on Growth, Yield, and Carbohydrate Distribution. Crop Science, 52(2), 836-848. https://doi.org/10.2135/cropsci2011.02.0100

Knight, T.G., Klieber, A., & Sedgley, M. (2002). Structural Basis of the Rind Disorder Oleocellosis in Washington Navel Orange (Citrus sinensis L. Osbeck). Annals of Botany, 90(6), 765–773. https://doi.org/10.1093/aob/mcf258

Kullaj E. (2018). Rootstocks for Improved Postharvest Quality of Fruits: Recent Advances. in: Siddiqui, MW (Ed.), Preharvest Modulation of Postharvest Fruit and Vegetable Quality. Academic Press. 8:189-207. https://doi.org/10.1016/B978-0-12-809807-3.00008-1

Lafuente, M.T., & Zacarias, L. (2006). Postharvest physiological disorders in citrus fruit. Stewart Postharvest Review, 2(1), 1-9. https://doi.org/10.2212/spr.2006.1.2

Lafuente, M.T., Zacarías, L., Martínez-Téllez, M.A., Sánchez-Ballesta, M.T., & Granell, A. (2003). Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biology and Technology, 29(3), 308–317. https://doi.org/10.1016/S0925-5214(03)00047-4

Magwaza, L.S., Opara, U.L., Cronjé, P.J.R., Landahl, S., Terry, L.A., & Nicola, I.B.M. (2013). Non-chilling physiological rind disorders in citrus fruit, J. Janick (eds.), Horticultural Reviews, John Wiley & Sons, Willey-Blackwell, New York. 41, 131-166. https://doi.org/10.1002/9781118707418.ch03

Marcilla, A., Zarzo, M., & Del Río, M.A. (2006). Effect of storage temperature on the flavour of citrus fruit. Spanish Journal of Agricultural Research, 4(4), 336-344. https://doi.org/10.5424/sjar/2006044-210

Martínez, J.M., Bañuls, J., Quiñones, A., Martín, B., Primo-Millo, E., & Legaz, F. (2002). Fate and transformations of 15N labelled nitrogen applied in spring to Citrus trees. Journal of Horticultural Science and Biotechnology, 77(3), 361-367. https://doi.org/10.1080/14620316.2002.11511506

Martínez-Cuenca, M.R., Primo-Capella, A., & Forner-Giner, M.A. (2017). Tolerance Response Mechanisms to Iron Deficiency Stress in Citrus Plants. Sarwat M., Ahmad A., Abdin M., Ibrahim M. (eds.). Stress Signaling in Plants: Genomics and Proteomics Perspective, 2:201-239. Springer, Cham. https://doi.org/10.1007/978-3-319-42183-4_9

Martínez-Jávega, J.M. (2004). Tratamientos postcosecha en mandarinas y naranjas. Vida rural. 197, 60-64.

Morinaga, K. & Ikeda, F. (1990). The effects of several rootstocks on photosynthesis, distribution of photosynthetic product, and growth of young satsuma mandarin trees. Journal of the Japanese Society of Horticultural Science, 59(1), 29-34. https://doi.org/10.2503/jjshs.59.29

Navarro, P., Gil, R., Guardado, A., & Salvador, A. (2013). Conservación frigorífica de naranja cv. Barberina. Levante Agrícola: Revista internacional de cítricos. 417, 206-209.

Obenland, D., Collin, S., Mackey, B., Sievert, J., & Arpaia, M.L. (2011). Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition. Postharvest Biology and Technology, 59(2), 187–193. https://doi.org/10.1016/j.postharvbio.2010.09.011

Ritenour, M.A., Stover, E., Boman, B.J., Dou, H., Bowman, K.D., & Castle, W.S. (2004). Effect of Rootstock on Stem-end Rind Breakdown and Decay of Fresh Citrus. American Society of Horticultural Science, 14(3), 315–319. https://doi.org/10.21273/HORTTECH.14.3.0315

Rodríguez-Gamir, J., Primo-Millo, E., Forner, J.B., & Forner-Giner, M.A. (2010). Citrus rootstock responses to water stress. Scientia Horticulturae, 126(2), 95-102. https://doi.org/10.1016/j.scienta.2010.06.015

Romero, P., Navarro, J.M., Perez-Perez, J., Garcia-Sanchez, F., Gomez-Gomez, A., Porras, I., Martinez, V., & Botía, P. (2006). Deficit irrigation and rootstock: their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin. Tree Physiology, 26(12), 1537-1548. https://doi.org/10.1093/treephys/26.12.1537

Roose, M., & Williams, T. (2007). Mandarin variety named 'Tango'. U.S Patent Application Nº 11/220,875.

Sala, J. (2000). Content, chemical composition and morphology of epicuticular wax of Fortune mandarin fruits in relation to peel pitting. Journal of the Science of Food and Agriculture, 80(13), 1887-1894. https://doi.org/10.1002/1097_0010(200010)80:13<1887::AID_JSFA730>3.0.CO;2-W

Salvador, A, Carvalho, CP, Monterde A, Martínez-Jávega, JM. Note. 1-MCP Effect on Chilling Injury Development in 'Nova' and 'Ortanique' Mandarins. Food Sci. Technol. Int. 12:165-170 (2006). https://doi.org/10.1177/1082013206063736

Sdiri, S., Navarro, P., Monterde, A., Benabda J., & Salvador A. (2012). New degreening treatments to improve the quality of citrus fruit combining different periods with and without ethylene exposure. Postharvest Biology and Technology, 63, 25–32. https://doi.org/10.1016/j.postharvbio.2011.08.005

Shi, J.X., Goldschmidt, E.E., Goren, R., & Porat, R. (2007). Molecular, biochemical and anatomical factors governing ethanol fermentation metabolism and accumulation of off-flavors in mandarins and grapefruit. Postharvest Biology and Technology, 46(3), 242–251. https://doi.org/10.1016/j.postharvbio.2007.05.009

Tietel, Z., Bar, E., Lewinsohn, E., Feldmesser, E., Fallik, E., & Porat, R. (2010). Effects of wax coatings and postharvest storage on sensory quality and aroma volatile composition of ‘Mor’ mandarins. Journal of Science of Food and Agriculture, 90 (6), 995-1007. https://doi.org/10.1002/jsfa.3909

Tietel, Z., Plotto, A., Fallik, E., Lewinsohn, E., & Porat, R. (2011a) Taste and aroma of fresh and stored mandarins. Journal of Science of Food and Agriculture, 91(1), 14–23 (2011a). https://doi.org/10.1002/jsfa.4146

Tietel, Z., Lewinsohn, E., Fallik, E., & Porat, R. (2011b). Elucidating the Roles of Ethanol Fermentation Metabolism in Causing Off-Flavors in Mandarins. Journal of Agriculture and Food Chemistry, 59 (21), 11779–11785. https://doi.org/10.1021/jf203037v

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., & Storey, R. (2007). Irrigation management and rootstock effects on navel orange [Citrus sinensis (L.) Osbeck] fruit quality. Agriculture Water Management. 91(1-3), 24–32. https://doi.org/10.1016/j.agwat.2007.04.002

Vercher, R., Tadeo, F.R., Almela, V., Zaragoza, S., Primo-Millo, E., & Agustí, M. (1994). Rind structure, epicuticular wax morphology and water permeability of 'Fortune' mandarin fruits affected by peel pitting. Annals of Botany, 74(6), 619-625.

III.3. BLOOD ORANGES

CHAPTER IV

Rootstock Effect on Fruit Quality, Anthocyanins, Sugars, Hydroxycinnamic Acids and Flavanones Content during the Harvest of Blood Oranges 'Moro' and 'Tarocco Rosso' Grown in Spain.

Julia Morales [a], Almudena Bermejo [b], Pilar Navarro [a], María Ángeles Forner-Giner [b] and Alejandra Salvador [a]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

Reference: *Food Chemistry, 2021, 342, 128305.*

Abstract

The physico-chemical quality parameters (external and internal color, firmness, acidity, total soluble solids, anthocyanins, sugars, hydroxycinnamic acids and flavanones) of 'Moro' and 'Tarocco Rosso' blood oranges grafted onto eight different rootstocks at three harvest time were studied. The rootstocks were 'Carrizo', 'C-35', 'Cleopatra' mandarin, 'Citrus volkameriana', 'Citrus macrophylla', 'Swingle' citrumelo, 'Forner-Alcaide 5' and 'Forner-Alcaide 13'. All studied parameters were highly rootstock/scion-dependent and showed changes throughout harvest. The content of the main anthocyanins revealed their relation with internal fruit color in both cultivars. The rootstocks that led to fruit with the lowest anthocyanins displayed the least sucrose content. The differences detected in the amount of hydroxycinnamic acids (chlorogenic, ferulic and sinapic) and flavanones (hesperidin, narirutin and didymin) related to anthocyanins content, explained phenylpropanoid pathway.

Keywords: Quality, citrus fruit, pigmented oranges, firmness, color, phenolic compounds

1. Introduction

In the Mediterranean citrus production area, the importance of blood oranges (*Citrus sinensis* L. Osbeck) is growing given their healthy qualities. Blood oranges are characterized by their high content of anthocyanins, water-soluble polyphenolic compounds which, apart from conferring fruit its characteristic red color, are related to human health properties due to their antioxidant activity (Habibi, Ramezanian, Guillén, Serrano & Valero, 2020). These anthocyanins exert potential action against certain diseases and reduce the risk of several cancer types, heart disease, and low-density lipoprotein (LDL) cholesterol accumulation (Hou, 2003). Indeed, the main aspect that consumers seek in blood oranges is their internal purple color as they associate natural red pigments with health benefits. However, color is related to the content and composition of anthocyanins present in blood oranges, which vastly vary depending on variety, maturity, cultivation region and many environmental conditions, overall ambient temperature and irradiation (Rapisarda, Bellomo & Intelisano, 2001; Crifò, Puglisi, Petrone, Reforgiato Recupero & Lo Piero, 2011). Much research has focused on studying the composition of anthocyanins of a wide variety of blood orange cultivars (Kelebek, Canbas & Selli, 2008), and on understanding the biosynthesis pathway and the factors regulating it as these pigments play an important physiological role in plants, such as protecting them against abiotic stress conditions and pathogen infections (Zhang, Butelli & Martin, 2014). Regarding the anthocyanins pathway, other important compounds markedly influence fruit quality, such as hydroxycinnamic acids, flavanones and sugars (Rapisarda, Carollo, Fallico, Tomaselli & Maccarone, 1998; Li, Van den Ende & Rolland, 2014; Cebadera-Miranda et al., 2019).

Although consuming blood oranges has traditionally been in the form of juice, the citrus market is increasingly demanding pigmented oranges for fresh fruit. This means that blood orange production focuses on obtaining fruit that meets the external and internal quality requirements demanded by consumers. Besides internal fruit color, attention must be paid to other quality attributes required by consumers. Firmness is a parameter that has gained much importance in citrus fruit commercialization in recent years. Indeed, blood oranges usually present low firmness values compared to blond oranges, which may be a quality limitation, especially when post-harvest fruit is submitted to long-term shipping or prolonged storage (Pallottino, Menesatti, Lanza, Strano, Antonucci & Moresi, 2012). As in most citrus fruit, sugars and acids content influences the sensory quality of blood oranges and changes during harvest. Most blood oranges maintain a higher acidity content than blond oranges during harvest (Fabroni, Amenta, Timpanaro, Todaro & Rapisarda, 2020).

In citrus, one important aspect to consider is the rootstock onto which a cultivar is grafted because it may influence several tree growth and development aspects, including yield, fruit quality, and tolerance to stress caused by biotic and abiotic factors (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007). The search for new better-performing citrus rootstocks than those normally used is one of the main challenges faced by the citrus industry in many countries. In this context, a citrus rootstocks breeding program is being developing at the Instituto Valenciano de Investigaciones Agrarias (IVIA) (Valencia, Spain) and one important objective of this program is to study the effect of rootstock on blood orange. Indeed, the influence of rootstock on blood oranges was recently addressed, with studies focusing on different aspects like yield and fruit quality (Reforgiato Recupero, Russo, Recupero, Zurru, Deidda & Mulas, 2009; Incesu, Çimen, Yesiloglu & Yilmaz, 2013; Continella et al., 2018). Nevertheless, the influence of a specific rootstock on fruit quality is highly dependent on both cultivar and climate conditions. In citrus fruit, and specifically in blood oranges, harvest can be a determining factor of fruit quality. Moreover, as anthocyanins are one of the most valuable parameters in blood oranges, studies about the effect of rootstocks on these pigments are needed, and more studies are necessary to evaluate the way that rootstock influences quality parameters to obtain a better rootstock/scion combination.

The objective of this study was to evaluate the effect of rootstock on the fruit quality of the two blood orange varieties cultivated in Spain, ‘Moro’ and ‘Tarocco Rosso’, throughout harvest. Besides evaluating changes in the main fruit quality attributes, an in-depth study into anthocyanins, hydroxycinnamic acids, sugars and flavanones composition was conducted.

2. Materials and Methods

2.1. Plant material and treatments

Fruit samples of ‘Tarocco Rosso’ and ‘Moro’ were collected from an experimental orchard in Museros (39º 57’ 70.95”N; -0º 36’ 06.65” W) on the Spanish Mediterranean coast with sandy soil. This orchard had EC_{1-5} of 0.407 dS m^{-1} (20ºC) and a pH of 7.05. Relative humidity was above 65%.

Both cultivars were grafted onto ‘Carrizo’ (CC) and ‘C-35’ (C35) citranges, ‘Cleopatra’ mandarin (CL), *Citrus volkameriana* (VK), *Citrus macrophylla* (M), ‘Swingle’ citrumelo (CT) and two hybrid selections, ‘Forner-Alcaide 5’ (FA5), ‘Forner-Alcaide 13’ (FA13), obtained in the rootstock breeding program carried out at the IVIA.

The fruit taken from five trees grafted onto each rootstock, collecting 20 fruits per tree, were harvested at three harvest times: February 7 (H1), February 25 (H2) and March 26 (H3). After each harvest time, fruit was transported to the IVIA where the following physico-chemical analyses were carried out: external and internal color, firmness, juice, total soluble solids, acidity, maturity index, sugars and phenolic compounds (anthocyanins, hydroxycinnamic acids and flavanones).

2.2. Determination of quality attributes

Color determination was made on rind and juice by a Minolta colorimeter (model CR-400, Minolta Co. Ltd, Osaka, Japan). Rind color was measured over 15 fruits per rootstock/scion combination. As blood orange rind coloration is not homogeneous in whole fruit, two measurements were taken in the dark area and two others in the bright area along the equatorial fruit part. Juice color was determined with three juices of five fruit each. The mean values for the ‘L’, ‘a’ and ‘b’ Hunter parameters were calculated with each fruit and expressed as the Citrus Color Index (CCI=1000a/Lb) (Jimenez-Cuesta, Cuquerella & Martínez-Jávega, 1981).

Firmness measurements were taken by a Universal Testing Machine (model 3343, Instron Limited, Buckinghamshire, England) using 15 fruits per rootstock/scion combination. The results were expressed as the percentage of millimeters of the fruit deformation that resulted from a 10 N force on the longitudinal axis at constant speed.

In each fruit lot, three samples of five fruit each were squeezed by an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution using phenolphthalein as the indicator and expressed as g citric acid/100mL of juice. The total soluble solid content (TSS) in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd, Tokyo, Japan) and data were expressed as ^{o}Brix.

2.3. Sugars analysis

Extraction and characterization was carried out following the procedure as previously described by Bermejo, Pardo, Morales, and Cano (2016). Carbohydrates were analyzed by HPLC equipped with a refractive index detector (Waters, Barcelona, Spain) using a 5-µm Tracer Carbohydrate column (250 mm x 4.5 mm) (Teknokroma, Barcelona, Spain). The mobile phase was acetonitrile:water (75:25) at a flow rate of 1 mL/min. Compounds were identified by comparing their retention time with standards and quantified using an external calibration curve. Fructose, glucose and sucrose sugars were obtained from Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g/L.

2.4. Phenolic compounds analysis

2.4.1. Anthocyanins

Sample extraction was performed following the procedure as previously described by Laribi et al. (2013). Anthocyanins were analyzed by HPLC equipped with a photodiode array detector (Merck Hitachi, Germany) in a Licospher 100 RP-18 column, preceded by a precolumn (4 mm x 4 mm) and confirmed by HPLC-MS using an HPLC equipped with a ZQ2000 mass detector (Waters, Barcelona, Spain). The mobile phase was 5% formic acid (A) and methanol (B) in a linear gradient starting with 15% B and reaching 35% B in 30 min at a flow rate of 1 mL/min. Chromatograms were recorded at 520 nm absorbance. The HPLC-MS analysis was run and worked under electrospray ion positive conditions. Full data acquisition was performed by scanning 200 to 800 uma in the centroid mode. Cyanidin 3-glucoside (RT=12.5 min $[MH]^+$ 449 m/z), was identified by comparing to an authentic pure standard obtained from Sigma (Sigma Co., Barcelona, Spain). Delphinidin 3-glucoside (RT=10.5 min, $[MH]^+$ 465 m/z), cyanidin 3-(6”-malonyl)-glucoside (RT=18.0 min $[MH]^+$ 535 m/z) and cyanidin 3-(6”-dioxalyl)-glucoside (RT=21.5 min $[MH]^+$ 593 m/z), were tentatively identified based on their retention times, absorption spectrum characteristics and mass spectrum data with available standards and the data described in the literature (Kelebek, et al., 2008). For the quantitative analysis, an external calibration curve with available standard cyanidin 3-glucoside was carried out. The results were expressed as mg/L of juice.

2.4.2. Hydroxycinnamic acids and flavanones

The extraction method was performed following the procedure as previously described by Bermejo et al. (2016). Compounds were analyzed by HPLC equipped with a photodiode array detector and a ZQ2000 mass detector (Waters, Barcelona, Spain) in a reverse-phase column C18. The gradient mobile phase was acetonitrile (A) and 0.6% acetic acid (B), starting with 10% A for 2 min, reaching 75% in 28 min and then back to the initial condition to be held for 5 min (total run time 35 min). The flow rate was 1 mL/min. Chromatograms were recorded at the absorbance of 200-400 nm. The HPLC-MS analysis was carried out and worked under electrospray ion negative and positive conditions. Full data acquisition was performed by scanning 150-800 uma in the centroid mode.

The major hydroxycinnamic acids in the fruit juice were quantified, two free acids (sinapic and ferulic) and a conjugated acid (chlorogenic).Chlorogenic acid (RT=10.6 min $[M-1]^+$ 353 m/z) was identified by comparing to an authentic pure standard purchased from Sigma-Aldrich (Barcelona, Spain). Ferulic acid

(RT=10.8 min $[M-1]^+$ 193 m/z) and sinapic (RT=11.2 min, $[M-1]^+$ 223 m/z) were tentatively identified by comparing their UV-vis and mass spectra based on available standards and the data described in the literature (Kelebek et al., 2008). For the quantitative analysis, an external calibration curve with available hydroxycinnamic acid standards was carried out. The results were expressed as mg/L of juice.

Flavanone compounds were identified on the basis of comparing their retention times, UV-Vis spectra and mass spectrum data with authentic standards using an external calibration curve with narirutin (RT=14.2 min $[MH]^+$ 581 m/z), hesperidin (RT=14.8 min [MH]+ 611 m/z) and didymin (RT=17.6 min $[MH]^+$ 595 m/z). Narirutin was purchased from Extrasynthesis (Genay, France), hesperidin from Sigma-Aldrich (Barcelona, Spain) and didymin from ChromaDex (Irvine, CA, USA). The results were expressed as mg/L of juice.

2.3. Statistical analysis

Data were subjected to an analysis of variance based on two factors (harvest time × rootstock) for each cultivar. The mean values of the evaluated parameters were compared by the least significant difference test (LSD) at a significance level of 5%. ($P < 0.05$). Principal component analysis (PCA) using Pearson’s correlation was also carried out. These analyses were performed using the statistical software Statgraphics Centurion XVII.II software (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1 Firmness, Total Soluble Solids and Acidity

Citrus fruit firmness is a quality parameter which the market is increasingly demanding, despite not being included in current quality standards. Hence one important weakness of some blood oranges cultivars is their low firmness, which makes their commercialization as fresh fruit difficult (Pallottino et al., 2012).

In this study, both evaluated cultivars ‘Tarocco Rosso’ and ‘Moro’ showed high firmness at all the harvest times (Table 1). Only ‘Moro’ grafted onto FA13, and ‘Tarocco Rosso’ grafted onto C35 and onto M, exhibited a significant loss of firmness at the third harvest. Nevertheless, all the fruit had commercial market values. In ‘Moro’, the maximum deformation values were 2.3%, shown for FA13 while in ‘Tarocco’ the highest value was 3.3% observed in M. In both cultivars, the fruit grafted onto CT had the highest firmness values with the least percentage deformation values close to 1.6%. Similar values were observed in C35 for ‘Moro’ and in FA5 and CC for ‘Tarocco Rosso’.

The influence of rootstock on sugars and acid content has been related to the inherent rootstock differences that affect plant water relations. These differences include root distribution, water uptake ability, hydraulic conductivity, and leaf or stem water potentials (Castle, 1995; Barry, Castle & Davies, 2004).

The TSS content in the blood orange juice fell within the of 9.3-10.8 ^{o}Brix range for ‘Moro’ and within the 9.9-11.8 ^{o}Brix range for ‘Tarocco Rosso’ (Table 1). These values were slightly lower than those measured in other studies done with blood oranges, such as ‘Tarocco Scirè’, ‘Tarocco Rosso’, ‘Tarocco Ippolito’ and ‘Sanguinello’, which ranged between 12.57 and 15.30 (Continella et al., 2018; Cebadera-Miranda et al., 2019).

In both cultivars, the TSS content was affected by harvest and rootstock, with significant interaction between these factors. In ‘Moro’, the TSS content at the first harvest ranged between 9.3 ^{o}Brix in FA13 and 10.4 ^{o}Brix in CC. At the following harvests all the fruit exhibited increased TSS, except for the fruit grafted onto CC and VK, without significant differences among harvests. At third harvest, minor differences in TSS content among rootstocks were shown, and ranged from 10.1 ^{o}Brix in the fruit from M to 10.8 ^{o}Brix in the fruit from FA5, CC and CL. In ‘Tarocco Rosso’, the rise in TSS content during harvest was significant in the fruit grafted onto FA5, CC and CL. In this cultivar, and similarly to ‘Moro’, the lowest TSS values were detected in the fruit from M, with average value close to 9.9 ^{o}Brix values, followed by C-35 fruit with average value close to 10.3 ºBrix. The highest values were recorded for the fruit from FA5.

An effect of harvest and rootstocks was also observed in fruit acidity. The acidity of ‘Moro’ fruit at first harvest, ranged from close to 1.35 g of citric acid/100 mL in VK and FA13 to 1.60 g of citric acid/100 mL in CT (Table 1). In all cases, fruit acidity lowered with harvest advance, overall after second harvest, except in the fruit from M whose levels remained until the third harvest. The most pronounced decline was recorded for the CT-fruit, but had the highest values at the third harvest, similarly to those for the M-fruit, close to 1.40 citric acid/100 mL. The acidity values of ‘Tarocco Rosso’ were lower than for ‘Moro’ and ranged at the first harvest from 1.00 g of citric acid/100 mL in the M-fruit to 1.21 g of citric acid/100 mL in the FA13 fruit. All the fruit also exhibited loss of acidity as harvest advanced. Similar to ‘Moro’, M-fruit acidity remained constant, but C35-fruit acidity did not vary in ‘Tarocco Rosso’. At third harvest the highest acidity values were for the fruit grafted onto CT and C35, with the lowest acidity for the fruit from FA13, M, and VK.

Table 1. Firmness, total soluble solids (TSS) and acidity (TA) of blood oranges ‘Moro’ and ‘Tarocco Rosso’ grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. The lowercase letters in each column represent the least significant difference (LSD) intervals (p≤0.05) when comparing the effect of rootstocks for each harvest time. The capital letters in each row represent the least significant difference (LSD) intervals (p≤0.05) when comparing the effect of harvest time per each rootstock.

		Moro			Tarocco Rosso		
		H1	H2	H3	H1	H2	H3
Firmness (% Def.)	FA13	1,77 $_{a}^{A}$	2,06 $_{b}^{AB}$	2,27 $_{c}^{B}$	1,86 $_{bcd}^{A}$	1,87 $_{bcd}^{A}$	1,94 $_{abc}^{A}$
	FA5	1,86 $_{a}^{A}$	2,11 $_{bc}^{A}$	2,04 $_{bc}^{A}$	1,61 $_{ab}^{A}$	1,77 $_{abc}^{A}$	1,60 $_{a}^{A}$
	CC	2,22 $_{b}^{A}$	1,97 $_{ab}^{A}$	2,00 $_{bc}^{A}$	1,91 $_{bcd}^{A}$	2,04 $_{cd}^{A}$	1,91 $_{ab}^{A}$
	C35	1,80 $_{a}^{A}$	1,93 $_{ab}^{A}$	1,73 $_{ab}^{A}$	1,66 $_{abc}^{A}$	1,70 $_{ab}^{A}$	2,30 $_{bc}^{B}$
	CL	2,21 $_{b}^{A}$	2,21 $_{bc}^{A}$	2,04 $_{c}^{A}$	2,05 $_{cd}^{A}$	1,97 $_{bcd}^{A}$	2,09 $_{abc}^{A}$
	M	2,23 $_{b}^{A}$	2,38 $_{c}^{A}$	2,07 $_{c}^{A}$	2,20 $_{de}^{A}$	2,40 $_{e}^{A}$	3,25 $_{d}^{B}$
	CT	1,60 $_{a}^{A}$	1,76 $_{a}^{A}$	1,60 $_{a}^{A}$	1,39 $_{a}^{A}$	1,50 $_{a}^{A}$	1,60 $_{a}^{A}$
	VK	2,23 $_{b}^{A}$	2,14 $_{bc}^{A}$	2,00 $_{bc}^{A}$	2,60 $_{e}^{A}$	2,40 $_{de}^{A}$	2,50 $_{c}^{A}$
TSS (ºBrix)	FA13	9,27 $_{a}^{A}$	9,80 $_{a}^{AB}$	10,47 $_{ab}^{B}$	11,22 $_{c}^{A}$	11,40 $_{cd}^{A}$	11,28 $_{bc}^{A}$
	FA5	10,33 $_{cd}^{A}$	10,60 $_{de}^{AB}$	10,83 $_{b}^{B}$	11,00 $_{bc}^{A}$	11,77 $_{d}^{B}$	11,67 $_{c}^{B}$
	CC	10,44 $_{d}^{A}$	10,80 $_{e}^{A}$	10,80 $_{b}^{A}$	10,40 $_{b}^{A}$	11,12 $_{cd}^{B}$	11,08 $_{bc}^{B}$
	C35	9,97 $_{bcd}^{A}$	10,47 $_{cde}^{B}$	10,67 $_{b}^{B}$	10,20 $_{ab}^{A}$	10,33 $_{ab}^{A}$	10,53 $_{b}^{A}$
	CL	10,10 $_{cd}^{A}$	10,40 $_{cde}^{AB}$	10,78 $_{b}^{B}$	10,68 $_{bc}^{A}$	11,10 $_{cd}^{B}$	11,10 $_{bc}^{B}$
	M	9,47 $_{ab}^{A}$	9,90 $_{ab}^{AB}$	10,13 $_{a}^{B}$	9,85 $_{a}^{A}$	10,03 $_{a}^{A}$	9,93 $_{a}^{A}$
	CT	9,83 $_{bc}^{A}$	10,30 $_{bcd}^{B}$	10,40 $_{ab}^{B}$	10,80 $_{bc}^{A}$	11,15 $_{cd}^{A}$	11,23 $_{bc}^{A}$
	VK	9,90 $_{bc}^{A}$	10,03 $_{abc}^{A}$	10,30 $_{ab}^{A}$	10,72 $_{bc}^{A}$	10,78 $_{bc}^{A}$	10,83 $_{b}^{A}$
TA (g citric acid/ 100 mL juice)	FA13	1,36 $_{a}^{B}$	1,31 $_{a}^{B}$	1,14 $_{a}^{A}$	1,21 $_{b}^{B}$	1,11 $_{bc}^{A}$	0,85 $_{a}^{A}$
	FA5	1,48 $_{bcd}^{B}$	1,29 $_{a}^{B}$	1,24 $_{a}^{A}$	1,13 $_{ab}^{B}$	1,08 $_{bc}^{A}$	0,90 $_{abc}^{A}$
	CC	1,44 $_{abc}^{B}$	1,34 $_{a}^{AB}$	1,21 $_{a}^{A}$	1,14 $_{ab}^{B}$	1,04 $_{ab}^{AB}$	0,90 $_{abc}^{A}$
	C35	1,54 $_{cd}^{B}$	1,49 $_{c}^{B}$	1,26 $_{ab}^{A}$	1,11 $_{ab}^{A}$	1,11 $_{bc}^{A}$	0,99 $_{c}^{A}$
	CL	1,48 $_{bcd}^{B}$	1,46 $_{bc}^{A}$	1,26 $_{ab}^{A}$	1,10 $_{ab}^{B}$	1,08 $_{bc}^{AB}$	0,94 $_{abc}^{A}$
	M	1,49 $_{bcd}^{A}$	1,41 $_{abc}^{A}$	1,40 $_{c}^{A}$	1,00 $_{a}^{A}$	0,92 $_{a}^{A}$	0,85 $_{a}^{A}$
	CT	1,60 $_{d}^{C}$	1,51 $_{c}^{B}$	1,37 $_{bc}^{A}$	1,15 $_{ab}^{B}$	1,17 $_{c}^{B}$	0,97 $_{c}^{A}$
	VK	1,35 $_{a}^{B}$	1,34 $_{a}^{A}$	1,14 $_{a}^{A}$	1,09 $_{ab}^{B}$	1,01 $_{ab}^{AB}$	0,87 $_{a}^{A}$

p-value	Firmness		TSS		TA	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
B: Harvest	0.160	0.001 *	0.000 *	0.054	0.000 *	0.000 *
AB	0.056	0.010 *	0.763	0.999	0.349	0.473

3.2 External and internal color

Blood oranges cultivars are characterized by the presence of phenolic compounds belonging to the anthocyanin class (Rapisarda et al., 2001). Although these compounds accumulate mainly in fruit flesh in some cultivars purple or red shaded peel may be observed (Rodrigo, Alquézar, Alós, Lado & Zacarías, 2013). This accumulation is sometimes due to the synthesis of anthocyanins when fruit is exposed to adverse environmental conditions, such as visible and UVB radiation, cold temperature and water stress (Muccilli et al., 2009). Therefore, this can lead to significant differences in external color between the peel area exposed to UVB radiation, whose coloration is the darkest, and the shaded peel area. For this reason, two different coloration areas were measured separately in this study: the most pigmented area (dark area) and the area with less color (bright area) (Fig. 1).

In both cultivars, in the two areas measured an important effect of the harvest and rootstock was detected and in any case significant interaction between these factors was found.

In ‘Moro’, the CCI of the bright area ranged between 12.0 and 14.7 at first harvest and a significant but slight increase took place as harvest advanced. The highest CCI values were observed during the second harvest in the fruit grafted onto FA5 and M. Moreover, at this last harvest, the lowest CCI was exhibited by the fruit form FA13, C35 and CT. The CCI of the dark area at the first harvest ranged from 16.1 in the CT-fruit to close to 28 in fruit from CL and VK. In general, an increase in the CCI values was observed in the second harvest before dropping at the third harvest. Fruit from FA13, C35 and CT, with the lowest values, exhibited slight changes throughout the harvest period.

In ‘Tarocco Rosso’, the bright area showed CCI values ranged between 13.1 in fruit from both C35 and FA13 fruit to 17.8 in the M-fruit at first harvest. This area exhibited an increase of CCI in the second harvest and dropped at the third harvest. The lowest values during the studied period were found in C35-fruit followed by VK-fruit. The highest values were shown in M-fruit that exhibited the smallest changes with the harvest advance. The CCI of the darkest area, at first harvest, ranged between 22.2 in C35-fruit to close to 33.8

in fruit from CL and VK. In fruit from all rootstocks, important coloration decrease occurred at the third harvest. The smallest reduction was shown in fruit from C35, CT and M. The C35-fruit exhibited the lowest CCI values.

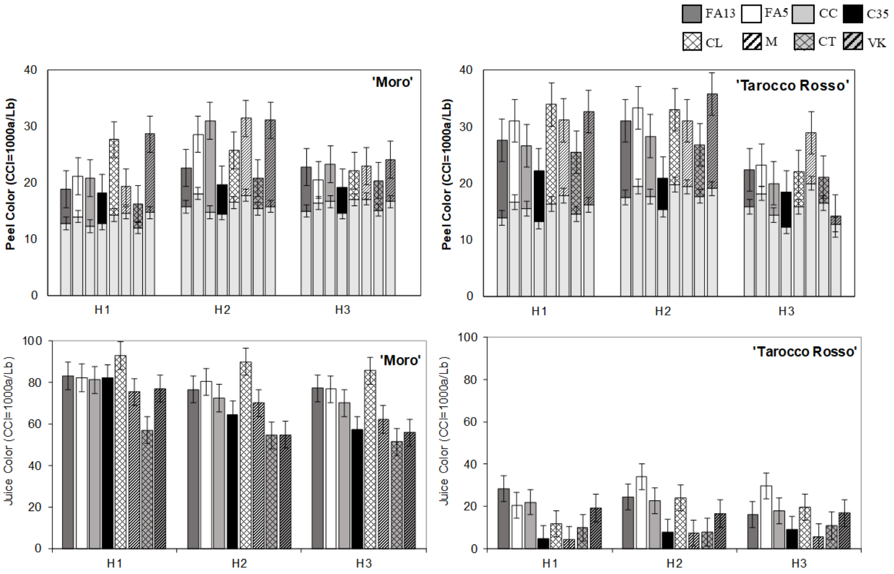
Although the anthocyanin content in peel was not measured, the increased peel color observed in ‘Moro’ at second harvest suggested that an increase in anthocyanin content took place. Likewise, in both cultivars, the significant reduction in peel color at the third harvest could be associated with anthocyanin degradation. In previous studies, an increase in total anthocyanins during harvest to a final reduction in late ripening stages has been reported in different blood orange cultivars (Barbagallo, Palmeri, Fabiano, Rapisarda & Spagna, 2007).

The external color changes herein observed could be related to the variation in environment temperature. It is known that cool nights and warm days are required to stimulate anthocyanin synthesis (Barry, Caruso & Gmitter, 2020). Figure supplementary 1 shows the maximum temperature and cold hours per day during the study period. Between the first and second harvest, the maximum temperature came close to 18^{o}C with an average 10 h of cold hours. These conditions could have induced anthocyanin synthesis in the ‘Moro’ fruit, overall in fruit from FA5, CC, M and VK, which would lead to the major color increase. The increased peel coloration observed in ‘Moro’ would corroborate that this cultivar is one that most strongly depends on prevailing climate conditions for full color development (Butelli et al., 2012).

After second harvest, the cold hour average fell to 5 h and the mean maximum temperature rose to 22^{o}C. This scenario could trigger anthocyanin degradation with the consequent drop in external color observed in both cultivars. It has been reported that fruit are apparently able to actively degrade anthocyanins under warm conditions (Steyn, 2008). The fact that the fruit from rootstocks like FA13, C35 and CT in Moro and C35, CT and M in ‘Tarocco Rosso’, show minor changes in external color during harvests suggests that these rootstocks induce low sensitiveness to temperature.

Regarding internal color, ‘Moro’ presented higher CCI values than ‘Tarocco Rosso’ (Fig. 1). Of the studied blood oranges, ‘Moro’ has been reported as a variety with the highest internal color values (Mondello, Cotroneo, Errante, Dugo & Dugo, 2000). A significant effect of harvest and rootstock was found in ‘Moro’. At first harvest, the color juice of the ‘Moro’ fruit grafted onto CL had the highest CCI values, close to 93.0, while the CT-fruit had the lowest values with 57.1. The CCI of the other fruit was between 75.4 and 83.1. As harvest advanced, CCI tended to lower. The CT-fruit, with the lowest values, exhibited the minor reduction. Nevertheless, the decline in fruit from C35, M and VK was more accused and achieved similar values of CT-fruit at third harvest. CL fruit had the maximum internal color values. In previous studies, it was reported that CT in blood orange cultivars induced the lowest internal fruit color values compared to seven different rootstocks (Continella et al., 2018).

In ‘Tarocco Rosso’ no significant effect of harvest was found in color juice although fruit from FA5 and CL increased the CCI values at the second harvest. Nevertheless, the influence of rootstock on internal CCI was relevant. The fruit from C35, M and CT had the lowest CCI values, between 4.2 and 11.0, while the highest values coming close to 30 for the FA5-fruit.



p-value	Darkest Area Color		Bright Area Color		Juice Color	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0,000 *	0,000 *	0,000 *	0,000 *	0,000 *	0,000 *
B:Harvest	0,000 *	0,000 *	0,000 *	0,000 *	0,022 *	0,364
AB	0,159	0,115	0,638	0,173	0,887	0,544

Figure 1. Peel (dark and bright areas) and juice color of blood oranges ‘Moro’ and ‘Tarocco Rosso’ grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test (p≤0.05).

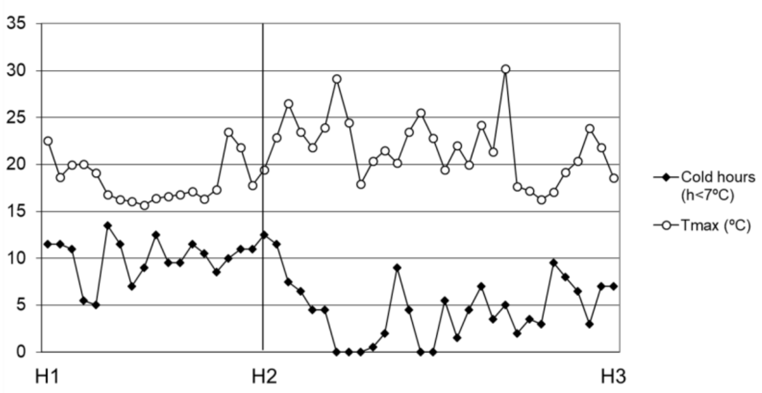
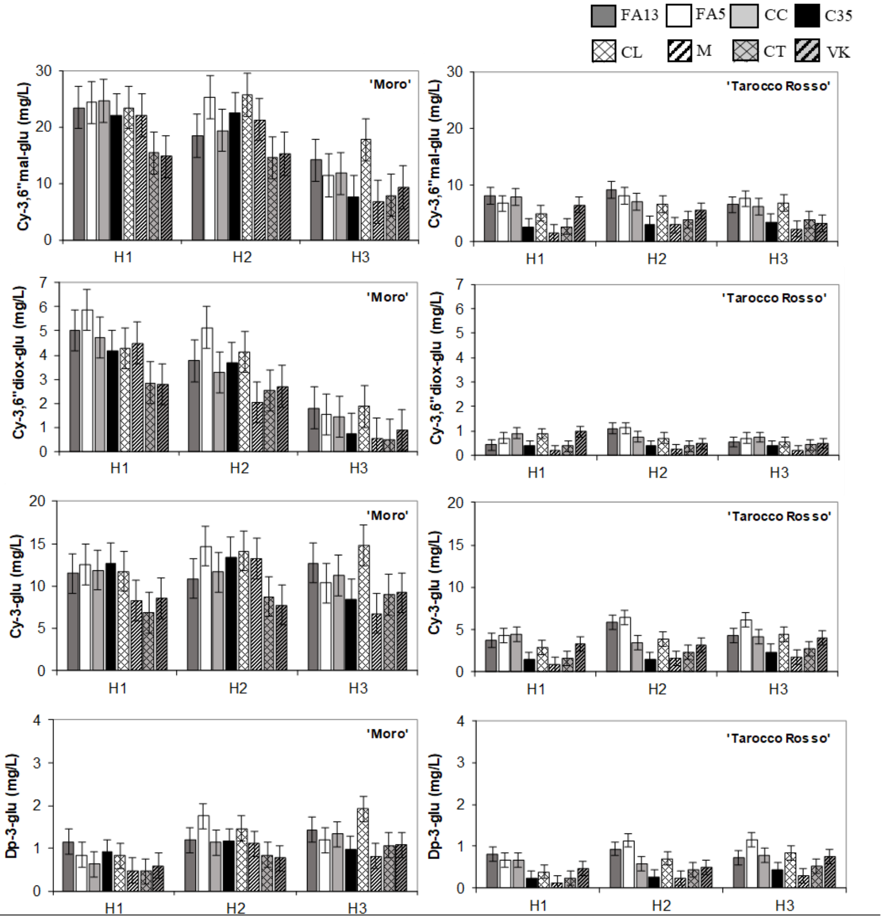


Figure supplementary 1. Peel (Evolution of temperatures during the three harvest times. White points represents the maximum temperature (^{o}C) each day. Black points represent the cold hours each day (T<7^{o}C).

3.3. Anthocyanin content in juice

As previously mentioned, blood oranges are rich in anthocyanins, the water-soluble compounds responsible for their distinctive purple coloration. Anthocyanins in blood oranges depend strongly on variety. In this study, four anthocyanins were determined in the juice of ‘Moro’ and ‘Tarocco Rosso’: delphinidin 3-glucoside (Dp-3-glu), cyanidin 3-glucoside (Cy-3-glu), cyanidin 3-(6”-malonyl)-glucoside (Cy-3,6”mal-glu) and cyanidin 3-(6”-dioxalyl)-glucoside (Cy-3,6”diox-glu) (Fig. 2). As reported in most blood oranges, Cy-3-glu and Cy-3,6”mal-glu were identified as the major anthocyanins in the juice of both cultivars (Dugo, Mondello, Morabito & Dugo, 2003; Ballistreri, Fabroni, Romeo, Timpanaro, Amenta & Rapisarda, 2019; Cebadera-Miranda et al., 2019). Anthocyanin content was higher in ‘Moro’ than in ‘Tarocco Rosso’, as previously reported by Kafkas, Ercisli, Kemal, Baydar and Yilmaz (2009), who established that ‘Moro’ is the richest in anthocyanins among blood oranges. In each cultivar, anthocyanin evolution during harvests differed depending on each individual compound.

In ‘Moro’, Cy-3,6”mal-glu content was affected by harvest and rootstock without significant interaction between these factors. Its content remained almost constant during the first two harvests. However, at the third one, the amount of this compound significantly dropped (Fig. 2). Cy-3,6”diox-glu content gradually decreased with harvest advance. Regarding the influence of rootstock on the content of these two anthocyanins, the fruit from CT and VK had the lowest values, which was specially observed during first and second harvest. After third harvest, when the content of these both compounds declined, minor differences among rootstocks appeared. It is important to remark that the smallest descent of both anthocyanins was observed in CL fruit, that exhibited the highest values of Cy-3,6”mal-glu in the third season. No significant effect of the harvest was found in Cy-3-glu, but an important influence of the rootstock was observed. The lowest values were found in fruit from CT and VK and the highest values was exhibited by CL-fruit. Finally, Dp-3-glu increased with the harvest advance and the rootstook also affect its content, without significant interaction between these factors. Similar to the other anthocyanins, the lowest content was for, CT and VK and the highest values for CL.



p-value	Cy-3,6"mal-glu		Cy-3,6"diox-glu		Cy-3-glu		Dp-3-glu	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0,000 *	0,000 *	0,000 *	0,000 *	0,004 *	0,000 *	0,002 *	0,000 *
B:Harvest	0,000 *	0,427	0,000 *	0,113	0,296	0,011 *	0,000 *	0,001 *
AB	0,188	0,830	0,350	0,053	0,345	0,440	0,213	0,362

Figure 2. Individual anthocyanins (delphinidin 3-glucoside (Dp-3-glu), cyanidin 3-glucoside (Cy-3-glu), cyanidin 3-(6”malonyl)-glucoside (Cy-3,6”mal-glu) and cyanidin 3-(6”-dioxalyl)-glucoside (Cy-3,6”diox-glu)) of blood oranges ‘Moro’ and ‘Tarocco Rosso’ grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test (p≤0.05).

Contrarily to observed in ‘Moro’, the harvest did not affect the content of Cy-3,6”mal-glu and Cy-3,6”diox-glu in ‘Tarocco Rosso’, nevertheless the rootstock had a significant influence. The fruit from C35, M and CT had the lowest content of these anthocyanins. Cy-3-glu and Dp-3-glu content increased throughout harvest. FA13 and FA5 were the rootstocks with more Cy-3-glu and Dp-3-glu content in fruit. Conversely, the fruit from C35, M and CT contained the smallest amount of both anthocyanins.

The relation between coloration and anthocyanin content has been previously reported (Rapisarda et al., 2001; Carmona, Alquézar, Marques & Peña, 2017). Accordingly, in this study, the higher juice color of ‘Moro’ compared to ‘Tarocco Rosso’ was explained by the content of the all the individual determined anthocyanins. Likewise, the changes in internal coloration exhibited in both cultivars throughout harvests were corroborated with those recorded in anthocyanins. Therefore in ‘Moro’, the drastic drop in Cy-3,6”mal-glu and Cy-3,6”diox-glu content was concomitant with the reduction in internal color, which would indicate that these anthocyanins were the pigments that mostly contributed to the juice color intensity of ‘Moro’. Similarly, the least internal coloration of the fruit grafted onto CT and VK would be associated with the lower content of the four determined anthocyanins. In the case of ‘Tarocco Rosso’, the fruit with the least internal coloration was that grafted on to the rootstocks with the lowest content of the four anthocyanins (C35, M and CT), and increased juice color was exhibited only at the second harvest in the fruit from FA5 and CL, which coincides with the increment in Cy-3-glu and Dp-3-glu content.

The descent of the Cy-3,6”mal-glu and Cy-3,6”diox-glu content observed at the third harvest in ‘Moro’ indicated the degradation of these anthocyanins. This coincides with previous studies in which the degradation of pigments in both rind and flesh has been observed in late ripening stages (Lo Piero, 2015). The catabolic process could be due to either the chemical instability of pigments or specific enzymatic activity lowering the pigment concentration in plant tissues, which often occurs in parallel to the synthesis process Oren-Shamir (2009). Regarding enzymatic activity, it has been reported that enzyme β-glucosidase correlates with the degradation of anthocyanins in late ripening stages (Barbagallo et al., 2007).

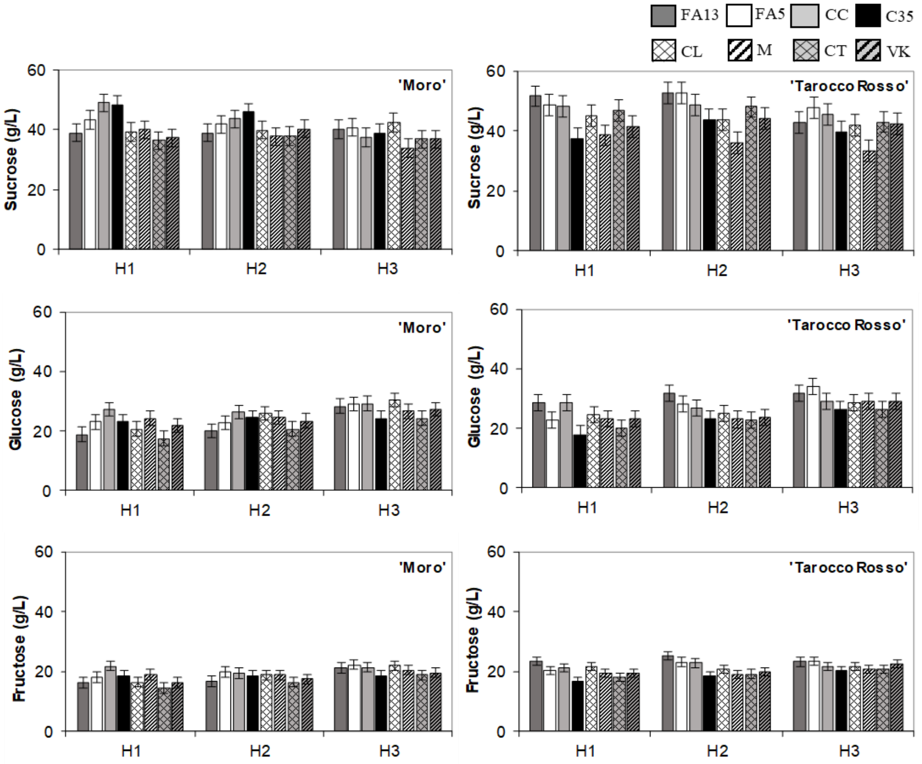
As mentioned above, an increase in the average maximum temperature from 18ºC to 22ºC after the second harvest, along with a drop in the average cold hours occurred (Fig. Supp. 1), which could cause the degradation of Cy-3,6”mal-glu and Cy-3,6”diox-glu in ‘Moro’. Nevertheless, Cy-3-glu did not alter as harvest advanced, and even an increase in Dp-3-glu was observed, which would indicate their lesser sensitivity to temperature changes. Previous studies on the thermal degradation of anthocyanins have shown a different stability to changes in environmental temperatures for each individual anthocyanin (Lo Piero, 2015).

In ‘Tarocco Rosso’, no evidence for degradation of the studied individual anthocyanins was found with advancing harvests. Contrarily, Dp-3-glu and Cy-3-glu slightly increased, which would suggest that the influence of the environment on a specific pigment strongly depends on cultivar.

3.4. Sugars, flavanones and hydroxycinnamic acids content in juice

Sugars are important components of the chemical composition of blood oranges. Apart from sugars having an effect on sensory properties, they are reported to be involved in anthocyanins biosynthesis (Solfanelli, Poggi, Loreti, Alpi & Perata, 2006; Li et al., 2014; Abdullah et al., 2018). The sugar profile of blood oranges depends on many factors, such as cultivar, harvest time and environment conditions (Kafkas et al., 2009). The effect of rootstock on the content of individual sugars has been addressed in different studies on citrus fruit (Legua, Forner, Hernández & Forner-Giner, 2014; Saini, Capalash, Kaur & Singh, 2019).

In the present study, as in most blood oranges, sucrose was the main sugar in both studied cultivars, followed by glucose and fructose (Fig. 3). In both cultivars the three sugars were influenced by harvest and rootstock and no significant interaction between these factors were detected.



p-value	Sucrose		Glucose		Fructose	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0,000 *	0,000 *	0,001 *	0,000 *	0,000 *	0,000 *
B:Harvest	0,011 *	0,003 *	0,000 *	0,000 *	0,000 *	0,002 *
AB	0,067	0,723	0,274	0,301	0,094	0,198

Figure 3. Individual sugars (sucrose, glucose and fructose) of blood oranges 'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test (p≤0.05).

In 'Moro', the sucrose content ranged from 36-37 g/L in the fruit from CT and VK to near 48.5 g/L in the fruit from CC and C35 at the first harvest. A gradual decrease during the following harvests were observed in all fruit. The rootstocks with the lowest mean of sucrose were M, CT and VK, and the highest ones were CC and C35. It is noteworthy that fruit from CC and C35 exhibited the most important decrease. The highest glucose and fructose content at the first harvest was detected in the CC-fruit with values of 27.3 g/L and 21.7 g/L, respectively, while the lowest values were for the CT-fruit, with 17.5 g/L of glucose and 14.5 g/L of fructose. In all cases, the content of both sugars gradually increased. Taking the mean of the three harvests, the CT and C35 fruit showed the lowest fructose and glucose content, with the highest values for the CC-fruit.

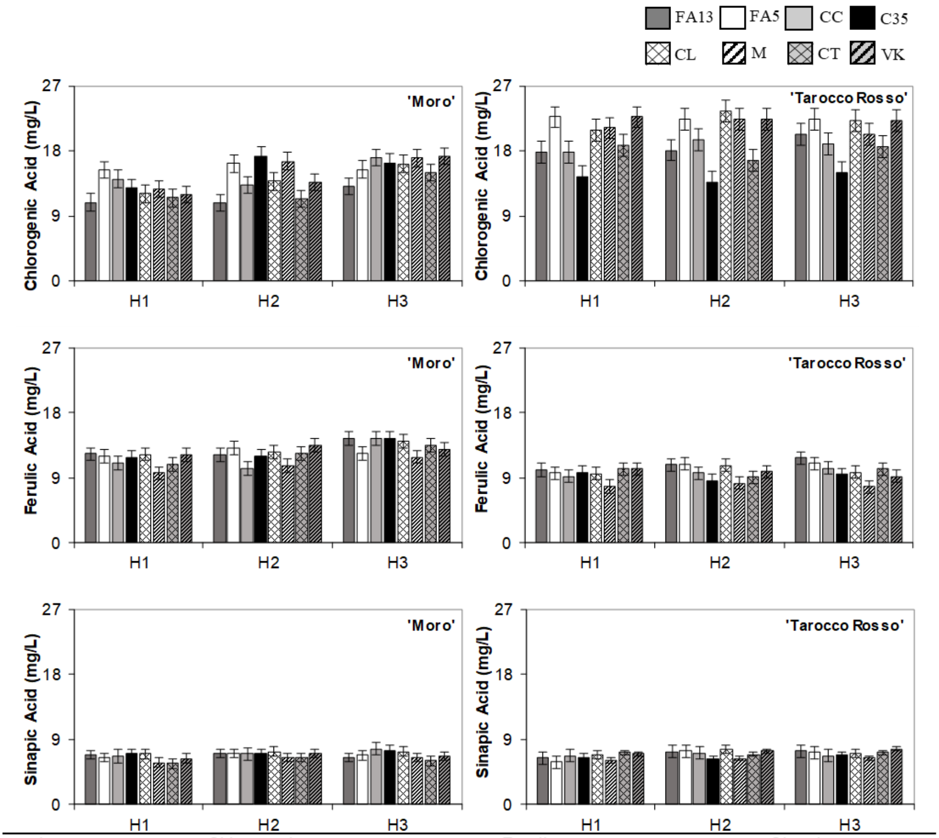
In 'Tarocco Rosso', sucrose content ranged from 37.5 g/L in C35 to 51.6 g/L in FA13 at the first harvest. Sucrose loss was exhibited in all fruit, overall in the third harvest. The highest sucrose mean was shown for the fruit from FA13 and FA5, and the M-fruit had the lowest value. Regarding glucose and fructose content in 'Tarocco Rosso', the C35-fruit had the lowest values, with 18.0 g/L and 16.9 g/L, respectively, for the first harvest. In the following harvests an increase in the content of these sugars were observed. The highest mean of both, glucose and fructose, was detected in FA13, while the fruit from C35 and CT had the lowest.

These results corroborate that the influence of rootstock on sugar content is cultivar-dependent. In blood oranges, a relation between sugar and anthocyanin accumulation has been reported as blood oranges require higher sugar metabolism to supply the need of carbon skeletons for anthocyanin biosynthesis (Muccilli et al., 2009; Carmona et al., 2019). Solfanelli et al. (2006) established that the sugar-dependent up-regulation of the anthocyanin synthesis pathway is sucrose-specific. In the same way, it was reported that sucrose induces anthocyanin biosynthesis (Li et al., 2014). Sucrose increases the stabilization of DELLA proteins and the degradation of gibberellins, and activates the structural genes of anthocyanin biosynthesis. So a positive correlation between sucrose concentration and anthocyanin accumulation has been found (Abdullah et al., 2018). In this study, a relation between sucrose content and anthocyanins was found in 'Tarocco Rosso'. In this cultivar, M and C35 supplied the lowest sugar content and lower TSS in the fruit compared to the other rootstocks, which could cause the lower anthocyanin content observed in the fruit from these rootstocks. In 'Moro', this relation between sugars and anthocyanins was not as evident. Nonetheless, the rootstocks that led to fruit having the lowest anthocyanin content were CT and VK. These rootstocks presented the least sucrose.

Hydroxycinnamic acids represent an important group of compounds that derive from the general phenylpropanoid pathway, which is the following step from individual sugars on the anthocyanins pathway. So blood oranges contain more hydroxycinnamic acids than blond ones, being proposed as varietal markers of blood orange juice (Rapisarda et al., 1998).

In this study, the main hydroxycinnamic acids detected in both blood orange cultivars were chlorogenic, ferulic and sinapic acids. Traces of caffeic acid and ρ-coumaric, was also identified (Data not shown). In both cultivars, chlorogenic acid was the most dominant hydroxycinnamic acid, followed by ferulic and sinapic acid. Nevertheless, Kelebek et al. (2008) found in two blood orange cultivars that ferulic acid was the most dominant hydroxycinnamic acid, followed by chlorogenic and sinapic acids. The differences found is due to the distribution of individual hydroxycinnamic acids is variety-dependent (Rapisarda et al., 1998; Kelebek et al., 2008).

In 'Moro', the content of three hydroxycinamic acids was affected by harvest and rootstock and only the interaction between these two factors was significant for chlorogenic acid. At the first harvest, chlorogenic acid ranged from 11 mg/L in the FA13-fruit to 15 mg/L in the FA5-fruit (Fig. 4). During the following harvests, the content of this acid increased in all the fruit, except for the fruit from FA5. The lowest chlorogenic acid mean values were found in the FA13-fruit, with the highest ones in the fruit from FA5, C35 and M. The ferulic acid content in all the fruit came close to 10-12 mg/L at the first harvest and, similarly to chlorogenic acid, it increased during the following harvests (Fig. 4). The lowest values were detected in M fruit.



p-value	Chlorogenic		Ferulic		Sinapic	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0,000 *	0,000 *	0,003 *	0,000 *	0,012 *	0,015 *
B:Harvest	0,000 *	0,680	0,000 *	0,434	0,032 *	0,016 *
AB	0,011 *	0,389	0,100	0,371	0,924	0,581

Figure 4. Individual hydroxycinnamic acids (chlorogenic, ferulic and sinapic acid) of blood oranges 'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test (p≤0.05).

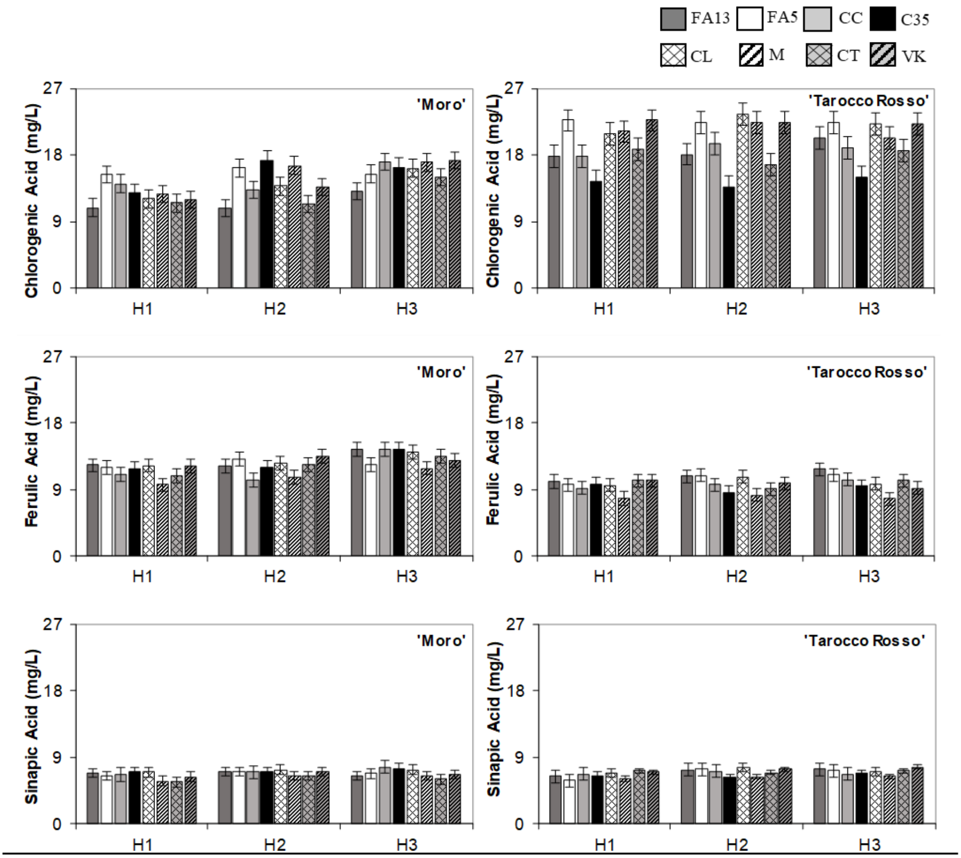
The amount of sinapic acid content was lower than the other acids, and ranged from 5.7 mg/L in the CT-fruit to 7 mg/L in both C35-fruit and CL-fruit at the first harvest with a significant increase during the following harvests. The lowest mean values were presented by the fruit from M and CT.

In 'Tarocco Rosso', no significant effect of harvest was observed chlorogenic and ferulic acid content (Fig 4). Nevertheless, major differences among rootstocks were observed. The lowest chlorogenic acid content during the studied period was exhibited by the C35-fruit with values close to 14 mg/L, followed by FA13, CC and CT with values between 18 mg/L and 20 mg/L. The maximum content was detected in the fruit from FA5, CL, M and VK with 22-23 mg/L. The lowest mean of ferulic acid was found for M-fruit, close to 8 mg/L. Finally, sinapic acid was significant increased with the harvest advance and the lowest amount was also shown by M-fruit, with mean value of 6.28 mg/L.

The increase in chlorogenic and ferulic acids observed in 'Moro', overall at the third harvest, was concomitant to anthocyanin Cy-3,6”mal-glu and Cy-3,6”diox-glu degradation. This could be explained by the general phenylpropanoid pathway. Phenylalanine is a direct precursor of anthocyanin synthesis through complex reactions. The first step is the transformation from phenylalanine to trans-cinnamic acid. In the following steps, cinnamic acid is hydroxylated by generating p-coumaric acid. Then p-coumaric is condensed by the specific enzyme of the anthocyanin biosynthetic pathway, chalcone synthase, to produce naringenin chalcone, which is implicated in anthocyanin biosynthesis. In 'Moro', anthocyanin degradation was observed at third harvest, which would stop the biosynthesis anthocyanin process, while the p-coumaric formed in the first steps of the phenylpropanoid pathway would be used to synthesize hydroxycinnamic acids, specifically chlorogenic and fumaric acids.

Flavanones are major flavonoids in citrus fruit. In both studied cultivars, hesperidin (HES) was the predominant flavanone, followed by narirutin (NAR) and didymin (DID), which agrees with what has been previously reported for other blood orange cultivars (Barreca, Bellocco, Leuzzi & Gattuso, 2014; Cebadera-Miranda et al., 2019).

In 'Moro', the three flavanones were affected by rootstock and harvest with significant interaction between these two factors. HES content at first harvest varied from 104 mg/L in the CT-fruit to 142 mg/L in the FA5-fruit (Fig. 5). In all cases, a significant increase during the following harvests was observed, except in the FA5-fruit, that exhibited the maximum HES values during first and second season to then dropped in the third until achieve the lowest amount of 134 mg/L. C35 was one of the rootstock that displayed higher HES content in the three harvest.



p-value	Chlorogenic		Ferulic		Sinapic	
	Moro	Tarocco Rosso	Moro	Tarocco Rosso	Moro	Tarocco Rosso
A:Rootstock	0,000 *	0,000 *	0,003 *	0,000 *	0,012 *	0,015 *
B:Harvest	0,000 *	0,680	0,000 *	0,434	0,032 *	0,016 *
AB	0,011 *	0,389	0,100	0,371	0,924	0,581

Figure 5 Individual flavanone (hesperidin (HES), narirutin (NAR) and didymin (DID)) of blood oranges 'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test (p≤0.05).

NAR content also rose in all the fruit while harvest advanced. At third harvest, the maximum level was also achieved by the C35-fruit (42 mg/L) and the minimum by the FA13-fruit (27 mg/L). An increase in DID content was also recorded as harvest advanced, except for the FA5-fruit, which maintained the same values during all three harvests. Although the differences among rootstocks depended on the harvest, similar to other flavanones, C35-fruit exhibited one of the highest DID content and the lowest was for both CT-fruit and FA13-fruit.

In 'Tarocco Rosso', the content of the three studied flavanones fell within a lower range than 'Moro'. Similar to 'Moro', HES and NAR content was affect by harvest and rootstock with significant interaction between them. The amount of HES varied from 51 mg/L in the FA13-fruit to 95 mg/L in the CT-fruit at the first harvest. A drop was observed in all the fruit during the following harvests. In the third harvest lower differences among rootstocks were found. NAR content ranged from close to 13 mg/L in FA13 and C35-fruit to 21 mg/L in CL and CT-fruit at first harvest. All fruit exhibited an increase the second harvest to then decrease in the third, except C35 that did not change with harvest advance and had the lowest values. In 'Tarocco Rosso', harvest time had no affect DID content. Nevertheless, the differences among rootstocks were evident. C35-fruit had the lowest values with a mean of 3.37 mg/L. The maximum values were displayed by the CC, CT and CL fruit with values close to 5-6 mg/L.

On the flavonoid pathway, it is noteworthy that naringenin chalcone, besides being involved in anthocyanins biosynthesis, is implicated in the formation of other phenolic compounds like flavanones. This could explain the increase in flavanones in the 'Moro' fruit that parallel the stop of the anthocyanins synthesis process which occurs when they are degraded. Contrary to that observed in 'Moro', the 'Tarocco Rosso' fruit did not exhibit anthocyanin degradation during the study period. Consequently, no relevant changes in the content of hydroxycinnamic acids or flavanones took place.

3.6 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) representing both cultivars, rootstocks and the internal parameters previously detailed are shown in Fig. 6 (A,B). The first two components explain 85.4% of the total variance data (Principal Component 1 (PC1): 63.2%; Principal Component 2 (PC2): 22.2%). PC1 are correlated positively with flavonoids and individual anthocyanins content, juice color, acidity and ferulic acid and separates the set of samples into two groups: 'Tarocco Rosso' and 'Moro'.

In order to further explore the effect of rootstock and harvest moment on the internal quality parameters a PCA was independently performed for each cultivar. In Moro the two first components explain 69.9% of the total variance Fig. 6 (C,D). The first component (PC1) is correlated positively with TA and negatively with individual flavonoids, hydroxycinnamic acids and sugars. This component explains the effect of the harvest moment, since separate the three maturity stages for all rootstocks. So, while the fruit from first harvest was related with the acidity and the anthocyanins Cy-3,6”mal-glu and Cy-3,6”diox-glu, as harvest advance the fruit was correlated with flavonoids, hydroxycinnamic acids and sugars. Moreover, M, CT and VK are located in Q3 and Q4, separated from the other cultivars by PC2, inversely correlated with juice color and individual anthocyanins.

In 'Tarocco Rosso', PCA shows that the internal quality was more influenced by rootstock effect than the harvest moment (Fig. 6. E,F). The two first components explain 65.9% of the total variance. PC1 is positively correlated with sugars, anthocyanins, juice color, ferulic and sinapic acids. The second component is positively correlated mainly with the content of the three flavonoids. PC1 corroborates that the lowest content of sugars and anthocyanins are shown by C35, M and CT (located in Q1 and Q3) while the highest content is shown by FA5 and FA13 (located in Q2 and Q4). Moreover, C35 is characterized by the low content of flavonoids and chlorogenic and sinapic acids. FA13 clearly is separated by PC2 also by the low flavonoids content.

(Flavonoids: HES=hesperidin, NAR=narirutin, DID=didymin; individual sugars: SUCR=sucrose, GLU=glucose; FRU=fructose; hydroxycinnamic acids: CHLOR=chlorogenic, FER=ferulic, SIN=sinapic; individual anthocyanins: Cy-mal-glu= cyanidin 3-(6”malonyl)-glucoside, Cy-diox-glu=cyanidin 3-(6”-dioxalyl)-glucoside, Cy-3-glu= cyanidin 3-glucoside, Dp-3-glu= delphinidin 3-glucoside).

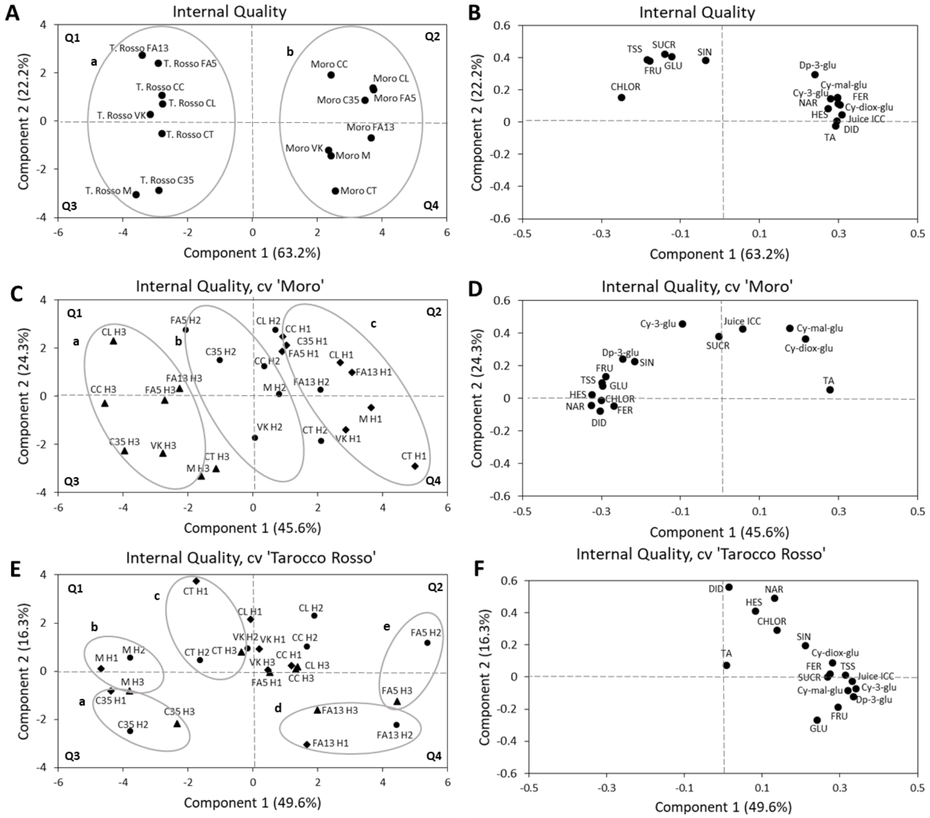


Figure 6. Principal component analysis of the internal quality parameters of ‘Moro’ and ‘Tarocco Rosso’ blood oranges grafted onto eight rootstocks. The score and loading plots for the first and second principal components are shown. A, B: Data set (two cultivars, eight rootstocks). C, D: Data set (‘Moro’ cultivar, eight rootstocks and three maturity stages). E, F: Data set (‘Tarocco Rosso’ cultivar, eight rootstocks and three maturity stages).

In conclusion, the results herein obtained point out the strong influence of rootstock on the quality parameters herein studied, which was highly scion-dependent. Furthermore, the changes that fruit undergoes during harvest are also affected by the rootstock onto which it was grafted.

Taking into account that in blood oranges, fruit firmness is currently one of the most important quality parameters from the commercial point of view, CT and C35 in 'Moro' and CT, FA13 and CC in 'Tarocco Rosso' were the rootstocks that induced the higher firmness values in fruit. The effect on the content of sugars and acids also depends on the rootstock/scion interaction. The evaluation of the four main anthocyanins revealed their relation to internal fruit color. In 'Moro', the fruit from M, CT and VK displayed the lowest juice color and the lowest content for all four anthocyanins. In 'Tarocco Rosso', C35, M and CT were the rootstocks that induced less color in fruit. The differences in anthocyanin content detected among rootstocks were also related to the supplied sucrose content. In addition, the degradation of Cy-3,6"mal-glu and Cy-3,6"diox-glu that took place in 'Moro' during the last harvest, as a response of increased temperature, contributed to increase the synthesis of hydroxycinnamic acids and flavanones. In 'Tarocco Rosso', no anthocyanin degradation was shown, which suggests that this cultivar is less sensitive to environmental changes.

Acknowledgements

The authors thank Anecoop S. Coop. for supplying the fruit herein used and for its technical support. This study was co-financed by FEDER and European Social Funds.

References

Abdullah, M., Cheng, X., Shakoor, A., Cao, Y., Lin, Y., Cai, Y., & Shangao, J. (2018). New Opinion of Sugar and Light Crosstalk in the Induction of Anthocyanins Biosynthesis in Fruits. *International Journal of Agriculture and Biology*, *20*(11), 2465-2474.

https://doi.org/10.17957/IJAB/15.0790

Ballistreri, G., Fabroni, S., Romeo, F. V., Timpanaro, N., Amenta, M., & Rapisarda, P. (2019). Anthocyanins and Other Polyphenols in Citrus Genus: Biosynthesis, Chemical Profile, and Biological Activity. In *Polyphenols in Plants* (pp. 191-215). Academic Press. https://doi.org/10.1016/B978-0-12-813768-0.00014-1

Barbagallo, R.N., Palmeri, R., Fabiano, S., Rapisarda, P., & Spagna, G. (2007). Characteristic of β-glucosidase from Sicilian blood oranges in relation to anthocyanin degradation. *Enzyme and microbial technology*, *41*(5), 570-575. https://doi.org/10.1016/j.enzmictec.2007.05.006

Barreca, D., Bellocco, E., Leuzzi, U., & Gattuso, G. (2014). First evidence of C- and O-glycosyl flavone in blood orange (Citrus sinensis (L.) Osbeck) juice and their influence on antioxidant properties. *Food Chemistry*, *149,* 244-252. https://doi.org/10.1016/j.foodchem.2013.10.096

Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. *Journal of the American Society for Horticultural Science, 129*(6), 881-889. https://doi.org/10.21273/JASHS.129.6.0881

Barry, G.H., Caruso, M., & Gmitter Jr, F.G. (2020). Commercial scion varieties. In *The Genus Citrus* (pp. 83-104). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-812163-4.00005-X

Bermejo, A., Pardo, J.L., Morales, J., & Cano, A. (2016). Comparative study of bioactive components and quality from juices of different mandarins: discriminant multivariate analysis of their primary and secondary metabolites. *Agricultural Sciences, 7(6),* 341-351. https://doi.org/10.4236/as.2016.76035

Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P. Reforgiato Recupero, G., & Martin, C. (2012). Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell, 24(3*), 1242-1255. https://doi.org/10.1105/tpc.111.095232

Carmona, L., Alquézar, B., Marques, V. V., & Peña, L. (2017). Anthocyanin biosynthesis and accumulation in blood oranges during postharvest storage at different low temperatures. *Food chemistry, 237,* 7-14. https://doi.org/10.1016/j.foodchem.2017.05.076

Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science, 23*(4), 383-394. https://doi.org/10.1080/01140671.1995.9513914

Cebadera-Miranda, L., Domínguez, L., Dias, M. I., Barros, L., Ferreira, I. C., Igual, M., Martínez-Navarrete, N., Fernández-Ruiz, V., Morales, P., & Cámara, M. (2019). Sanguinello and Tarocco (Citrus sinensis [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. *Food Chemistry, 270*, 395-402. https://doi.org/10.1016/j.foodchem.2018.07.094

Continella, A., Pannitteri, C., La Malfa, S., Legua, P., Distefano, G., Nicolosi, E., & Gentile, A. (2018). Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange. *Scientia horticulturae*, *230*, 62-67. https://doi.org/10.1016/j.scienta.2017.11.006

Crifò, T., Puglisi, I., Petrone, G., Reforgiato Recupero, G., & Lo Piero, A.R. (2011). Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. *Gene*, *476*(1-2), 1-9. https://doi.org/10.1016/j.gene.2011.02.005

Dugo, P., Mondello, L., Morabito, D., & Dugo, G. (2003). Characterization of the anthocyanin fraction of sicilian blood orange juice by micro-HPLC-ESI/MS. *Journal of Agricultural and Food Chemistry*, *51*(5), 1173-1176. https://doi.org/10.1021/jf026078b

Fabroni, S., Amenta, M., Timpanaro, N., Todaro, A., & Rapisarda, P. (2020). Change in taste-altering non-volatile components of blood and common orange fruit during cold storage. *Food Research International, 131,* 108916.
https://doi.org/10.1016/j.foodres.2019.108916

Filho, F.D.A.A., Espinoza-Núñez, E., Stuchi, E.S., & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. *Scientia Horticultura. 114(1),* 45-49.
https://doi.org/10.1016/j.scienta.2007.05.007

Habibi, F., Ramezanian, A., Guillén, F., Serrano, M., & Valero, D. (2020). Blood oranges maintain bioactive compounds and nutritional quality by postharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate during cold storage. *Food Chemistry*, 306, 125634.
https://doi.org/10.1016/j.foodchem.2019.125634

Hou, D. X. (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine*, 2003, 3(2), 149–159.
https://doi.org/10.2174/1566524033361555

Incesu, M., Çimen, B., Yesiloglu, T., & Yilmaz, B. (2013). Rootstock effects on yield, fruit quality, rind and juice color of Moro blood orange. *Journal of food, Agriculture & Environment*, *11*(3&4), 867-871.

Jimenez-Cuesta M., Cuquerella J., & Martínez-Jávega J. (1981) Determination of a color index for citrus fruit degreening. *Proceedings of the International Society of Citriculture*, 2: 750–753.

Kafkas, E., Ercisli, S., Kemal, K.N., Baydar, K., & Yilmaz, H. (2009). Chemical composition of blood orange varieties from Turkey: A comparative study. *Pharmacognosy Magazine*, *5(20),* 329. https://doi.org/10.4103/0973-1296.58155

Kelebek, H., Canbas, A., & Selli, S. (2008). Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (Citrus sinensis (L.) Osbeck) grown in Turkey. *Food Chemistry*, *107*(4), 1710-1716. https://doi.org/10.1016/j.foodchem.2007.10.004

Laribi, A.I., Palou, L., Intrigliolo, D.S., Nortes, P.A., Rojas-Argudo, C., Taberner, V., Bartual, J., & Pérez-Gago, M.B. (2013). Effect of sustained and regulated deficit irrigation on fruit quality of pomegranate cv. ‘Mollar de Elche’ at harvest and during cold storage. *Agricultural Water Management*, 125, 61-70. https://doi.org/10.1016/j.agwat.2013.04.009

Legua, P., Forner, J. B., Hernández, F., & Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. *Scientia Horticulturae*, *174*, 60-64. https://doi.org/10.1016/j.scienta.2014.05.004

Li, Y., Van den Ende, W., & Rolland, F. (2014). Sucrose induction of anthocyanin biosynthesis is mediated by DELLA. *Molecular Plant, 7*(3), 570-572.

Lo Piero, A.R. (2015). The state of the art in biosynthesis of anthocyanins and its regulation in pigmented sweet oranges [(Citrus sinensis) L. Osbeck]. *Journal of agricultural and food chemistry*, *63*(16), 4031-4041. https://doi.org/10.1021/acs.jafc.5b01123

Mondello, L., Cotroneo, A., Errante, G., Dugo, G., & Dugo, P. (2000). Determination of anthocyanins in blood orange juices by HPLC analysis. *Journal of pharmaceutical and biomedical analysis*, *23*(1), 191-195.

https://doi.org/10.1016/S0731-7085(00)00269-7

Muccilli, V., Licciardello, C., Fontanini, D., Russo, M.P., Cunsolo, V., Saletti, R., Reforgiato Recupero, G., & Foti, S. (2009). Proteome analysis of Citrus sinensis L. (Osbeck) flesh at ripening time. *Journal of Proteomics, 73(1),* 134-152. https://doi.org/10.1016/j.jprot.2009.09.005

Oren-Shamir, M. (2009). Does anthocyanin degradation play a significant role in determining pigment concentration in plants? *Plant Science, 177*(4), 310-316. https://doi.org/10.1016/j.plantsci.2009.06.015

Pallottino, F., Menesatti, P., Lanza, M. C., Strano, M. C., Antonucci, F., & Moresi, M. (2012). Assessment of quality-assured Tarocco orange fruit sorting rules by combined physicochemical and sensory testing. *Journal of the Science of Food and Agriculture,* 93(5), 1176-1183. https://doi.org/10.1002/jsfa.5871

Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., & Maccarone, E. (1998). Hydroxycinnamic acids as markers of Italian blood orange juices. *Journal of Agricultural and Food Chemistry*, *46*(2), 464-470. https://doi.org/10.1021/jf9603700

Rapisarda, P., Bellomo, S.E., & Intelisano S. (2001). Storage temperature effects on blood orange fruit quality. *Journal of Agricultural and Food Chemistry, 49*(7), 3230-3235. https://doi.org/10.1021/jf010032l

Reforgiato Recupero, G.R., Russo, G., Recupero, S., Zurru, R., Deidda, B., & Mulas, M. (2009) Horticultural evaluation of new citrus latipes hybrids as rootstocks for citrus. *HortScience*, 44(3), 595-598. https://doi.org/10.21273/HORTSCI.44.3.595

Rodrigo, M.J., Alquézar, B., Alós, E., Lado, J., & Zacarías, L. (2013). Biochemical bases and molecular regulation of pigmentation in the peel of Citrus fruit. *Scientia Horticulturae*, *163*, 46-62. https://doi.org/10.1016/j.scienta.2013.08.014

Saini, M.K., Capalash, N., Kaur, C., & Singh, S.P. (2019). Comprehensive metabolic profiling to decipher the influence of rootstocks on fruit juice metabolome of Kinnow (C. nobilis× C. deliciosa). *Scientia Horticulturae*, *257*, 108673. https://doi.org/10.1016/j.scienta.2019.108673

Solfanelli, C., Poggi, A., Loreti, E., Alpi, A., & Perata, P. (2006). Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant physiology*, *140*(2), 637-646. https://doi.org/10.1104/pp.105.072579

Steyn, W.J. (2008). Prevalence and functions of anthocyanins in fruits. In *Anthocyanins* (pp. 86-105). Springer, New York, NY. https://doi.org/10.1007/978-0-387-77335-3_4

Zhang, Y., Butelli, E., & Martin, C. (2014). Engineering anthocyanin biosynthesis in plants. *Current opinion in plant biology,* 19, 81-90. https://doi.org/10.1016/j.pbi.2014.05.011

CHAPTER V

Effect of Cold Storage on Fruit Quality of Blood Oranges

Julia Morales [a], Pilar Navarro [a], Cristina Besada [a], Almudena Bermejo [b], and Alejandra Salvador [a]

[a]Departamento de Tecnología Postcosecha, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.
[b]Centro de Citricultura y Producción Vegetal, Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain.

Reference: *Acta Horticulturae. 1256. ISHS 2019. Proc. VI International Conference Postharvest Unlimited (Oct, 2017). Eds.: D. Valero et al.*

Abstract

Traditionally the production of blood oranges has been destined to the juice industry. However, in the last years the demand for this citrus fruit for fresh consumption is considerably increasing. Nowadays there is not enough information about the optimum storage conditions of 'Sanguinelli' and 'Tarocco Rosso', the most important cultivars in the Spanish Mediterranean area. In this study the effect of cold storage at different temperatures on fruit quality was evaluated in both cultivars. In two different seasons, after harvest, the fruit was stored up to 45 days at 1, 5, or 9°C. Periodically a sample of fruit was transferred to 20°C during 6 days simulating shelf-life conditions. The following quality parameters were evaluated: maturity index, external colour, firmness, ethanol concentration and physiological disorders. Although there were slight differences between seasons, 'Sanguinelli' showed higher sensitiveness to low temperatures than 'Tarocco Rosso', which manifested chilling injury symptoms after 30 days of cold storage at the three studied temperatures. The lower temperature storage the higher disorders incidence was observed. During storage the acidity slightly decreased and total soluble solids remains constant in both cultivars. The increase in the external colour was affected by the storage temperature; fruit stored at 5 and 9°C exhibited the highest colour index. The increase of ethanol concentration throughout the storage period was not associated with a sensory quality loss.

Keywords: Blood orange, physiological disorders, postharvest, cold storage

1. Introduction

Blood oranges production in Spain had been decreasing until it almost disappeared during the last decades. However, their demand has recently increased (Sanfeliu, 2016) mainly due to the fact that nowadays the consumer is interested in healthier products. Blood oranges, characterized by their unique flesh and rind color (Maccarone, Maccarrone, Perrini & Rapisarda, 1983), stand out for their compound profile rich in secondary metabolites involved in the nutritional and healthy quality of this fruits and have been associated with potentially beneficial effects in various human diseases (Rapisarda, Bellomo & Intelisano, 2001; Prior & Wu, 2006; Wang & Stoner, 2008; Fallico, Ballistreri, Arena, Brighina& Rapisarda, 2017).

Traditionally, blood oranges have been destined to the juice industry but the demand as fresh fruit is growing. Most important blood orange cultivars (Citrus sinensis (L.) Osbeck) are ‘Sanguinelli’, ‘Moro’ and ‘Tarocco’. ‘Sanguinelli’ is original from Spain while ‘Tarocco’ and ‘Moro’ are from Italy.

The production increase leads to the need of storing the fruit in order to supply the market according to the demand level. Cold storage is a common technique used to store citrus fruit. However, some cultivars are susceptible to manifest chilling injury (CI) when exposed to low temperature for extended periods. Skin damages are well-known to be among the main chilling injury symptoms of citrus fruit and may lead to non-commercial fruit or enhancing susceptibility of fruit to decay (Rab, Sajid, Khan, Nawab, Arif & Khattak, 2012; Lindhout, Treeby & Parish., 2005). Blood oranges have been reported to be susceptible to chilling injury when stored below 7-8°C (Pratella, Tonini & Cessari, 1969; Schirra, D’hallewin, Cabras, Angioni & Garau, 1998).

Different studies have approached the effect of cold storage in different blood orange cultivars, most of them focused on juice quality changes and pathological alterations during storage (Pannitteri et al., 2017; Hamedani, Rabiei, Moradi, Ghanbari & Azimi, 2012). However, there is not enough information about physiological alterations in the skin related with low temperatures which may limit the commercialization for fresh consumption. The objective of this study is to analyze the behaviour during the cold storage period of two of the most important cultivars of blood oranges, ‘Tarocco

Rosso' and 'Sanguinelli', under the cultivation conditions of Comunidad Valenciana (Spain).

2. Materials and Methods

2.1. Plant material and treatments

'Tarocco Rosso' and 'Sanguinelli' blood oranges were harvested in March from an experimental farm (Anecoop S. Coop.) located in Museros, Valencia (Spain) during two different seasons. After harvest, fruit was selected, removing damaged fruits, washed in foam curtain with biodegradable detergent (Fruit-Cleaner®, Fomesa Fruitech SLU), cleared up and waxed with a commercial coating of 14% of total solids with 2000 ppm of Imazalil as fungicide (Waterwax TTT-2I®, Fomesa Fruitech SLU). Finally, fruit was separated into lots of 30-40 fruits to be stored up, to 45 days maximum, at 1, 5 or 9°C.

Periodically, each 15 days, a lot per temperature and cultivar was transferred to 20°C during 6 days to simulate shelf-life period. Determination of external color, firmness, total soluble solids, titratable acidity, juice yield, ethanol content and sensory evaluation of the flavour were carried out at harvest and after each shelf-life period. Physiological disorders were also evaluated after each cold storage period.

2.2. Quality attributes determination

The external color of the fruit, was measured with a Minolta colorimeter (model CR400, Minolta Co. Ltd, Osaka, Japan), over 20 fruits per each treatment, taking 2 measurements in the equatorial zone of each fruit. The mean values for 'L', 'a' and 'b' Hunter parameters were calculated for each fruit and expressed as citrus color index (CCI) (CCI=1000a/Lb). Firmness measurements were made with a Universal Testing Machine (model 3343, Intron Limited, Buckinghamshire, UK) using, as well, 20 fruits per treatment; the results were expressed as the percentage of mm of fruit deformation resulting from a 10 N force on the longitudinal axis at a constant speed. The parameters of internal quality were analyzed with three samples of juice, using

5 fruits per each juice. Total acidity (TA) was determined by volumetric titration with 0.1 NaOH solution on 5 mL of juice. Total soluble solids content (TSS) in the juice was determined by measuring the refractive index of juice and the dates were expressed as °Brix. Maturity index was calculated by the rate TSS/TA. The juice yield was expressed as a percentage. The ethanol concentration was quantified using the headspace method (Ke & Kader, 1990) by gas chromatography. Sensory evaluation was assessed by eight to ten semi-trained judges. Flavour was determined using a 9-point scale, where 1 was equivalent to extremely unpleasant, 5 fair, and 9 excellent. Physiological disorders appeared during the cold storage were evaluated visually, over the total of fruits per lot, according to the rind surface affected with the following scale of intensity: 0, no alterations; 1, light (50%). Results were expressed as percentage of damaged fruits and the intensity of damage with an index of alterations (IA), using the weighted average of the data obtained.

2.3. Statistical analysis

Statistical procedures were performed using a statistical software (Statgraphics plus 5.1. Manugistics, Inc., Rockville, MD, USA). All data were subjected to analysis of variance, and means were compared using LSD test at $P<0.05$.

3. Results and discussion

3.1. Fruit quality

Fruit from the second season exhibited more advanced mature state (Tables 1 and 2). It can be appreciated in a higher CCI and lower firmness and TA at harvest moment, being more noticeable in ‘Sanguinelli’ than in ‘Tarocco Rosso’.

In both cultivars, during the storage period, an increase of external color was observed. The increase of CCI was higher in the fruit stored at 5 and 9°C than the fruit stored at 1°C. This is according to Lo Piero, Puglisi, Rapisarda & Petrone (2005) and Butelli et al. (2017) who reported that anthocyanin accumulation, biocompound characteristic from peel and flesh in blood oranges, can be promoted by keeping the fruit at temperatures between 4 and 10°C. ‘Sanguinelli’ showed a higher CCI than ‘Tarocco Rosso’ due to the presence of zones with a deep purple color over the homogeneous orange color peel.

The fruit firmness decreased during storage period at the three assayed temperatures. Nevertheless, it was the fruit stored at 9°C which showed a high percentage of deformation. Previous studies in other citrus cultivars have reported that the lowest firmness is detected in fruit stored at high temperature between the cold studied temperatures (Sdiri, Navarro, Monterde, Salvador, Cuenca, Aleza & Ben Abda, 2012; Sdiri, Navarro, Cuenca, Pardo & Salvador, 2015).

During storage studied period, MI (TSS/TA) did not suffer important changes at the three studied temperatures, which agrees with Rapisarda et al. (2001), who reported slight changes in TA and TSS only after 50 days of storage.

In all the cases, juice yield remained higher than 35% throughout the storage period, the loss of juice not being influenced by the temperature of storage. According to the quality standards for blood oranges, the required juice yield is 30% (Regulation UE N° 543/2011).

During the studied period, the ethanol content increased with storage duration and higher temperatures. However, this increase was not negatively reflected in the sensory evaluation of the fruits since presence of off-flavours were no detected (data not shown).

Table 1. Quality parameter changes after storage of 'Sanguinelli' oranges at 1, 5 or 9°C for 15, 30 or 45 days followed by 6 days shelf-life at 20°C. Each column, per each season, with different letters indicates significant differences (P<0.05).

Day	Temp(^{o}C)	Color Index (1000a/Lb)	Firmness (% def.10N)	TA (g/100 mL)	TSS (^{o}Brix)	Juice yield (%)	Ethanol (mg/100mL)
1st Season							
0		20.9	1.51	1.34	11.5	42.2	127.1
15	1	27.2a	1.91a	1.27ab	11.3a	36.8a	128.5a
	5	28.0ab	1.82a	1.24ab	11.7ab	38.5a	157.0a
	9	30.1ab	2.02a	1.24b	11.6ab	39.5a	161.3a
30	1	29.4a	1.88a	1.26ab	11.8ab	38.4a	169.6a
	5	37.8b	1.84a	1.18a	11.3a	36.7a	156.2a
	9	38.3b	2.34b	1.31b	12.1b	39.6a	245.9b
45	1	35.5b	1.97a	1.30b	11.9b	38.4a	162.4a
	5	37.5b	1.92a	1.17a	11.4ab	37.7a	276.5bc
	9	37.4b	2.29b	1.24ab	11.7ab	40.2a	302.5c
2nd Season							
0		25.3	2.26	1.24	11.9	43.1	192.3
15	1	24.0ab	2.85a	1.16b	11.7a	42.6b	214.2a
	5	27.2b	2.90a	1.09ab	11.7a	41.5ab	273.4ab
	9	24.1ab	3.30b	1.07ab	11.7a	43.0b	255.4ab
30	1	21.9a	2.90a	1.16b	12.2a	37.7a	303.8ab
	5	32.0b	2.91a	1.15b	11.8a	39.8ab	306.9ab
	9	30.4b	3.36b	1.08ab	11.8a	40.3ab	374.5b
45	1	22.0a	2.94a	1.08ab	11.5a	39.8ab	262.7ab
	5	31.8b	2.87a	1.16b	11.9a	39.9ab	357.9b
	9	32.4b	3.67b	1.01a	11.4a	40.5ab	375.3b

Table 2. Quality parameter changes after storage of 'Tarocco Rosso' oranges at 1, 5 or 9°C for 15, 30 or 45 days followed by 6 days shelf-life at 20°C.

Day	Temp(°C)	Color Index (1000a/Lb)	Firmness (% def.10N)	TA (g/100 mL)	TSS (°Brix)	Juice yield (%)	Ethanol (mg/100mL)
1st Season							
0		19.6	3.17	1.27	12.8	44.5	121.7
15	1	15.7a	3.99ab	1.21a	12.9a	43.7a	152.7a
	5	19.0b	3.77a	1.28a	13.0a	44.0a	159.5a
	9	19.4b	3.99ab	1.18a	12.9a	44.7a	216.6ab
30	1	17.2ab	4.20b	1.19a	12.8a	44.3a	204.6ab
	5	20.9bc	3.94ab	1.18a	12.7a	45.3a	232.6ab
	9	19.1b	4.19b	1.20a	12.6a	45.9a	268.1b
45	1	19.4b	3.92ab	1.23a	13.0a	44.1a	191.6ab
	5	21.6c	4.45b	1.13a	12.7a	44.8a	307.4b
	9	19.8bc	4.52b	1.14a	12.6a	45.2a	239.1b
2nd Season							
0		21.7	3.93	1.22	12.5	46.9	106.3
15	1	18.5a	4.56a	1.20b	12.7a	44.5c	196.5a
	5	19.7a	4.38a	1.19b	12.9a	43.9bc	231.6ab
	9	22.8b	5.27b	1.15ab	12.8a	43.3bc	209.8a
30	1	18.3a	4.53a	1.20b	13.1a	41.7b	213.9a
	5	24.6b	4.51a	1.19b	12.9a	42.4bc	289.2b
	9	24.8b	5.41b	1.16ab	13.1a	39.7a	294.8b
45	1	23.5b	5.32b	1.22b	12.8a	40.3a	227.7a
	5	31.1c	5.42b	1.13a	12.9a	42.1bc	384.0c
	9	25.5b	6.02c	1.11a	12.7a	41.2ab	336.8bc

3.2. Physiological disorders

During cold storage, fruit shown physiological alterations at all studied temperatures (Figure 1); the lower storage temperature, the higher the incidence. Most of the symptoms were manifested after shelf-life period, and the incidence was higher in the second season. Moreover, 'Sanguinelli' showed higher incidence of physiological alterations than 'Tarocco Rosso' in both seasons. Physiological alterations shown by fruit stored at 1°C were related with high sensibility to low temperatures. The symptoms were exhibited as peel browning depressions. They were localized at the top of the fruits, near to the calyx (Figure 2A). At 9°C storage, fruits showed alterations mainly associated to dehydration around the calyx (Figure 2C). Finally, at 5°C, alterations observed in fruit did not have a clear symptomatology, showing damages related to low temperature and also to dehydration (Figure 2B). In 'Sanguinelli', although the symptoms of disorders were not present after the first 15 days of storage at 1°C, these were visible after shelf-life period with values of 40 and 30%, respectively, at two seasons. The percentage of altered fruit reached values closer to 90% after shelf-life of 45 days in the second season. The severity of damages, expressed as index of alterations, was increased with storage time, overall after shelf-life periods. The incidence of damages arrived until maximum values as 1.5 in the second season. This would represent that all fruits showed disorder symptoms with a moderate intensity degree and they may not be commercial. 'Tarocco Rosso' stored at 1°C showed less sensibility to the low temperatures than 'Sanguinelli', reaching at the end of storage values of alteration index close to 1 and a percentage of altered fruit of 50%.

Fruits from 5°C suffered less incidence of alteration than that stored at 1°C. All fruit kept up good quality with values of percentages of damaged fruit and index alteration commercially acceptable after 30 days of storage. 'Sanguinelli', at second season, achieved higher percentages of damaged fruit than 60% with an index of 1 (slight degree) after shelf-life period that followed to 45 days at cold storage. 'Tarocco Rosso' showed low incidence of damage, with low values of intensity of damage (IA=0.3), even after 45 days of storage.

During cold storage at 9°C, alterations observed were related with dehydration in the peripeduncular zone. 'Sanguinelli' exhibited higher incidence of these symptoms during the second season, where the percentage was close to 40% of affected fruit after shelf-life period that followed 30 days of cold storage, however, the values of alteration were below 1. 'Tarocco Rosso' fruit showed low incidence of alterations during all the storage period at 9°C.

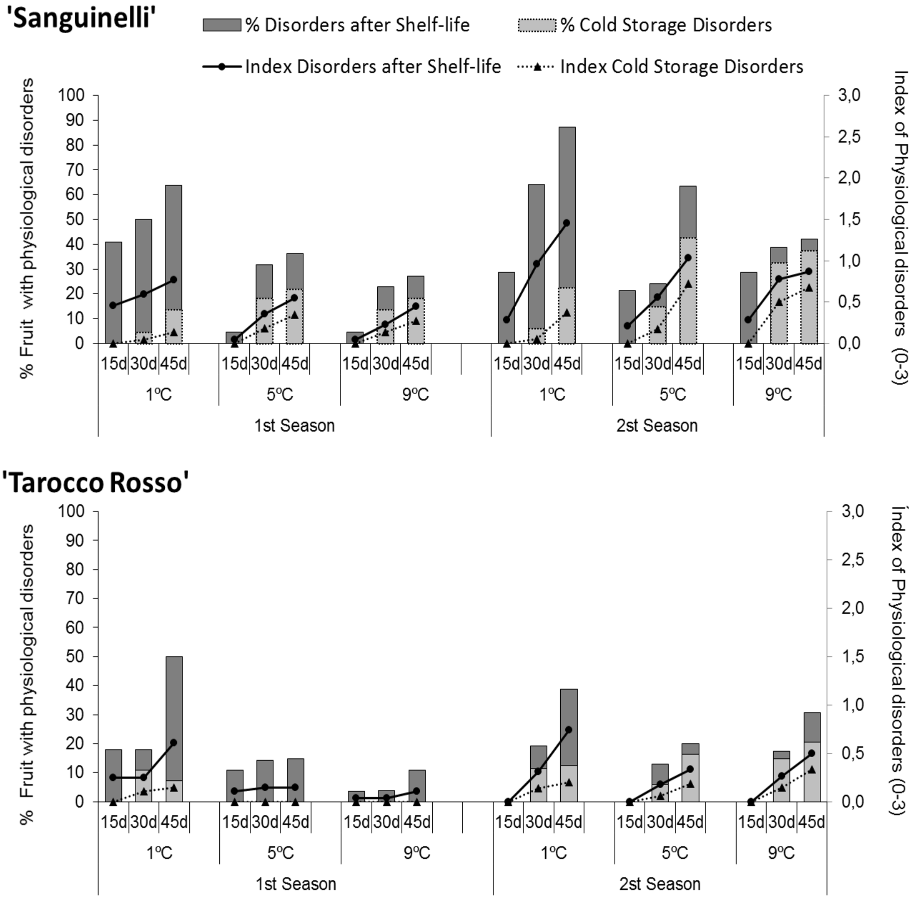


Figure 1. Physiological alterations (% of fruit affected) and index of alterations of 'Sanguinelli' and 'Tarocco Rosso' at 1, 5 or 9°C for 15, 30 or 45. The left axis with bars show the percentage of disorders, bright grey is the percentage of fruit affected after cold storage and dark grey the alterations appeared after Shelf-life periods, 6 days at 20°C. Right axe and lines show the intensity of the damages with the index alteration, the values are since 0 (without damages) to 3 (severe damage).

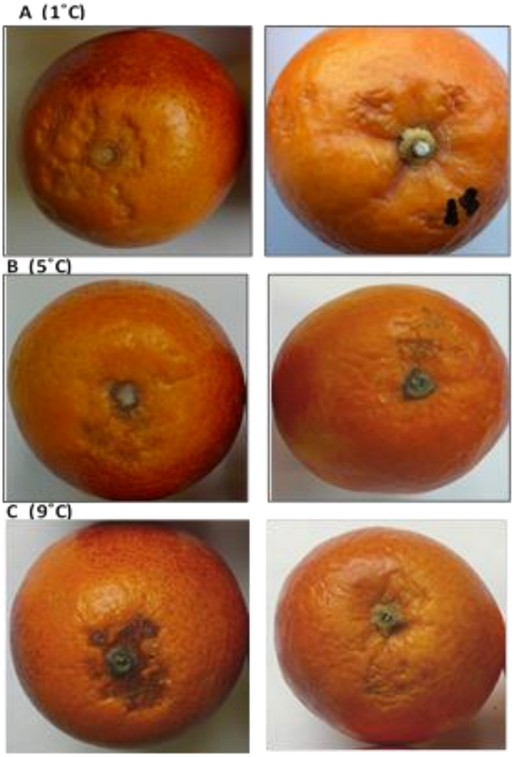


Figure 2. Physiological disorders shown during cold storage in 'Sanguinelli' (left) and 'Tarocco Rosso' (right).

4. Conclusions

According to the obtained results, none of the three studied temperatures affected the internal quality during the storage up to 45 days. However, cold storage caused external physiological disorders, 'Sanguinelli' being more sensitive than 'Tarocco Rosso' to manifest symptoms associated with low temperatures exposure. Both cultivars could be stored at temperatures between 5 and 9°C with a minimum index of alterations during a maximum period of 30 days or 45 days in case of 'Sanguinelli' and 'Tarocco Rosso', respectively.

Acknowledgements

The authors acknowledge Anecoop S. Coop. for the collaboration provided in the development of this work.

References

Butelli, E., Garcia-Lor, A., Licciardello, C., Las Casas, G., Hill, L., Recupero, G.R., Keremane, M.L., Ramadugu, C., Krueger, R., Xu, Q., Deng, X., Fanciullino, A.L., Froelicher, Y., Navarro, L., & Martin, C. (2017). Changes in anthocyanin production during domestication of Citrus. *Plant Physiology, 173 (4),* 2225–2242. https://doi.org/10.1104/pp.16.01701

Fallico, B., Ballistreri, G., Arena, E., Brighina, S., & Rapisarda, P. (2017). Bioactive compounds in blood oranges (Citrus sinensis (L.) Osbeck): level and intake. *Food Chemistry, 215,* 67–75. https://doi.org/10.1016/j.foodchem. 2016.07.142

Hamedani, M., Rabiei, V., Moradi, H., Ghanbari, A., & Azimi, M.R. (2012). Determination of storage duration and temperature effects on fruit quality parameters of blood orange (Citrus sinensis cv. Tarocco). *Biharean Biologist, 6(1),* 10–13

Ke, D., & Kader, A. (1990). Tolerance of 'Valencia' oranges to controlled atmospheres as determined by physiological responses and quality attributes. *Journal of the American Society for Horticultural Science,* 115 (5), 779–783 https://doi.org/10. 21273/JASHS.115.5.779

Lindhout, K., Treeby, M.T., & Parish, R.W. (2005). Chill out: chilling-related injuries in navel oranges. *Acta Horticulturae, 687*, 77–84 https://doi.org/10.17660/ActaHortic.2005.687.8

Lo Piero, A.R., Puglisi, I., Rapisarda, P., & Petrone, G. (2005). Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. *Journal of agricultural and food chemistry*, 53 (23), 9083–9088. https://doi.org/10.1021/jf051609s

Maccarone, E., Maccarrone, A., Perrini, G.E.A., & Rapisarda, P. (1983). Anthocyanins of the Moro orange juice. *Annali di Chimica, 73 (9–10),* 533–539.

Pannitteri, C., Continella, A., Lo Cicero, L., Gentile, A., La Malfa, S., Sperlinga, E., Napoli, E.M., Strano, T., Ruberto, G., & Siracusa, L. (2017). Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice. *Food Chemistry, 230*, 441–447. https://doi.org/10. 1016/j.foodchem.2017.03.041

Pratella, G., Tonini, G., & Cessari, A. (1969). Postharvest disease problems of Italian citrus fruit. Paper presented at: First International Citrus Symposium (University of California at Riverside).

Prior, R.L., & Wu, X. (2006). Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free radical research, 40(10),* 1014–1028. https://doi.org/10.1080/10715760600758522

Rab, A., Sajid, M., Khan, N.U., Nawab, K., Arif, M., & Khattak, M.K. (2012). Influence of storage temperature on fungal prevalence and quality of citrus fruit (cv. Blood Red). *Pakistan Journal of Botany, 44(2),* 831–836.

Rapisarda, P., Bellomo, S.E., & Intelisano, S. (2001). Storage temperature effects on blood orange fruit quality. *Journal of agricultural and food chemistry, 49(7),* 3230–3235. https://doi.org/10.1021/jf010032l

Regulation of Execution (UE) N°543/2011. June 7, 2011 (Official Journal of the European Union).

Sanfeliu, I. (2016). La Citricultura en España: Presente y Futuro (Comité de Gestió n de Cítricos). http://www.agronegocios.es/digital/files/planstar/Sanfeliu_pstar_citricos_valencia.pdf

Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., & Garau, V.L. (1998). Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. *Journal of agricultural and food chemistry,* 46(3*), 1177–1180.* https://doi.org/10.1021/jf970776s

Sdiri, S., Navarro, P., Monterde, A., Salvador, A., Cuenca, J., Aleza, P., & Ben Abda, J. (2012). Postharvest behavior of 'Garbi' and 'Safor' - new triploid mandarins. Acta Horticulturae, 945, 255–262. https://doi.org/10.17660/ActaHortic.2012. 945.34

Sdiri, S., Navarro, P., Cuenca, J., Pardo, J., & Salvador, A. (2015). Postharvest behavior of new mandarin cultivars obtained in the IVIA. *Acta Horticulturae, 1065*, 1663–1668. https://doi.org/10.17660/ActaHortic.2015.1065.213

Wang, L.S., & Stoner, G.D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269(2),* 281– 290. https://doi.org/10.1016/j.canlet.2008.05.020

IV. GENERAL DISCUSSION

GENERAL DISCUSSION

The objective of this Thesis was to evaluate the effect that the most widely used rootstocks in Mediterranean citrus production areas had on external and internal fruit quality of different varieties of commercial interest.

The proposed studies complement the research line of the Rootstock Breeding Programme that is developing at the IVIA (Spain). This programme focuses on obtaining rootstocks with good adaptability to the environment and are resistant to different biotic and abiotic factors, such as diseases, salinity, water stress or calcareous soils, among others. Nevertheless, as citrus production in Spain is intended mainly for fresh consumption, it is necessary to be able to meet current market demands to know the effect those rootstocks have on internal and external fruit quality.

The influence of rootstock on fruit quality has been widely addressed by different authors. In this Thesis, a broad review on the effect of rootstocks on main fruit quality parameters, and the factors that may cause these effects, are included in the Introduction. It is reported that rootstocks can influence physico-chemical quality parameters, as well as the nutritional profile of bioactive compounds (Castle, 1995). Rootstocks can also influence the appearance of physiological disorders in citrus fruit, which can compromise fruit commercialisation.

The influence of rootstocks on citrus quality strongly depends on the rootstock-scion interaction as the compatibility between both bud unions determines certain characteristics, such as water uptake ability, loss of fibrous roots, or the translocation of nutrients and other metabolites (Albrecht, Tripathi, Kim & Bowman, 2019). We must bear in mind that climate conditions and cultural practices can also influence fruit quality. Therefore, it is necessary to evaluate the effect of rootstock on a specific cultivar under its agronomic production conditions.

It is noteworthy that most of the studies that have addressed the effect of rootstocks on citrus fruit quality have been conducted at a single maturity point, and very few studies have considered if these effects remain throughout the season (Cardeñosa, Barros, Barreira, Arenas, Moreno-Rojas & Ferreira, 2015; Emmanouilidou & Kyriacou, 2017).

In the present Thesis, the effect of rootstocks on the fruit quality of the different studied varieties (‘Clemenules’ and ‘Tango’ mandarins, and blood oranges ‘Tarocco Rosso’ and ‘Moro’) was studied in different commercial maturity stages.

The influence of harvest time on how rootstocks affect quality parameters is clearly revealed in **Chapter I** of the present Thesis, in which the characterization of the physico-chemical and nutritional quality of ‘Clemenules’ mandarins grafted onto eight rootstocks at three commercial harvest times was conducted during two seasons. In this study, most of the evaluated quality parameters were influenced by rootstock and this effect was harvest time-dependent. One of the most remarkable results was that Forner-Alcaide 13 and C-35 citrange induced early breakthrough in color change. This is an important aspect to take into account in ‘Clemenules’ mandarins because, as in most early varieties, their fruit reach internal maturity before external full colouration. Hence they can be harvested at the beginning of the season to be submitted to ethylene degreening postharvest treatment to achieve commercial coloration (Carvalho, Salvador, Navarro, Monterde & Martínez-Jávega, 2008; Sdiri, Navarro, Monterde, Ben Abda, & Salvador, 2012). An important factor to consider in the degreening process is the time required to reach the desired fruit colour, which depends on the initial fruit colour. As the degreening treatment should be as short as possible to avoid physiological disorders (Porat, 2008), the rootstocks with advanced external fruit are a good option in the fruit that have to be degreened.

Moreover, in this study, Forner-Alcaide V17 stands out for maintaining higher acidity levels throughout the evaluated harvest, which is very useful for prolonging the commercial period and leaving fruit with high fruit quality on trees until the season ends. Loss of acidity can bring about an “empty taste” and consumers’ interest in fruit can dwindle (Jemriić & Pavičić, 2004).

In recent years, increased consumer health awareness has influenced consumer interest in eating healthier and functional food (Barba et al., 2017). This desired nutritional profile is often linked with the antioxidant functions that promote many citrus compounds, such as vitamin C, and carotenoids and phenolic compounds like flavonoids. Vitamin C is the main contributor to total antioxidant capacity in mandarin fruit (80.5%) (Putnik et al., 2017). In the present study, the high acidity levels shown by the 'Clemenules' mandarins from trees grafted onto Forner-Alcaide V17 were related to the higher citric acid concentration, and this rootstock also induced high concentrations of vitamin C, flavonoids, glucose and fructose in fruit. Carrizo citrange also led to high concentrations of sucrose and vitamin C in fruit.

One important aspect to be taken into account is that environmental factors and management systems can vary particular rootstock-related attributes (Bowman & Joubert, 2020). When introducing a new variety into a specific production area, it is important to know its behaviour in rootstocks that, given their traits, can be beneficial. The varietal group of hybrid mandarins has gradually made its way in Spanish citrus production through the commercial space left open by clementines later (De-Miguel, Caballero & Fernández-Zamudio, 2019). Of these, 'Tango' is a recent cultivar that is being introduced as an interesting mid-late season mandarin into the Mediterranean production area. Nowadays, most 'Tango' plots are located in Andalucía (South Spain), and production is expected to exponentially grow in forthcoming years in other Mediterranean areas. In this production region, Carrizo citrange is the most widespread, although Forner-Alcaide 5 is becoming important for its better tolerance to CTV, *Phytophthora*, flooding and salinity (Forner, Forner-Giner & Alcaide, 2003; Martínez-Cuenca, Primo-Capella & Forner-Giner, 2017). In this context in **Chapter II** of the present Thesis**,** the physico-chemical, sensorial and nutritional qualities of 'Tango' mandarins from the trees grafted onto Carrizo citrange or Forner-Alcaide 5 were evaluated at seven harvest times in two orchards with different characteristics. One orchard was located near the sea with sandy soil in Huelva, while the other orchard was located inland with loamy soil in Sevilla.

This study revealed that both growth location and rootstocks strongly influenced the fruit quality of 'Tango' mandarins. The Huelva fruit had higher sugars and acids than those from Seville, and in both orchards Forner-Alcaide 5 induced the maximum values of these parameters. This fact has been related to root distribution and water uptake ability. The sandy soil of Huelva led to higher fibrous roots density than the loamy soil of Seville and, therefore, to a higher water status due to the more effective water uptake and great hydraulic conductivity that induced higher sugars and acids (Barry, Castle & Davies, 2004). Regarding the effect of rootstock, the higher sugars and acids content induced by Forner-Alcaide 5, compared to Carrizo citrange, can be explained by their higher photosynthetic rates, which would cause more photoassimilate compounds, which are transported from leaves to fruit (González-Mas, Llosa, Quijano & Forner-Giner, 2009).

In this study, flavonoids were also affected by location as the fruit from Huelva had higher levels of herperidin, naruritin and didymin. Rootstocks did not affect hesperidin levels, but Forner-Alcaide 5 induced higher naruritin and didymin in fruit than Carrizo citrange. Vitamin C was also higher in the fruit from the trees grafted onto Forner-Alcaide 5.

The sensorial evaluation corroborated a rise in sugars and a drop in acids, which were observed well into the season and allowed the optimum harvest time to be established depending on the different studied conditions. So while the fruit from both studied rootstocks achieved the highest liking scores in the second half of February in the Huelva orchard, the fruit from Carrizo citrange in the Sevilla orchard exhibited the best organoleptic quality early in January, as did the fruit from Forner-Alcaide 5 at the end of January. All this reveals, once again the importance that rootstock may have on the optimal fruit harvest time.

Bearing in mind that ‘Tango’ is a variety whose postharvest behaviour is unknown under Mediterranean conditions, a study to evaluate susceptibility to chilling injury and quality changes of ‘Tango’ mandarins was conducted in the present Thesis in **Chapter III**. This study is very important because Spain is the main country to export fresh mandarins worldwide. In most marketing scenarios, the use of low temperatures is necessary to maintain fruit quality during transport and marketing. However, some citrus fruit varieties are susceptible to develop chilling injury when exposed to low temperatures. Chilling injury symptoms depend on citrus fruit species and varieties, and also on their maturity stage (Sdiri et al., 2011; Lo’ay & Dawood, 2019). Moreover, postharvest citrus quality can be influenced by preharvest biotic and abiotic factors (Arpaia, 1994; Treeby, Henriod, Bevington, Milne & Storey, 2007). Of these factors, rootstock can play an important role in the development of some physiological alterations, such as rind breakdown (Agusti, Almela, Juan, Mesejo & Martinez-Fuentes, 2003; Treeby et al., 2007), olocellosis (Zheng et al., 2011; Zheng et al., 2018), peel pitting (Alferez, Alquezar, Burns & Zacarias, 2010) or stem-end rind breakdown (Ritenour, Stover, Boman, Dou, Bowman, Castle; 2004). This has been related to rootstocks' ability to uptake water and to provide an optimal peel water status.

In this context, as in the previous study of 'Tango', the fruit grown at two locations (Huelva and Seville) and grafted onto two rootstocks (Carrizo citrange and Forner-Alcaide 5) were evaluated under different cold storage conditions.

The results revealed that 'Tango' mandarins developed chilling injury disorders, manifested as pitting lesions in the equatorial area when fruit were stored at 1°C or 5°C for 20 days. Incidence was higher as harvest advanced. No remarkable effect was found for location, but the fruit from the trees grafted onto Forner-Alcaide 5 had fewer peel disorders than the fruit from Carrizo citrange. The effect of rootstocks on different postharvest disorders has been previously reported (Cronjé, 2013; Kullaj, 2018), and has been related to its influence on the mineral nutrition, water balance and plant growth regulators that can affect water and osmotic potentials in fruit rind (Alferez et al., 2010; Magwaza, Opara, Cronjé, Landahl, Terry & Nicolaï, 2013). In this study, rind disorders were characterised by optical stereo microscope observations, and also Cryo-Sem evaluations of rind fruit were carried out. The microstructural study revealed that only the upper flavedo layer was affected in damaged fruit, in which epidermal and hypodermal tissues had dramatically collapsed. The changes noted in sugars, acids and ethanol content during storage did not compromise final fruit quality in any case, which was corroborated by the fruit sensorial evaluation. By way of conclusion, the sensitiveness of 'Tango' mandarin to manifest rind disorders when stored at 1°C or 5°C must be taken into account to commercialise this cultivar. Nevertheless, storage at 9°C did not pose a risk of internal or external fruit quality loss.

In recent years, the growing demand for blood oranges on European markets has led to some pigmented varieties of commercial interest being incorporated into the Rootstock Breeding Programme conducted at the IVIA to evaluate which rootstock confers better conditions towards fruit yield and quality. One of the main problems of blood oranges in fields is the marked fruit drop before they reach the optimum maturity stage. Consumer preferences for blood oranges stem from their high content of anthocyanin and hydroxycinnamic acids, which are related to antioxidant activity and, therefore, to healthy properties (Rapisarda, Carollo, Fallico, Tomaselli & Maccarone, 1998; Rapisarda, Bellomo & Intelisano, 2001). Although blood oranges have been traditionally consumed as juice, the citrus market is increasingly demanding these pigmented varieties for fresh consume. Anthocyanins content is determined by the genotype, but is also highly cold-dependent, and is influenced by environmental conditions and storage temperature (Cultrone, Cotroneo & Reforgiato Recupero, 2010). The Mediterranean climate is appropriate for growing blood oranges as this group of oranges needs the warm days and low night temperatures, typical of this geographical area, to acquire their dark red colour. To date most studies that have addressed the effect of rootstock on the quality of blood oranges have been carried out in Italy, where pigmented varieties are very much in demand (Continella et al., 2018). Nevertheless, information acquired under Spanish conditions is scarce, which was why the effect of rootstock on the fruit quality of two of the main blood orange varieties, 'Moro' and 'Tarocco Rosso', was studied in the present thesis (**Chapter IV**). Besides evaluating changes in the main fruit quality attributes at three harvest times, an in-depth study into anthocyanins, hydroxycinnamic acids, sugars and flavanones composition was conducted.

The result revealed major differences between both studied varieties. In 'Moro', internal quality parameters were strongly influenced by harvest time. Flavonoids, hydroxycinnamic acids and rose as harvest advanced. This effect was not as evident for 'Tarocco Rosso'.

In both cultivars, one major effect of rootstock was found on most of the evaluated parameters. Of the eight studied rootstocks, Forner-Alcaide 5 and Forner-Alcaide 13 in 'Tarocco Rosso' were those that induced the highest content of sugars and anthocyanins. It is worth observing the relation between sugar and anthocyanin accumulation as it is known that blood oranges need a higher sugar metabolism to provide the carbon skeleton for anthocyanin biosynthesis (Muccilli et al., 2009). Although this relation was not as evident in the 'Moro' cultivar, the rootstocks that induced the lowest anthocyanin content ('Swingle' citrumelo and Volkameriana) also led to fruit containing less sucrose.

In the present study, degradation of anthocyanins, specifically of cyanidin 3-(6"-malonyl)-glucoside and cyanidin 3-(6"-dioxalyl)-glucoside, at the last harvest time took place in 'Moro', and was related as a response of increased temperature as previously reported (Steyn, 2008). This anthocyanin degradation was concomitant with the synthesis of hydroxycinnamic acids and flavanones, which has been explained by the general phenylpropanoid pathway. In 'Tarocco Rosso', no anthocyanin degradation was observed, which suggests that this cultivar is less sensitive to environmental changes.

It is noteworthy that another quality parameter that can limit the commercialisation of blood oranges is firmness as pigmented varieties present less firmness than blond oranges. This parameter can compromise fruit quality, especially when fruit are stored for a long time or are submitted to long-term post-harvest shipping (Pallottino, Menessatti, Lanza, Strano, Antonucci & Moresi, 2013). In the present study, 'Swingle' citrumelo and C-35 citrange in 'Moro' and 'Swingle' citrumelo, Forner-Alcaide 13 and Carrizo citrange in 'Tarocco Rosso', were the rootstocks that induced the most fruit firmness.

Given the importance of blood oranges in today's citrus market, the effect of cold storage on the fruit quality of 'Tarocco Rosso' and 'Sanguinelli' was evaluated in **Chapter V** in this thesis. This study was carried out during two seasons. However, in this case, a study about the effect of rootstock could not be conducted because fruit were unavailable in the different rootstocks.

The results revealed that none of the studied temperatures (1°C, 5°C or 9°C) negatively affected internal fruit quality during storage times of up to 45 days. Even external fruit colour increased when fruit were exposed to 5°C and 9°C, and the external aspect improved. This effect coincides with previous studies, which established that temperatures between 4°C and 10°C promoted anthocyanin synthesis (Lo Piero, Puglisi, Rapisarda & Petrone, 2005; Butelli et al., 2012). Storage at 1°C did not suffice to activate the anthocyanin pathway.

Cold storage caused external physiological disorders in ‘Sanguinelli’ to be more sensitive than ‘Tarocco Rosso’ to develop symptoms related to low temperature exposure. The lower the storage temperature, the higher the incidence of disorders became. This can be linked with the increased colour exhibited at 5°C and 9°C because it has been reported that an increase in anthocyanin content in response to cold stress could be one of the mechanisms by which plant cells can ameliorate osmotic control and enhance chilling tolerance (Chalker-Scott, 1999). Therefore, the fruit from both cultivars could be stored at temperatures between 5°C and 9°C with a minimum risk of alterations for a 30-day period for ‘Sanguinelli’ and for 45 days for ‘Tarocco Rosso’. Future research is necessary to investigate the influence of rootstock on sensitivity to low temperatures in pigmented varieties.

References

Albrecht, U., Tripathi, I., Kim, H., & Bowman, K.D. (2019). Rootstock effects on metabolite composition in leaves and roots of young navel orange (Citrus sinensis L. Osbeck) and pummelo (C. grandis L. Osbeck) trees. Trees, 33(1), 243-265. https://doi.org/10.1007/s00468-018-1773-1

Alferez, F., Alquezar, B., Burns, J.K., & Zacarias, L. (2010). Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology, 56(1), 44-49. https://doi.org/10.1016/j.postharvbio.2009.12.007.

Agusti, M., Almela, V., Juan, M., Mesejo, C., & Martinez-Fuentes, A. (2003). Rootstock influence on the incidence of rind breakdown in 'Navelate'sweet orange. The journal of horticultural science and biotechnology, 78(4), 554-558. https://doi.org/10.1080/14620316.2003.11511662

Arpaia M.L. (1994). Preharvest factors influencing postharvest quality of tropical and subtropical fruit. HorScience, 29(9), 982–985.

Barba, F.J., Putnik, P., Bursac Kovacevic, D., Poojary, M.M., Roohinejad, S., Lorenzo, J.M., Koubaa, M. (2017). Impact of conventional and non-conventional processing on prickly pear (Opuntia spp.) and their derived products: From preservation of beverages to valorization of by-products. Trends in Food Science & Technologies, 67, 260–70. https://doi.org/10.1016/j.tifs.2017.07.012

Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. Journal of the American Society for Horticultural Science, 129(6), 881–889. https://doi.org/10.21273/JASHS.129.6.0881

Bowman, K.D., & Joubert, J. (2020). Citrus rootstocks. In The Genus Citrus, 1st ed.; Talón, M., Caruso, M., Gmitter, F.G., Eds.; Woodhead Publishing: Duxford, United Kingdom, 105-127. https://doi.org/10.1016/B978-0-12-812163-4.00006-1

Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P. Reforgiato Recupero, G., & Martin, C. (2012). Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell, 24(3), 1242-1255. https://doi.org/10.1105/tpc.111.095232

Cardeñosa, V., Barros, L., Barreira, J.C., Arenas, F., Moreno-Rojas, J.M., Ferreira, I.C. (2015). Different citrus rootstocks present high dissimilarities in their antioxidant activity and vitamins content according to the ripening stage. Journal of Plant Physiology, 174, 124–130. https://doi.org/10.1016/j.jplph.2014.10.013

Carvalho, C.P., Salvador, A., Navarro, P., Monterde, A., & Martínez-Jávega, J.M. (2008). Effect of auxin treatments on calyx senescence in the degreening of four mandarin cultivars. HortScience, 43(3), 747-752. https://doi.org/10.21273/HORTSCI.43.3.747

Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. New Zealand Journal of Crop and Horticultural Science, 23(4), 383-394. https://doi.org/10.1080/01140671.1995.9513914

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. Photochemistry and photobiology, 70(1), 1-9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

Continella, A., Pannitteri, C., La Malfa, S., Legua, P., Distefano, G., Nicolosi, E., & Gentile, A. (2018). Influence of different rootstocks on yield precocity and fruit quality of ‘Tarocco Scirè’ pigmented sweet orange. Scientia horticulturae, 230, 62-67. https://doi.org/10.1016/j.scienta.2017.11.006

Cronjé P.J.R. (2013). Postharvest rind disorders of 'Nadorcott' mandarin are affected by rootstock in addition to postharvest treatments. Acta Horticulturae, 1007, 111–117. https://doi.org/10.17660/ActaHortic.2013.1007.9

Cultrone, A., Cotroneo, P.S., & Reforgiato Recupero, G. (2010). Cloning and molecular characterization of R2R2-MYB and bHLH-MYC transcription factors from Citrus sinensis. Tree Genetics & Genomes, 6, 101–112. https://doi.org/10.1007/s11295-009-0232-y

De-Miguel, M.D., Caballero, P., & Fernández-Zamudio, M.A. (2019). Varietal Change Dominates Adoption of Technology in Spanish Citrus Production. Agronomy, 9(10), 631-645. https://doi.org/10.3390/agronomy9100631

Emmanouilidou, M.G., & Kyriacou, M.C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of 'Lane Late' and 'Delta' sweet orange. Scientia horticulturae, 225, 112-121. https://doi.org/10.1016/j.scienta.2017.06.056

Forner, J.B., Forner-Giner, M., & Alcaide, A. (2003). Forner-Alcaide 5 and Forner-Alcaide 13: two new citrus rootstocks released in Spain. HortScience, 38(4), 629-630. https://doi.org/10.21273/HORTSCI.38.4.629

González-Mas, M.C., Llosa, M.J., Quijano, A., & Forner-Giner, M.A. (2009). Rootstock effects on leaf photosynthesis in 'Navelina' trees grown in calcareous soil. HortScience, 44(2), 280–283. https://doi.org/10.21273/HORTSCI.44.2.280

Jemriić, T., & Pavičić, N. (2004). Postharvest treatments of Satsuma mandarin (Citrus unshiu Marc.) for the improvement of storage life and quality. In Production practices and quality assessment of food crops, 1st ed.; Dris, R., & Jain, S.M., Eds.; Springer, Dordrecht, Helsinki, Finland, 213-227. https://doi.org/10.1007/1-4020-2535-1_8

Kullaj, E. (2018). Rootstocks for improved postharvest quality of fruits: recent advances. In Preharvest Modulation of Postharvest Fruit and Vegetable Quality, 1st ed.; Siddiqui M.W., Eds.; Elsevier Inc. Academic Press, London. United Kingdom, 189–207. https://doi.org/10.1016/B978-0-12-809807-3.00008-1

Lo'ay, A.A., & Dawood, H.D. (2019). Chilling injury, fruit color maturity stages, and antioxidant enzyme activities of lemon 'baladi CV' fruits under cold storage stress. Scientia Horticulturae, 257, 108676. https://doi.org/10.1016/j.scienta.2019.108676

Lo Piero, A.R., Puglisi, I., Rapisarda, P., & Petrone, G. (2005). Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. Journal of agricultural and food chemistry, 53(23), 9083-9088. https://doi.org/10.1021/jf051609s

Magwaza, L.S., Opara, U.L., Cronjé, P.J., Landahl, S., Terry, L.A., & Nicolaï, B.M. (2013). Non-chilling physiological rind disorders in citrus fruit. In Horticultural Reviews, vol. 41, 1st Ed.; Janick J., Eds; John Wiley & Sons, Willey-Blackwell, New York, NY, 131-176. https://doi.org/10.1002/9781118707418.ch03

Martínez-Cuenca, M.R., Primo-Capella, A., & Forner-Giner, M.A. (2017). Tolerance Response Mechanisms to Iron Deficiency Stress in Citrus Plants. In Stress Signaling in Plants: Genomics and Proteomics Perspective, Vol. 2, 1st Ed.; Sarwat, M., Ahmad, A., Abdin, M.Z., & Ibrahim, M.M., Eds; Springer, Cham. Switzerland. 201-239. https://doi.org/10.1007/978-3-319-42183-4_9.

Muccilli, V., Licciardello, C., Fontanini, D., Russo, M. P., Cunsolo, V., Saletti, R., Reforgiato Recupero G & Foti, S. (2009). Proteome analysis of Citrus sinensis L. (Osbeck) flesh at ripening time. Journal of Proteomics, 73(1), 134-152. https://doi.org/10.1016/j.jprot.2009.09.005

Pallottino, F., Menesatti, P., Lanza, M. C., Strano, M. C., Antonucci, F., & Moresi, M. (2013). Assessment of quality-assured Tarocco orange fruit sorting rules by combined physicochemical and sensory testing. Journal of the Science of Food and Agriculture, 93(5), 1176-1183. https://doi.org/10.1002/jsfa.5871

Porat, R. (2008). Degreening of citrus fruit. Tree and Forestry Science and Biotechnology, 2(1), 71-76.

Putnik, P., Barba, F.J., Lorenzo, J.M., Gabrić, D., Shpigelman, A., Cravotto, G., & Bursać Kovačević, D. (2017). An integrated approach to mandarin processing: Food safety and nutritional quality, consumer preference, and nutrient bioaccessibility. Comprehensive reviews in food science and food safety, 16(6), 1345-1358. https://doi.org/10.1111/1541-4337.12310

Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., & Maccarone, E. (1998). Hydroxycinnamic acids as markers of Italian blood orange juices. Journal of Agricultural and Food Chemistry, 46(2), 464-470. https://doi.org/10.1021/jf9603700

Rapisarda, P., Bellomo, S.E., & Intelisano, S. (2001). Storage temperature effects on blood orange fruit quality. Journal of Agricultural and Food Chemistry, 49(7), 3230–3235. https://doi.org/10.1021/jf010032l

Ritenour, M.A., Stover, E., Boman, B.J., Dou, H., Bowman, K.D., & Castle, W.S. (2004). Effect of rootstock on stem-end rind breakdown and decay of fresh citrus. HortTechnology, 14(3), 315-319. https://doi.org/10.1016/j.scienta.2018.04.058

Sdiri, S., Navarro, P., Monterde, A., Salvador, A., Cuenca, J., Aleza, P., & Ben Abda, J. (2011). Postharvest behavior of 'Garbi' and 'Safor' new triploid mandarins. In IV International Conference Postharvest Unlimited 2011 945, 255-262. https://doi.org/10.17660/ActaHortic.2012.945.34

Sdiri, S., Navarro, P., Monterde, A., Ben Abda, J., & Salvador, A. (2012). New degreening treatments to improve the quality of citrus fruit combining different periods with and without ethylene exposure. Postharvest Biology and Technology, 63(1), 25-32.
https://doi.org/10.1016/j.postharvbio.2011.08.005

Steyn, W.J. (2008). Prevalence and functions of anthocyanins in fruits. In Anthocyanins, 1st ed.; Winefiel, C., Davies, K., Gould, K., Eds.; Springer, New York, NY, 86-105.
https://doi.org/10.1007/978-0-387-77335-3_4

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., & Storey, R. (2007). Irrigation management and rootstock effects on navel orange [Citrus sinensis (L.) Osbeck] fruit quality. Agricultural Water Management, 91(1-3), 24-32.
https://doi.org/10.1016/j.agwat.2007.04.002

Zheng, Y., Deng, L., He, S., Zhou, Z., Yi, S., Zhao, X., & Wang, L. (2011). Rootstocks influence fruit oleocellosis in ‘Hamlin’ sweet orange (Citrus sinensis (L.) Osbeck). Scientia horticulturae, 128(2), 108-114.
https://doi.org/10.1016/j.scienta.2011.01.005

Zheng, Y., Wang, Y., Yang, Q., Liu, Y., Xie, R., He, S., Deng, L., Yi, S.; Lv, Q; & Ma, Y. (2018). Modulation of tolerance of “Hamlin” sweet orange grown on three rootstocks to on-tree oleocellosis by summer plant water balance supply. Scientia Horticulturae, 238, 155-162.
https://doi.org/10.1016/j.scienta.2018.04.058

V. GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

1. Rootstock effect on ‘Clemenules’ mandarin

The changes in the main physico-chemical and nutritional quality during the harvest time of the ‘Clemenules’ mandarins grafted onto eight different rootstocks was reported in two seasons.

Forner-Alcaide 13 and C-35 Citrange induced early breakthrough in color change. This is an important aspect to take into account in ‘Clemenules’ mandarins as the fruit from both rootstocks can be harvested earlier in the season and be submitted to a shorter ethylene degreening postharvest treatment. The fruit from these rootstocks also stood out for their higher firmness values.

Forner-Alcaide V17 stands out for undergoing the greatest color increase and maintaining higher acidity levels throughout the season.

The highest contents of vitamin C, flavonoids, glucose and fructose were found in the fruit from the trees grafted onto Forner-Alcaide V17. Carrizo Citrange also led to high concentrations of sucrose and vitamin C in fruit.

2. Rootstock effect on ‘Tango’ mandarin

The physico-chemical, sensorial and nutritional quality of ‘Tango’ mandarins from the trees grafted onto Carrizo Citrange or Forner-Alcaide 5 at seven harvest times in two orchards with different characteristics (Seville and Huelva) was provided.

During the harvest period, fruit quality was strongly influenced by location and by the rootstock onto the cultivar was grafted.

The fruit from Huelva had higher sugars and acids than those from Seville, which was related to the higher sandy soil percentage in the Huelva orchard.

At both evaluated locations, Forner-Alcaide 5 induced higher sugar and acid content compared to Carrizo Citrange, which was associated with the higher photosynthetic rate reported for Forner-Alcaide 5.

The nutritional profile of ‘Tango’ mandarins was determined for the first time, which was influenced by rootstock. In both orchards, Forner-Alcaide 5 induced higher sucrose and vitamin C levels. Whereas rootstocks did not affect hesperidin levels, Forner-Alcaide 5 induced higher naruritin and didymin contents than Carrizo Citrange, but this effect was found only in Huelva. Regarding organic acids, while higher citric acid was observed in fruit from Forner-Alcaide 5 in both orchards, no robust effect on malic and succinic acid contents was found for rootstock.

The sensorial evaluation allowed us to establish the optimal harvest time for this cultivar under each studied condition. In the Seville orchard, the fruit from Carrizo Citrange exhibited the best organoleptic quality early in January, while the fruit from Forner-Alcaide 5 did so at the end of January. Nevertheless, in Huelva orchard, regardless of the rootstock onto trees was grafted, fruit achieved maximum sensorial quality in the second half of February.

The studies that addressed the postharvest behavior of ‘Tango’ mandarins in two plots revealed that this cultivar exhibited chilling injury symptoms when stored at 1°C or 5°C for 20 days onward. Regarding the rootstock effect, the fruit from the trees grafted onto Forner-Alcaide 5 had less peel disorder incidence than the fruit from Carrizo Citrange. Cold storage did not compromise final fruit quality, as corroborated by the fruit sensorial evaluation.

The rind disorders developed by ‘Tango’ mandarin at low temperature were characterized by optical stereo microscope and Cryo-Sem. This study revealed that only the upper flavedo layer was affected in damaged fruit, whose epidermal and hypodermal tissues had dramatically collapsed.

Storage at 9°C for up 30 days did not affect the internal and external commercial quality of ‘Tango’ mandarins.

3. Rootstock effect on blood oranges

Changes in physico-chemical and nutritional quality of ‘Moro’ and ‘Tarocco Rosso’ blood oranges grafted onto eight rootstocks during harvest season was provided.

In ‘Moro’, the internal quality parameters were strongly influenced by harvest time, while this effect was no as evident for ‘Tarocco Rosso’. In both cultivars, rootstock had a very strong effect.

The higher juice color of ‘Moro’ compared to ‘Tarocco Rosso’ was explained by the higher content of all the determined individual anthocyanins. Moreover, a reduction in internal color was observed in ‘Moro’ as harvest advanced, which was related to the degradation of cyanidin 3-(6”-malonyl)-glucoside and cyanidin 3-(6”-dioxalyl)-glucoside as a response to the increment in temperature during the season. The most sensitive rootstocks to temperature changes were C-35 Citrange, Macrophylla and Volkameriana. Anthocyanin degradation was concomitant with the synthesis of hydroxycinnamic acids and flavanones, which has been explained by the general phenylpropanoid pathway. In this cultivar, the rootstocks with the lowest juice color and, therefore, the lower content of all anthocyanins, were Macrophylla, ‘Swingle’ Citrumelo and Volkameriana.

In ‘Tarocco Rosso’, no relevant effect of harvest was found on juice color and there was no evidence for anthocyanin degradation, which indicates that this cultivar is less sensitive to environmental changes. Nor were there any relevant changes in the contents of hydroxycinnamic acids and flavanones throughout the season. C-35 Citrange, Macrophylla and ‘Swingle’ Citrumelo induced the smallest content of anthocyanins and sugars, while the highest content was found in the fruit from Forner-Alcaide

5 and Forner-Alcaide 13. C-35 Citrange and Forner-Alcaide 13 were characterized by their low flavonoid contents.

The effect of cold storage on the fruit quality of ‘Tarocco Rosso’ and ‘Sanguinelli’ was studied during two seasons. Storage at 1°C, 5°C and 9°C for 45 days did not compromise the internal quality of both blood orange cultivars. Nevertheless, storage at 1°C caused chilling injury disorders, and ‘Sanguinelli’ was more sensitive than ‘Tarocco Rosso’. Both blood orange cultivars could be stored between 5°C and 9°C with a low risk of disorders being manifested for 30 days for ‘Sanguinelli’ and up to 45 days with ‘Tarocco Rosso’.