

## Overall Fruit Quality of 'Lane Late' Orange on Sub-Standard and Semi-Dwarfing Rootstocks

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### ABSTRACT

Appearance, taste, and bioactive compounds of 'Lane Late' orange fruits on three sub-standard or semi-dwarfing rootstocks [Forner-Alcaide no. 5, Forner-Alcaide no. 13 and Forner-Alcaide no. 41] were evaluated in Spain against three more traditional rootstocks [Carrizo citrange, *Citrus macrophylla* and 'Cleopatra' mandarin]. Different harvesting times were identified per rootstock. The most suitable harvesting time for 'Lane Late' orange fruits on 'Cleopatra' mandarin, Forner-Alcaide no. 13 or Forner-Alcaide no. 5 was March, between one and two months later than *Citrus macrophylla*, Carrizo citrange or Forner-Alcaide no. 41, more relevant date for this late-season navel orange. Despite harvesting 'Lane Late' orange fruits on the most suitable date for each rootstock, significant differences in overall fruit quality were observed among them. Thus, *Citrus macrophylla* induced the largest size but also the lowest organoleptic quality, Carrizo citrange induced the lowest bioactive compounds content, while Forner-Alcaide no. 5, Forner-Alcaide no. 13 and 'Cleopatra' mandarin induced the highest internal quality. Lane Late orange fruits on Forner-Alcaide no. 41 showed an intermediate behavior.

**Keywords:** Harvesting time, Ripening index, Traditional rootstocks, Physiological disorders, Polyphenols content

### INTRODUCTION

Spain, the sixth producer of citrus fruits worldwide and the leading exporter of fresh citrus fruits, encompasses 294,258 hectares of citrus crop. Sweet orange [*Citrus sinensis* (L.) Osb] is the most important citrus species, for which the most widely cultivated cultivars in Spain belong to the Navel group (MAPAMA, 2017).

Most navel oranges fruits in Spain are used for fresh consumption (MAPAMA, 2017), thus, both the external and organoleptic fruit quality are of great importance. In addition, considering the current healthier lifestyle, the fruit's health promoting properties should be taken into account. Flavonoids, group of polyphenolic

compounds, appear to be associated with a lower risk of major diseases (Gattuso *et al.*, 2007).

Citrus fruits overall quality not only depends on the species or cultivar (Gorinnstein *et al.*, 2001; Cardenosa *et al.*, 2015), but culture conditions (Hervalejo *et al.*, 2007; Navarro *et al.*, 2010), rootstock (Forner-Giner *et al.*, 2003; Hervalejo *et al.*, 2010; Legua *et al.*, 2014) or fruit ripening stage (Xu *et al.*, 2008) may also have a major impact.

In Spain, Carrizo citrange is the main rootstock used in citrus, over 80% of nursery production (Forner-Giner *et al.*, 2003). Therefore, new citrus rootstocks are necessary not only to adapt the citrus crop to different environmental conditions but to

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improve the agronomical behavior of the grafted citrus cultivar or reduce the production cost (reduced vegetative growth rootstocks with less pruning needs and easier harvesting).

In this sense, in 1974, J.B. Forner began a program for breeding citrus rootstocks by traditional hybridization at the Instituto Valenciano de Investigaciones Agrarias (IVIA) in Spain (Forner and Alcaide, 1993, 1994; Forner-Giner *et al.*, 2003). New interesting citrus rootstocks to reduce tree size, such as Forner-Alcaide no. 5, Forner-Alcaide no. 13 and Forner-Alcaide no. 41, emerged from this program (Forner *et al.*, 2003).

In this context, this work aimed to evaluate the overall fruit quality of 'Lane Late' orange (appearance, taste and bioactive compounds) grafted on six rootstocks according to ripening stage, over five consecutive seasons. The study rootstocks included three sub-standard or semi-dwarfing rootstocks, and the other three were more traditional. This study discusses the most appropriate harvesting time of 'Lane Late' orange for each citrus rootstocks.

## MATERIALS AND METHODS

### Plant Material and Experimental Design

The study was carried out throughout five seasons (2009/2010-2013/2014) in an experimental plot in Sevilla (Spain) with Mediterranean climate, 18.1 °C average annual temperature, 586 mm annual rainfall, and 1292 mm reference evapotranspiration ( $ET_0$ ), Loamy soil texture (25% clay, 32% sand and 43% silt), with organic matter level around 1.0%, 0.12 dS/m electrical conductivity of a 1:5 soil water extract, 4.8% active  $CaCO_3$  and pH of 8.0.

Standard cultural practices were applied, including drip irrigation, chemical weed-control and hand-pruned annually after harvest. Irrigation was applied by two drip-

lines for each tree row, with 2.20 L/h drippers every 60 cm. Seasonal water requirements were calculated based on reference evapotranspiration ( $ET_0$ ) and citrus crop coefficient ( $K_c$ ) (Doorenbos and Pruitt, 1977).

The experimental plot consisted of 4800 m<sup>2</sup> with 'Lane Late' orange trees on *Citrus macrophylla* Wester, Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.], 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tan.) and three hybrid selections, sub-standard or semi-dwarfing rootstocks: Forner-Alcaide no. 5 [*Citrus reshni* Hort. ex Tan. x *Poncirus trifoliata* (L.) Raf.], Forner-Alcaide no. 13 [*Citrus reshni* Hort. ex Tan. x *Poncirus trifoliata* (L.) Raf.] and Forner-Alcaide no. 41 [*Citrus reshni* Hort. ex Tan. x *Poncirus trifoliata* (L.) Raf.].

The 'Lane Late' orange trees were 6 years old at the beginning of the experiment. Tree spacing was 6 m x 4 m. The design consisted of 4 randomized blocks, including a primary plot with 6 trees (N=24).

### Samples

Three fruit samplings were conducted from January to April to establish the effect of the ripening stage on quality parameters at the beginning (January) and the end (April) of the 'Lane Late' orange harvesting period, taking March as an intermediate date. Samples consisting of 25 fruits were collected from each rootstock and replication. Morphological and organoleptic quality parameters were measured during five seasons (2009/2010-2013/2014). Bioactive compounds determination was performed during 2012/2013 and 2013/2014.

### Morphological Parameters

Fruit morphological parameters evaluated were color index, weight (g), equatorial diameter (D, mm), height (H, mm), shape (D/H) and peel thickness (mm).

Color index was measured with a colorimeter (Konica Minolta, CR-300,

Ramsey, NJ, USA) at three points around the fruit equatorial plane. Using Hunter parameters, Color index (C.I.) was calculated as  $C.I. = a \times 100 / (L \times b)$ , where L indicates lightness and a and b are chromaticity coordinates (Jiménez-Cuesta *et al.*, 1981).

Samples were weighed using a digital scale, and fruit weight was determined by dividing fruit sample weight (g) by the total number of fruits in the sample.

Equatorial diameter (D), height (H) and peel thickness were determined using an electronic digital slide gauge (Mitutoyo, Absolute digimatic caliper, East Kilbride, UK).

### Organoleptic Parameters

The juice parameters analyzed were juice content %, density (g/mL), titrable acidity (TA; g/100 mL), total soluble solids (TSS, °Brix) and ripening index ( $RI = TSS/TA$ ).

The juice from each fruit sample was extracted using an electric squeezer, and juice percentage (w/w) was calculated by the juice weight over fruit sample weight ratio.

Juice density was determined by a Hydrometer 1000-1100 calibrated at 20 °C (Nahita, Madrid, Spain). The hydrometer was inserted into a 100 mL test tube full to overflowing with juice, being the density (g/cc) the reading of the point where the surface of the juice touches the stem of the hydrometer. Before measuring density, the juice was tempered at the reference temperature indicated in the hydrometer (20 °C).

TSS was measured using a digital refractometer (Atago, PR100, Bellevue, WA, USA) and TA was determined by titration of 5 mL of juice with 0.1 N NaOH using phenolphthalein as indicator (Forner-Giner *et al.*, 2003; Legua *et al.*, 2011a, b).

### Physiological Disorders and Inner Firmness

The inner firmness and the degree of fruit creasing and granulation were evaluated.

Inner firmness was measured as the maximum penetration force (N) reached during tissue breakage and was determined with a 5 mm diameter flat probe. Penetration depth was 5 mm and the cross-head speed was 40 mm/s using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK). 'Lane Late' orange fruits were peeled and firmness was measured in the equatorial zone.

The degree of creasing and granulation were determined using a 4-point rating scale: 0 = no disorder in the fruit, 1 = mild disorder, 2 = moderate disorder, 3 = severe disorder.

### Total Polyphenolic and Total Flavonoid Content

Fresh 'Lane Late' orange fruit (1 g) was extracted with 3 mL dimethyl sulfoxide:methanol (50:50 v/v). The mixtures were vortexed for 2 minutes and, later, centrifuged at 5000 rpm for 15 minutes at 4 °C. Supernatants were transferred to vials, stored at -80 °C, and subsequently used to analyze total phenolics and flavonoids.

Total phenolics were determined with Folin-Ciocalteu reagent using the Slinkard and Singleton (1977) method. An aliquot of the extract (40 µL) was mixed with 1.9 mL of distilled water and subsequently with 120 µL of Folin-Ciocalteu reagent. After 3 minutes, 400 µL of a 20% aqueous sodium carbonate solution was added. Samples were allowed to stand for 2 hours and subsequently read at 765 nm with a spectrophotometer (Shimadzu UV 2401 PC, Columbia, MD, USA) and compared with a known concentration range of similarly prepared gallic acid standards. Results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight of orange fruits (mg EG/100 g FW).

Total flavonoids were determined by using a colorimetric method previously described by Dewanto *et al.* (2002). An aliquot of the previously diluted extract (250 µL) was

mixed with 1.5 mL of distilled water and subsequently with 75  $\mu$ L of a 5% NaNO<sub>2</sub> solution. After 6 minutes, 150  $\mu$ L of a 10% AlCl<sub>3</sub>-6H<sub>2</sub>O solution was added and allowed to stand for 5 minutes before further addition of 0.5 mL of 1 M NaOH. Samples were subsequently read 30 minutes at 415 nm against a prepared blank using a spectrophotometer (Shimadzu UV 2401 PC, Columbia, MD, USA) and compared with a known concentration range of quercetin standards similarly prepared. Results are expressed as micrograms of quercetin equivalents per gram of fresh weight of orange fruits ( $\mu$ g EQ/g FW).

### Statistical Analysis

Data were statistically analyzed using variance analysis (ANOVA) procedures. Means were separated through Tukey's multiple range test, using the STATISTICA 7.0 software package (Statsoft Inc. 2004). Normality and homogeneity assumptions were tested before ANOVA, using the Kolmogorov-Smirnov and Cochran's tests, respectively. In the case of non-observance of the normality and homogeneity assumptions, a nonparametric Kruskal-Wallis test was adopted.

## RESULTS AND DISCUSSION

In this study, both ripening stage and rootstock affected the overall fruit quality: appearance, taste, and bioactive compounds. Thus, this effect needs to be established in order to properly identify the most suitable fruit harvesting time per rootstock and establish the best overall 'Lane Late' orange fruit quality.

### Suitable Harvesting Time of 'Lane Late' Orange Fruits per Rootstock

All rootstocks showed a similar trend throughout the different sampling dates in

terms of morphological (Table 1) and bioactive compounds (Table 4), but not fruit organoleptic quality (Table 2) or physiological alterations (Table 3).

### Morphological Parameters

Fruit size increased with fruit ripening (Table 1). These results are in line with those reported by Fattahi *et al.* (2011) and Merino *et al.* (2015). Thus, April might be more interesting in terms of obtaining larger fruits. Yet, by January, 'Lane Late' orange fruit on *Citrus macrophylla* had already reached significant diameter and weight (84.89 mm and 305 g), surpassed only by Carrizo citrange (85.00 mm and 321 g, respectively) in April (Table 1). The evolution towards a more elongated fruit and thicker peel (Table 1) does not have a major impact on fruit quality in itself. However, extremes in peel thickness are not desirable. Fruits with a thick peel are rougher fruits, whereas those of thin peel are more sensitive to peel disorders during postharvest handling and storage. Despite the fact that 'Lane Late' orange fruit partially reverted to a greener peel color in April (lower CI values; Table 1), which is known as 'regreening', 'Lane Late' orange fruit color index recorded in April was similar to the one obtained in January.

### Organoleptic Parameters

Ripening index, the total soluble solids to acid ratio, is the basis for establishing commercial maturity (R (EU) no. 543/2011 of European Commission, of 7 June 2011) and fruit taste. In this respect, the "Pritchett tongue" (Baier, 1954) associated juice taste with the relationship between total soluble solids and the ripening index (RI). A minimum RI of 6.5 is required for commercial maturing of navel oranges fruits, while a RI greater than 16 requires a minimum total soluble solids content of 12 °Brix to preserve an acceptable organoleptic

**Table 1.** Effect of rootstock and sampling date on morphological 'Lane Late' orange fruit quality.<sup>a</sup>

Rootstock	D(mm) <sup>a</sup>												H (mm)												D/H																							
	January			March			April			Mean			January			March			April			Mean			January			March			April			Mean														
	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr												
CA	83.31	cd	83.81	b	85.00	bc	84.04	c	83.05	cd	85.17	bc	86.70	cd	84.97	cd	1.01	a	0.99	a	0.98	abc	0.99	a	83.05	cd	85.17	bc	86.70	cd	84.97	cd	1.01	a	0.99	a	0.98	abc	0.99	a	1.01	a	0.99	a	0.98	abc	0.99	a
CL	78.99	ab	80.78	a	81.27	a	80.43	ab	79.05	ab	82.58	ab	84.08	ab	82.04	ab	1.00	a	0.98	a	0.97	a	0.98	a	79.05	ab	82.58	ab	84.08	ab	82.04	ab	1.00	a	0.98	a	0.97	a	0.98	a	1.00	a	0.98	a	0.97	a	0.98	a
FA13	77.84	a	80.21	a	82.01	ab	80.06	a	76.81	a	80.73	a	83.01	a	80.19	a	1.02	a	1.00	a	0.99	bc	1.00	a	76.81	a	80.73	a	83.01	a	80.19	a	1.02	a	1.00	a	0.99	bc	1.00	a	1.02	a	1.00	a	0.99	bc	1.00	a
FA41	80.92	bc	81.90	ab	83.62	ab	82.20	bc	80.84	bc	83.65	ab	85.99	abc	83.49	bc	1.00	a	0.98	a	0.98	ab	0.99	a	80.84	bc	83.65	ab	85.99	abc	83.49	bc	1.00	a	0.98	a	0.98	ab	0.99	a	1.00	a	0.98	a	0.98	ab	0.99	a
FA5	80.97	bc	82.71	ab	83.31	ab	82.33	bc	80.49	bc	83.31	ab	85.42	abc	83.07	bc	1.01	a	1.00	a	0.98	abc	0.99	a	80.49	bc	83.31	ab	85.42	abc	83.07	bc	1.01	a	1.00	a	0.98	abc	0.99	a	1.01	a	1.00	a	0.98	abc	0.99	a
MP	84.89	d	87.27	c	87.70	c	86.62	d	84.27	d	88.60	c	88.63	d	87.16	d	1.01	a	0.99	a	0.99	c	1.00	a	84.27	d	88.60	c	88.63	d	87.16	d	1.01	a	0.99	a	0.99	c	1.00	a	1.01	a	0.99	a	0.99	c	1.00	a
Mean	81.15	A	82.78	B	83.82	C	82.61	ns	80.75	A	84.01	B	85.64	C	83.49	ns	1.01	B	0.99	A	0.98	A	0.99	ns	80.75	A	84.01	B	85.64	C	83.49	ns	1.01	B	0.99	A	0.98	A	0.99	ns	1.01	B	0.99	A	0.98	A	0.99	ns
Rootstock*Date	ns																																															
Rootstock	Tp (mm)												CI												W (g)																							
	January			March			April			Mean			January			March			April			Mean			January			March			April			Mean														
	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr									
CA	5.53	a	5.91	a	6.12	ab	5.85	b	12.31	a	13.62	a	12.30	a	12.74	a	294	cd	297	ab	321	b	304	bc	12.31	a	13.62	a	12.30	a	12.74	a	294	cd	297	ab	321	b	304	bc	294	cd	297	ab	321	b	304	bc
CL	5.37	a	5.75	a	5.80	a	5.64	ab	12.13	a	13.65	a	12.31	a	12.70	a	250	ab	274	a	281	a	270	a	12.13	a	13.65	a	12.31	a	12.70	a	250	ab	274	a	281	a	270	a	250	ab	274	a	281	a	270	a
FA13	5.26	a	5.49	a	5.98	a	5.58	ab	11.37	a	12.48	a	12.47	a	12.11	a	244	a	267	a	286	a	266	a	11.37	a	12.48	a	12.47	a	12.11	a	244	a	267	a	286	a	266	a	244	a	267	a	286	a	266	a
FA41	5.55	a	5.83	a	6.31	ab	5.90	b	11.95	a	12.47	a	11.93	a	12.12	a	274	bc	287	a	301	ab	287	ab	11.95	a	12.47	a	11.93	a	12.12	a	274	bc	287	a	301	ab	287	ab	274	bc	287	a	301	ab	287	ab
FA5	5.21	a	5.60	a	5.50	a	5.44	a	12.17	a	13.65	a	11.97	a	12.60	a	276	c	290	a	297	ab	288	ab	12.17	a	13.65	a	11.97	a	12.60	a	276	c	290	a	297	ab	288	ab	276	c	290	a	297	ab	288	ab
MP	6.48	b	6.92	b	6.90	b	6.76	c	12.41	a	14.13	a	12.70	a	13.08	a	305	d	326	b	328	b	320	c	12.41	a	14.13	a	12.70	a	13.08	a	305	d	326	b	328	b	320	c	305	d	326	b	328	b	320	c
Mean	5.56	A	5.92	B	5.95	B	5.86	ns	12.06	A	13.33	B	12.28	A	12.56	ns	274	A	290	B	303	C	289	ns	12.06	A	13.33	B	12.28	A	12.56	ns	274	A	290	B	303	C	289	ns	274	A	290	B	303	C	289	ns
Rootstock*Date	ns																																															

<sup>a</sup> Mean values in the same column followed by different lower case letters indicate significant differences between rootstocks, by Tukey's test ( $P < 0.05$ ). Mean values in the same row followed by different upper case letters indicate significant differences between sampling dates. ns: no significant interaction. CA: Carrizo citrange; CL: Cleopatra mandarin; FA13: Former-Alcaide no. 13; FA41: Former-Alcaide no. 41; FA5: Former-Alcaide no. 5; MP: C. macrophylla Wester.

**Table 2.** Effect of rootstock and sampling date on 'Lane Late' orange organoleptic fruit quality. <sup>a</sup>

Rootstock	Juice (%) <sup>b</sup>				Density (g/cc)									
	January	March	April	Mean	January	March	April	Mean						
CA	49.17	bc B	47.17	b B	43.14	b A	46.49	b	1.035	ab	1.043	a	1.043	ab
CL	47.79	bc A	47.56	b A	45.51	b A	46.98	bc	1.040	ab	1.049	a	1.049	b
FA13	49.35	c A	49.46	b A	46.85	b A	48.55	c	1.046	b	1.049	a	1.048	b
FA41	47.15	b A	47.46	b A	45.71	b A	46.77	bc	1.037	ab	1.052	a	1.042	b
FA5	47.66	bc A	48.87	b A	47.34	b A	47.96	bc	1.048	b	1.046	a	1.050	b
MP	43.17	a B	39.56	a A	38.51	a A	40.42	a	1.025	a	1.039	a	1.039	a
Mean	47.38	B	46.68	B	44.51	A	46.20	*	1.038	A	1.04	A	1.045	A
Rootstock*Date	ns													

  

Rootstock	TSS (°Brix)				TA (g/100cc)									
	January	March	April	Mean	January	March	April	Mean						
CA	10.54	b	11.45	b	11.51	b	11.14	b	0.85	ab	0.64	a	0.58	b
CL	10.98	bc	11.93	bc	12.22	cd	11.71	cd	0.97	bc	0.77	b	0.63	bc
FA13	11.81	d	12.53	d	12.59	d	12.31	e	0.98	bc	0.83	b	0.68	c
FA41	11.02	bc	11.58	b	11.67	bc	11.39	bc	0.80	a	0.65	a	0.57	b
FA5	11.31	c	12.16	cd	12.44	d	11.97	de	0.99	c	0.80	b	0.70	c
MP	9.36	a	9.80	a	10.36	a	9.85	a	0.80	a	0.57	a	0.47	a
Mean	10.83	A	11.58	B	11.80	B	11.39		0.90	C	0.71	B	0.60	A
Rootstock*Date	ns													

  

Rootstock	RI							
	January	March	April	Mean				
CA	13.13	ab	18.14	b	20.60	bcd	17.45	b
CL	11.51	a	15.89	a	20.03	abc	15.77	a
FA13	12.33	ab	15.61	a	19.34	ab	15.76	a
FA41	13.70	b	18.31	b	21.40	cd	17.95	b
FA5	11.50	a	15.59	a	18.53	a	15.20	a
MP	12.20	ab	17.20	ab	22.44	d	17.24	ab
Mean	12.39	A	16.79	B	20.39	C	16.56	
Rootstock*Date	ns							

<sup>a</sup> Mean values in the same column followed by different lower case letters indicate significant differences between rootstocks, by Tukey's test (P < 0.05). Mean values in the same row followed by different upper case letters indicate significant differences between sampling dates. ns: no significant interaction. <sup>b</sup> Juice content (Juice), juice density (Density), total soluble solids (TSS), titratable acidity (TA) and ripening index (RI), during 2009/2010 to 2013/2014 seasons. CA: Carrizo citrange; CL: 'Cleopatra' mandarin; FA13: Former-Alcaide no. 13; FA41: Former-Alcaide no. 41; FA5: Former-Alcaide no. 5; MP: C. macrophylla Wester.

**Table 3.** Effect of rootstock and sampling date on 'Lane Late' inner orange fruit firmness, during 2012/2013 and 2013/2014 seasons, and physiological alterations: creasing and granulation, during 2009/2010, to 2013/2014 seasons.

Rootstock	Firmness (N)						Creasing <sup>a</sup>						Granulation <sup>a</sup>							
	January		March		April		January		March		April		January		March		April		Mean	
	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns	Mean	ns
CA	7.72 a	6.92 a	5.41 a	6.68 ab	0.06 a	0.21 a	0.17 a	0.63 b A	0.70 ab A	0.91 ab A	0.13 a	0.63 b A	0.70 ab A	0.91 ab A	0.75 ab					
CL	7.53 a	7.45 a	5.70 ab	6.89 abc	0.06 a	0.36 a	0.23 a	0.57 b A	0.71 ab A	0.84 ab A	0.13 a	0.57 b A	0.71 ab A	0.84 ab A	0.72 ab					
FA13	8.27 a	7.13 a	6.46 b	7.28 c	0.00 a	0.16 a	0.22 a	0.30 a A	0.40 a A	0.74 a B	0.18 a	0.30 a A	0.40 a A	0.74 a B	0.48 a					
FA41	7.73 a	7.21 a	6.42 b	7.12 bc	0.06 a	0.16 a	0.16 a	0.45 ab A	0.60 a A	0.86 ab A	0.18 a	0.45 ab A	0.60 a A	0.86 ab A	0.64 a					
FA5	8.02 a	6.48 a	6.33 ab	6.94 abc	0.01 a	0.28 a	0.24 a	0.51 ab A	0.77 ab AB	0.89 ab B	0.22 a	0.51 ab A	0.77 ab AB	0.89 ab B	0.72 ab					
MP	7.23 a	6.64 a	5.95 ab	6.61 a	0.21 a	0.64 a	0.50 a	0.59 b A	1.23 b B	1.15 b B	0.45 a	0.59 b A	1.23 b B	1.15 b B	0.99 b					
Mean	7.75 B	6.97 B	6.04 A	6.92	0.07 A	0.30 B	0.25 B	0.51 A	0.74 B	0.90 B	0.21	0.51 A	0.74 B	0.90 B	0.72					
Rootstock*Date	ns																			

<sup>a</sup> <1: mild, 1-2: moderate and >2: severe alteration. Mean values in the same column followed by different lower case letters indicate significant differences between rootstocks, by Tukey's test (P < 0.05). Mean values in the same row followed by different upper case letters indicate significant differences between sampling dates. ns: no significant interaction. \*: significant interaction. ns: no significant interaction; CA: Carrizo citrange; CL: Cleopatra mandarin; FA13: Forner-Alcaide no. 13; FA41: Forner-Alcaide no. 41; FA5: Forner-Alcaide no. 5; MP: C. macrophylla Wester.

juice quality (the "Pritchett tongue"). During 'Lane Late' orange fruit ripening, total soluble solids increased while titratable acidity decreased (Table 2), evolving in line with the process noticed by Kahn *et al.* (2007) in certain Navel orange cultivars. Evolution of these parameters resulted in an increase in the ripening index (Table 2), which has also been observed for other navel orange cultivars or even other citrus species (Fattahi *et al.*, 2011; Merino *et al.*, 2015). Since January, the 'Lane Late' orange fruit recorded a RI that was much higher than the minimum required for its commercial maturity. Nevertheless, since March, *Citrus macrophylla*, Carrizo citrange and Forner-Alcaide no. 41 induced bland juices taste as a result of a high RI together without enough total soluble solids contents (Table 2). Just like Kahn *et al.* (2007) observation, 'Lane Late' orange fruits experienced a reduction in juice content (Table 2), which was only significant on *Citrus macrophylla* in March (39.56%) and Carrizo citrange (43.14%) in April. Based on both parameters (juice content and RI), January provided the most appropriate timing to harvest 'Lane Late' orange fruit on *Citrus macrophylla*, Carrizo citrange, and Forner-Alcaide no. 41. Conversely, in 'Cleopatra' mandarin, Forner-Alcaide no. 13, and Forner-Alcaide no. 5, the timing to harvest could be postponed until March (Table 2).

### Physiological Disorders and Inner Firmness

Granulation is a serious pre-harvest fruit disorder, wherein juice sacs become hard, dry and enlarged (Sharma and Saxena, 2004). Granulation occurs most often in fruit picked late in the season (Table 3), as other authors point out (Kahn *et al.*, 2007). Degree of granulation of 'Lane Late' orange fruits increased from January to April (Table 3), but only significantly on *Citrus macrophylla* in March and on Forner-Alcaide no. 13 and Forner-Alcaide no. 5 in April. Hence, as reported in other research (Kumar *et al.*,

**Table 4.** Effect of rootstock and sampling date on total polyphenolic (TP) and flavonoid (TF) content in 'Lane Late' orange fruit during 2012/2013 and 2013/2014 seasons.

Rootstock	TP (mg EG/100g FW) <sup>a</sup>				TF (mg EQ/100g FW) <sup>b</sup>			
	January	March	April	Mean	January	March	April	Mean
CA	68.88 a	63.89 a	64.39 a	65.72 a	46.57 a	47.10 a	48.60 a	47.43 a
CL	83.97 c	75.95 ab	67.21 a	75.71 c	56.99 b	62.80 b	55.63 ab	58.47 b
FA13	83.49 bc	80.26 b	67.78 a	77.18 c	58.85 b	56.07 ab	53.12 a	56.01 b
FA41	80.32 abc	72.16 ab	70.03 a	74.17 bc	56.80 ab	54.65 ab	64.91 b	58.79 b
FA5	81.76 bc	71.59 ab	66.61 a	73.32 abc	63.66 b	51.99 ab	58.01 ab	57.89 b
MP	71.56 ab	64.69 a	63.46 a	66.57 ab	57.51 b	52.95 ab	57.50 ab	55.99 b
Mean	78.33 C	71.42 B	66.58 A	72.11	56.73 A	54.26 A	56.30 A	55.76
Rootstock*Date	ns				ns			

<sup>a</sup> mg of gallic acid equivalents per 100 g of fresh weight of oranges (mg EG/100g FW); <sup>b</sup> mg of quercetin equivalents per 100 g of fresh weight of oranges (mg EQ/100g FW); Mean values in the same column or row followed by different lower case letters indicate significant differences between, respectively, rootstocks or sampling dates, by Tukey's test ( $P < 0.05$ ). ns: no significant interaction; CA: Carrizo citrange; CL: 'Cleopatra' mandarin; FA13: Forner-Alcaide no. 13; FA41: Forner-Alcaide no. 41; FA5: Forner-Alcaide no. 5; MP: *C. macrophylla* Wester.

1994; Sharma and Saxena, 2004; Kahn *et al.*, 2007), the highest granulation corresponded to a large fruit and vigorous rootstock (*Citrus macrophylla*; Table 3). Creasing is another pre-harvest fruit disorder, consisting of the albedo breakdown. Creasing increased in 'Lane Late' orange fruits during ripening (Table 3). Despite differences obtained among sampling dates, the maximum values reached did not exceed a slight degree in either creasing or granulation (Table 3). The inner firmness of the 'Lane Late' orange fruit decreased during ripening (Table 3). The lowest values reached in April could probably be consequence of lower juice content obtained at the same time (Table 2). Together, these two parameters are a good indicator of the loss of fruit flesh texture on the palate.

#### Total Polyphenolic and Total Flavonoid Content

The ripening stage has an effect on the bioactive compounds (Table 4). Large variations of the bioactive compounds in

relation to the ripening stage have also been observed in other citrus species and even plum cultivars (Fattahi *et al.*, 2011; Vlaic *et al.*, 2017). While total polyphenolic content in 'Lane Late' orange fruits progressively decreased during fruit ripening, flavonoids content (which is associated with a lower risk of major human diseases) did not change throughout the trial (Table 4).

A different effect of the ripening stage on bioactive compounds was reported by Fattahi *et al.* (2011) in other orange species. Not only were the results obtained by Fattahi *et al.* (2011) for cultivars other than 'Lane Late' navel orange, but also they were tested at a rather early fruit ripening stage, with a ripening index below 6.5 in most cases (R (EU) no. 543/2011 of European Commission, of 7 June 2011).

Considering all quality parameters collectively, the most suitable harvesting times for 'Lane Late' orange fruits per rootstock were different. Thus, January was the most suitable harvesting time for 'Lane Late' orange fruits on *Citrus macrophylla*, Carrizo citrange and Forner-Alcaide no. 41 and March for 'Lane Late' orange fruits on 'Cleopatra' mandarin, Forner-Alcaide no. 13



and Forner-Alcaide no. 5. Considering that the actual interest in 'Lane Late' navel orange cultivar lies in delaying fruit harvesting as much as possible, *Citrus macrophylla* or Carrizo citrange proved to be less interesting citrus rootstocks. This aspect must be considered by citrus producing countries for fresh market where these citrus rootstocks are either widely used or the acreage of trees on these citrus rootstocks is increased, such as Saudi Arabia (Al-Jaleel *et al.*, 2005) and Spain (Forner-Giner *et al.*, 2003).

### Overall Quality of 'Lane Late' Orange Fruits on the Different Rootstocks

The overall quality of 'Lane Late' orange fruit was affected by the rootstock, which was found to significantly influence fruit size and peel thickness (Table 1). *Citrus macrophylla* produced significantly the biggest (diameter and height) and heaviest 'Lane Late' orange fruits (86.62 mm, 87.16 mm and 320 g, respectively; Table 1). Carrizo citrange showed an intermediate behavior, inducing 'Lane Late' orange fruits that were significantly larger and heavier than those on Forner-Alcaide no. 13 and 'Cleopatra' mandarin. The lowest diameter and weight values recorded on Forner-Alcaide no. 13 and 'Cleopatra' mandarin did not differ significantly from those recorded on Forner-Alcaide no. 41 or Forner-Alcaide no. 5 on any of the sampling dates. On the other hand, *Citrus macrophylla* induced significantly the highest peel thickness (6.76 mm; Table 1). It was observed that the largest fruits tend to have the thickest peels, as previously identified by Kahn *et al.* (2007). Forner-Alcaide no. 13 and Forner-Alcaide no. 41 induced higher fruit inner firmness (6.46 and 6.42 N, respectively), which was even statistically different from Carrizo citrange (5.41 N) in April (Table 3). There was no significant effect by rootstock in terms of color, shape index (Table 1) or fruit creasing (Table 3). On the contrary, 'Lane Late' orange fruit on *Citrus*

*macrophylla* experienced the highest granulation, which was statistically different from those on Forner-Alcaide no. 13 and Forner-Alcaide no. 41. Regardless of the rootstock, neither the physiological disorders nor creasing or granulation exceeded a slight degree in April (Table 3). 'Lane Late' orange fruits on *Citrus macrophylla* or Carrizo citrange showed lower internal quality than those on the other rootstocks (Tables 2 and 3). 'Lane Late' orange fruits on *Citrus macrophylla* recorded the lowest total soluble solids (9.85 °Brix; Table 2) and the lowest juice content (40.42%, Table 2), while Carrizo citrange induced the lowest polyphenolic and flavonoid contents (65.72 mg EG/100g FW and 47.43 mg EQ/100g FW, respectively; Table 4). Similar results in terms of the low internal fruit quality induced by *Citrus macrophylla* have also been reported by other authors (Al-Jaleel *et al.*, 2005; Hervalejo *et al.*, 2010; Legua *et al.*, 2011b). Conversely, 'Lane Late' orange fruits on Forner-Alcaide no. 13, followed by Forner-Alcaide no. 5 and 'Cleopatra' mandarin, induced the highest internal fruit quality (Table 2), recording the highest soluble solids (12.31 °Brix) and juice content (48.55%). Moreover, 'Cleopatra' mandarin and Forner-Alcaide no. 13 showed the highest values in bioactive compounds contents (Table 4).

### CONCLUSIONS

Different harvesting times were identified as the most suitable for 'Lane Late' orange fruits per rootstock in order to determine the best overall fruit quality. In 'Lane Late' orange fruits on *Citrus macrophylla*, Carrizo citrange and Forner-Alcaide no. 41, January was the recommended harvesting time. However, the most suitable harvesting time for 'Lane late' orange fruit on 'Cleopatra' mandarin, Forner-Alcaide no. 13 and Forner-Alcaide no. 5 was March, a more interesting timing since 'Lane Late' orange is a late-season navel orange.

Despite harvesting 'Lane Late' orange fruits on the most suitable date for each rootstock, *Citrus macrophylla* induced the largest fruit size but also the poorest organoleptic juice quality, while Carrizo citrange showed the lowest bioactive compounds contents. However, Forner-Alcaide no. 5, Forner-Alcaide no. 13, and 'Cleopatra' mandarin recorded the highest internal quality, while Forner-Alcaide no. 41 showed an intermediate behavior.

Among the three citrus rootstocks more suitable for 'Lane Late' orange fruits, Forner-Alcaide no. 5, Forner-Alcaide no. 13, and 'Cleopatra' mandarin, the only slight differences found were that 'Cleopatra' mandarin induced higher flavonoids content, Forner-Alcaide no. 13 induced greater organoleptic quality and higher polyphenolic contents, and Forner-Alcaide no. 5 induced higher fruit size and weight. In the choice between the three latter citrus rootstocks, other aspects such as local biotic and abiotic conditions should also be considered.

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## کیفیت کلی میوه پرتقال Lane Late روی پایه های زیر-استاندارد و نیمه کوتاه

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### چکیده

هدف این پژوهش ارزیابی شکل ظاهری، طعم، و مواد زیست فعال میوه پرتقال Lane Late روی سه پایه زیراستاندارد یا نیمه کوتاه شامل *Forner-Alcaide no.5*، *Forner-Alcaide no.13*، *Citrus Carrizo citrange* و *Forner-Alcaide no.41* در مقایسه با سه پایه سنتی شامل *Citrus Cleopatra´ mandarin* و *macrophylla* در اسپانیا بود. برای هر پایه زمان برداشت متفاوتی شناسایی شد. مناسبترین زمان برداشت برای میوه پرتقال Lane Late روی پایه های *Cleopatra´ mandarin*، *Forner-Alcaide no. 13* یا *Forner-Alcaide no. 5* در ماه مارس بود که بین یک تا دو ماه دیرتر از *Citrus macrophylla*، *Carrizo citrange*، یا *Forner-Alcaide no. 41* بود و برای این پرتقال ناول دیررس مناسب تر است. با وجود آنکه میوه پرتقال Lane Late روی هر پایه در مناسبترین زمان برداشت شد، تفاوت های معناداری در مورد کیفیت کلی میوه میان آنها مشاهده شد. از این قرار، *Citrus macrophylla* بزرگترین اندازه را القا کرد ولی در عین حال میوه کمترین کیفیت ارگانولپتیک را داشت، در حالیکه پایه *Carrizo citrange* کمترین محتوای مواد زیست فعال را القا کرد و پایه های *Forner-Alcaide no. 5*، *Forner-Alcaide no. 13* و *Cleopatra´ mandarin* بیشترین کیفیت درونی را القا کردند. میوه پرتقالهای Lane Late روی پایه *Forner-Alcaide no. 41* رفتاری میانه نشان دادند.