



Article

Fruit Position on Tree Canopy Affects Fruit Quality Traits in ‘Sanguinelli’ Blood Oranges

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Abstract: In modern orchard systems, the tree canopy is designed to ensure homogeneity in fruit quality. However, even in those crops there are some variables that affect the fruit maturation process and fruit quality properties. The aim of this work was to determine if canopy layer (upper vs. lower), fruit shoot position (grouped vs. individual) and orientation (west vs. east) affect fruit quality attributes of ‘Sanguinelli’ blood oranges. Thus, different quality traits, such as weight, internal colour (IC), external colour (EC), total soluble solids (TSS), titratable acidity (TA) and maturity index (MI) were determined for this purpose. Results showed that fruit weight, internal colour, TA and MI were influenced by the number of fruits per shoot. In this sense, the highest values of weight, IC and MI were found in the grouped fruits, while the highest values in TA were in the individual fruits. Regarding the EC and TSS, they were strongly related to the canopy layer, since the highest values were found in fruit located at the upper parts of the canopy. On the contrary, the orientation did not have a significant effect on fruit quality properties. Therefore, consistent differences in quality traits of ‘Sanguinelli’ blood oranges fruits were observed depending on canopy layer and number of fruits per shoot.

Keywords: blood oranges; canopy position; total soluble solids; titratable acidity; colour



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1. Introduction

Sweet oranges (*Citrus sinensis* L. Osbeck) are the main citrus species produced worldwide. Sweet oranges can be divided into two groups: white or blonde oranges, which are grown in most citrus-producing countries, and blood oranges, which are grown in a few regions where the cold temperatures at nights allow the synthesis of the red pigment [1]. In the Mediterranean area, the most common blood orange cultivars produced are ‘Sanguinelli’ in Spain and ‘Tarocco’ and ‘Moro’ in Italy. Blood oranges are characterized by their high anthocyanin content, conferring on the fruit the typical red colour. Moreover, this fruit is rich in phenolic compounds, such as flavones, flavanones and phenolic acids. Both anthocyanins and phenolics are related to the high antioxidant capacity of the fruit and the human health benefits [2]. Therefore, consumers demand blood oranges with a strong purple-red colour in the peel and flesh, since they associate this sensory parameter with health properties, affecting considerably fruit acceptance and marketing. In fact, skin and juice colour have become key quality factors affecting the consumers’ buying decisions and, therefore, the grower’s profit. Thus, modern agriculture has to be focused on developing

more efficient and productive rootstocks and cultivars and new strategies for the crop management in order to achieve deep-coloured fruits. In this sense, planting distances, tree architecture, crop load or orchard location are variables that considerably affect the final fruit quality [3].

Fruit position in the canopy has been reported to be strongly related to the final fruit quality in many fruit species. Sunlight, temperature and humidity are the main factors to be considered. The increase in light exposure affected positively the total soluble solids content and reduced the titratable acidity in grapes [4]. This is an important factor in blood oranges, since the flavour quality and shelf life of citrus is largely determined by the sugar–acid ratio [5]. Other results showed that the total soluble solids content in apples and pears was strongly influenced by the fruit canopy position, being higher in the outer fruits than in the inner ones, while the effects of the canopy position on titratable acidity in apples and grapes were not clear [6–8]. On the other hand, the canopy position affected the size of starfruit (*Averrhoa carambola*), being higher in the inner fruits than in the outer ones [9]. Contrarily, in apples, fresh weight was higher in fruit harvested from the outer canopy positions compared to fruit from the inner parts of the canopy [10,11]. The colour is another relevant quality trait that is impacted by the fruit canopy position. According to previous results, the peel of the apples from the top of the tree canopy had a nine-times-higher anthocyanin concentration than the inner fruits [12]. These results were in agreement with previous ones, where the lower amounts of anthocyanins were measured in apples from the inner parts of the tree canopy [10,13,14]. Light promotes anthocyanin biosynthesis in red fruit species, such as grapes, and these compounds act as radical scavengers protecting fruit tissues from reactive oxygen species (ROS) produced by UV radiation and high light intensity [8]. Furthermore, previous results showed that ethylene has an important key role in increasing the biosynthesis of light-induced anthocyanins in apples [15]. On the contrary, altered light conditions in the field, providing shaded zones, showed an inhibition of the anthocyanin accumulation in grapes [16]. Both high and low temperatures can lead to modifications in the anthocyanin content. In this sense, it was reported that cold temperatures induced the accumulation of anthocyanins in the flesh of pigmented red orange fruit, and this enhancement was accomplished by the up-regulation of phenylalanine ammonia-lyase (PAL) [1]. However, as far as we know, there is no available literature regarding the effects of the fruit position in the tree canopy on their quality properties in blood oranges. Therefore, this study aimed to elucidate the influence of the canopy layer (upper vs. lower), the grouping of fruit in shoots (grouped vs. individuals) and the orientation (west vs. east) in the main quality traits of ‘Sanguinelli’ blood oranges.

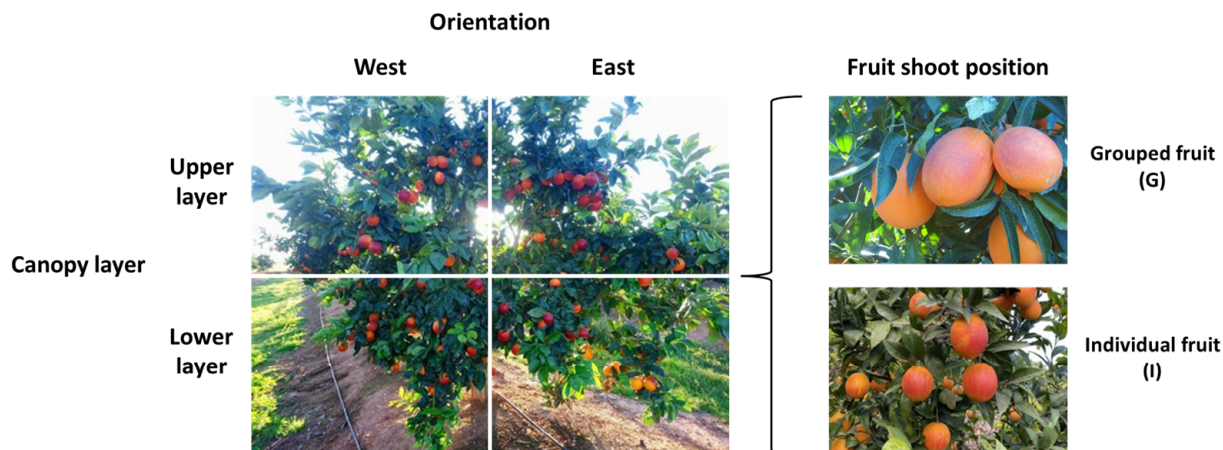
2. Materials and Methods

2.1. Plant Material and Experimental Design

The study was conducted in 2023, in a commercial field located in Orihuela (Alicante, Spain) under climatic Mediterranean conditions (with a 19 °C average yearly temperature and 300 mm/year of precipitation) and standard agronomical practices for blood oranges. Four adult trees (14 years) of ‘Sanguinelli’ blood oranges grafted onto *Citrus macrophylla* were selected at random. All the fruit were harvested at the commercial maturation stage and labelled according to their canopy tree position, as shown in Figure 1. In total, 1400 oranges were harvested from the four trees and transferred to the laboratory within 2 h. Then, the following quality parameters were measured in each individual fruit.

2.2. Fruit Quality Parameters

Blood oranges were visually assessed into 5 maturation stages according to the flavedo external colour, as follows. Stage 1: light orange-green colour (more than 50% of the fruit surface). Stage 2: light orange-green colour (less than 50% of the fruit surface). Stage 3: dark orange with absence of green colour. Stage 4: orange-red colour. Stage 5: red-brown colour (Figure 2).



- (1) Individual fruits from the upper layer of the canopy in the west side.
- (2) Grouped fruits from the upper layer of the canopy in the west side.
- (3) Individual fruits from the upper layer of the canopy in the east side.
- (4) Grouped fruits from the upper layer of the canopy in the east side.
- (5) Individual fruits from the lower layer of the canopy in the west side.
- (6) Grouped fruits from the lower layer of the canopy in the west side.
- (7) Individual fruits from the lower layer of the canopy in the east side.
- (8) Grouped fruits from the lower layer of the canopy in the east side.

Figure 1. Classification of ‘Sanguinelli’ blood oranges according to orientation (west vs. east), canopy layer (upper vs. lower) and fruit shoot position (grouped vs. individual).



Figure 2. Classification of ‘Sanguinelli’ blood oranges according to the flavedo colour from stages 1 to 5.

Fruits were individually weighed by using a Radwag WLC 2/A2 balance (Radwag wagi Elektroniczne: Radom, Poland) with a precision to two decimal places. The results were expressed in grams (g).

Total soluble solids (TSS) in juice were individually measured using a digital refractometer (Hanna Instruments, Woonsocket, RI, USA), and the results were expressed as °Brix. In addition, titratable acidity (TA) was also measured individually using automatic titration (785 DMP Titrino, Metrohm AG, Herisau, Switzerland) where 1 mL of juice was neutralized with NaOH 0.1 mM until a pH of 8.1 was reached. The maturity index (MI) of the blood oranges was shown as an absolute value obtained from the ratio between TSS and TA.

In addition, a total of 20 fruits of each maturation stage, classified according to the flavedo colour scale shown in Figure 2, were taken for colour measures. Peel colour was measured at three equidistant points of the equatorial fruit perimeter, and flesh colour was measured at three equidistant points of the segment at the equatorial surface by using a

Minolta colourimeter (CRC400; Konica Minolta, Osaka, Japan). Results were expressed as hue angle ($\arctg b^*/a^*$).

2.3. Statistical Analysis

Results were expressed as the mean \pm SE. Data were subjected to an analysis of the variance (ANOVA), and a multiple-range test (Tukey's test) was applied to determine significant differences between treatments (p -value < 0.05) when the variables analysed were more than 2 (orientation, canopy layer and shoot position). In addition, the chi-square test was applied to determine the significant differences between the frequency distribution of different categorical variables. Those statistical analyses were performed using SPSS, version 22 (IBM Corp., Armonk, NY, USA). The heatmap matrix was performed using the ClustVis web tool [17]. The PCA model was constructed with normalized data using Unscrambler 11 software (CAMO AS, Oslo, Norway).

3. Results and Discussion

The results showed that 56% of the fruits were harvested from the east side of the tree and 44% from the west side. Additionally, 62% of the fruit harvested belonged to the lower canopy layer, while 38% was harvested from the upper canopy layer. Finally, most of the fruits harvested, at 60%, were individuals (Table 1). Therefore, those results showed that the lower canopy layer of the east side was the highest-yielding part of the tree. Thus, fruit number was influenced by the canopy layer and the number of fruits per shoot (Table 1).

Table 1. Total number of fruits harvested from the four trees in each canopy layer, fruit shoot position and orientation.

Fruit Zone	Number of Fruits	ANOVA	F-Value
EUI	171	Canopy layer (C)	10.086 ***
EUG	119	Shoot position (P)	8.984 ***
ELI	293	Orientation (O)	1.300 ns
ELG	202		
WUI	159	C \times S	0.286 ns
WUG	73	C \times O	0.232 ns
WLI	245	S \times O	0.199 ns
WLG	138	C \times S \times O	0.026 ns
TOTAL	1400		

Samples were abbreviated as follows: west (W), east (E), lower canopy layer (L), upper canopy layer (U), individual (I) and grouped (G). Significant differences are presented with F-value, and asterisks denote significant differences (* $p < 0.1$, ** $p < 0.05$ and *** $p < 0.01$). When no significant differences were found, 'ns' was used.

The influence of orientation, canopy layer and fruit shoot position on the fruit weight was evaluated. Fruit weight ranged from 39 to 273 g, and the mean results showed no significant ($p < 0.05$) effect of any of these variables on fruit weight (Table 2). However, previous results published on apples showed the importance of the canopy layer in fruit weight, the fruits of the greatest size being harvested from the upper layers of the canopy [18]. On the contrary, in the present experiment, 62% of fruits were harvested from the lower layer of the tree (Table 1), and those fruits were not different in weight compared with the fruits from the upper layer (Table 2). This is the first study where the effect on weight of the individual and grouped fruits per shoot has been assessed, and an in-depth study of their frequency distribution shows that the highest fruit weight was found in the grouped fruits,

independently of the canopy layer. Thus, the weight of 60% of the grouped fruits was higher than 143 g, while only 40% of the individual fruit reached this weight (Figure 3A,B). Fruit growth is the result of dry matter and water accumulation. This process is related to the net contribution of phloem download in fruit tissues, xylem flow and transpiration. In this sense, an efficient phloem download in fruit cells would lead to increased pressure gradients from sources to sinks, and, in turn, to enhanced osmotic potential in fruit cells and a water-deficit potential, driving water movement into cells and leading to cell growth. Thus, grouped fruit would have higher sink sources than individual ones and higher amount of photosynthates would be imported to grouped fruits, contributing to their increase in fruit size and weight.

Table 2. Effect of canopy layer, fruit shoot position and orientation in weight, external colour (EC), internal colour (IC), total soluble solids (TSS), titratable acidity (TA) and maturity index (MI) of ‘Sanguinelli’ blood oranges.

Fruit Zone	Weight (g)	EC	IC	TSS (°Brix)	TA (g 100 mL ⁻¹)	MI (°Brix: TA)
EUI	134.08 ± 4.54	2.60 ± 0.08	2.79 ± 0.09	12.61 ± 0.11	1.48 ± 0.04	8.25 ± 0.14
EUG	143.12 ± 4.62	2.62 ± 0.07	3.05 ± 0.10	12.66 ± 0.08	1.45 ± 0.02	8.82 ± 0.08
ELI	133.12 ± 3.63	1.76 ± 0.09	2.82 ± 0.07	12.02 ± 0.15	1.46 ± 0.03	8.40 ± 0.09
ELG	150.67 ± 4.20	1.84 ± 0.08	3.07 ± 0.08	12.02 ± 0.11	1.37 ± 0.03	8.68 ± 0.11
WUI	140.01 ± 4.29	2.73 ± 0.07	2.88 ± 0.06	12.46 ± 0.12	1.46 ± 0.02	8.67 ± 0.10
WUG	140.33 ± 5.53	2.83 ± 0.09	3.25 ± 0.07	12.40 ± 0.10	1.47 ± 0.03	8.65 ± 0.08
WLI	132.86 ± 3.32	2.10 ± 0.07	2.69 ± 0.06	11.75 ± 0.09	1.51 ± 0.03	7.91 ± 0.09
WLG	159.70 ± 4.93	2.08 ± 0.06	3.08 ± 0.09	11.33 ± 0.06	1.44 ± 0.03	7.98 ± 0.10
ANOVA				F-value		
Canopy layer (C)	0.415 ns	14.441 ***	0.398 ns	12.717 ***	1.061 ns	5.047 **
Shoot position (S)	4.292 **	0.050 ns	6.386 **	0.268 ns	4.012 *	0.233 ns
Orientation (O)	0.166 ns	1.396 ns	0.104 ns	2.640 ns	1.882 ns	1.424 ns
C × S	1.440 ns	0.007 ns	0.001 ns	0.244 ns	1.388 ns	0.154 ns
C × O	0.037 ns	0.090 ns	0.639 ns	0.428 ns	1.585 ns	1.406 ns
S × O	0.000 ns	0.001 ns	0.242 ns	0.390 ns	0.396 ns	0.335 ns
C × S × O	0.380 ns	0.050 ns	0.040 ns	0.141 ns	0.029 ns	0.004 ns

Samples are abbreviated as follows: west (W), east (E), lower canopy layer (L), upper canopy layer (U), individual (I) and grouped (G). Data are the mean ± ES. Significant differences are presented with F-value, and asterisks denote significant differences (* $p < 0.1$, ** $p < 0.05$ and *** $p < 0.01$). When no significant differences were found, ‘ns’ was used.

Regarding the evaluation of the external (EC) and internal colour (IC), a scale with five maturation stages levels was designed. The flavedo colour changed from light orange-green (stages 1 and 2) to dark orange (stage 3) and orange with red-brown tones (stage 4 and 5) (Figure 2). The hue angle of the flavedo decreased from 80.35 ± 1.44 in stage 1 to 46.04 ± 1.33 in stage 5, showing that external fruit colour varied highly among individual fruits and that the maturation stages performed according to visual colour appreciation suit the hue colour index well. Regarding the fruit flesh, the colour changed from light orange in stage 1 (68.01 ± 1.25) to orange in stage 2 (62.15 ± 1.31) and a red-orange colour was maintained during stages 3, 4 and 5 with hue angle values of 51 (Table 3). The green colour is related to the presence of chlorophylls in the peel. Those compounds are naturally degraded as fruit matures as a consequence of temperatures below 13 °C in lemon fruit species harvested from the southeast of Spain [19]. Furthermore, cold temperatures also promote the synthesis of carotenoids (mainly β -carotene) and anthocyanins

in flavedo and flesh, those compounds being responsible for the orange and red colours, respectively [20]. Results in Table 2 about the fruit EC showed a close influence of the canopy layer on this parameter, the most coloured fruits being harvested from the upper layer, without significant differences according to the fruit shoot position and orientation. Moreover, those results were in accordance with the frequency of the maturation stages described in the scale, where the blood oranges at the external stage 1 were found in high numbers in the lower layer of the tree (Figure 3C), while fruits at maturation stages 4 and 5 were harvested at high frequency from the upper canopy layer (Figure 3D). In this sense, the accumulation of carotenoids and anthocyanins would be related to the role of those compounds in the plants. Currently, it is well known that these molecules are involved in defensive processes such as protection against UV-B and high light intensities [21]. An important key role of anthocyanins in the antioxidant capacity of the plant organs, including fruits, has been also described [22]. Thus, apples with low light availability due to the canopy position showed lower red colour compared to ones with more hours of sunlight per day [12]. This effect has been observed in mandarins harvested from the inner parts of the tree canopy, which showed lighter colouration than the outer ones [23]. On the contrary, in the present experiment, no significant differences were found between fruits from west- and east-orientated canopies, while higher red colour and maturation stage were recorded for fruit harvested from the upper tree canopy (Table 3). Thus, under the climatic conditions of the southeast of Spain, anthocyanin biosynthesis seems to be more dependent on the lower temperature of the upper canopy parts than on the high light exposure of the east-oriented fruits.

Table 3. External (EC) and internal colour (IC) of each stage expressed as hue angle.

Maturation Stages	EC	IC
1	80.35 ± 1.44 a	68.01 ± 1.25 a
2	72.52 ± 1.37 b	62.15 ± 1.31 b
3	54.78 ± 1.50 c	51.91 ± 1.38 c
4	51.95 ± 1.41 d	51.01 ± 1.41 c
5	46.04 ± 1.33 e	50.79 ± 1.37 c

Data are the mean ± ES. Different lower-case letters indicate significant differences according to Tukey's multiple range test at the 95% confidence level between fruits from different maturation stages.

Results about the fruit IC showed that this parameter was related to the fruit shoot position (Table 2), the grouped fruits being more coloured compared to the individual ones (Figure 3E,F), while canopy layer and orientation had no influence on the fruit IC (Table 2). Those results were confirmed with the evaluation of the maturation stage frequency distribution, since ca. 45% of the grouped fruits were in maturation stage 4, while most of the individual fruits showed maturation stage 3.

Total soluble solids (TSS) were strongly related to the canopy layer from where the fruits were harvested and not by shoot position or orientation (Table 2). Thus, 'Sanguinelli' blood oranges harvested from the upper layer had ca. 7% more TSS, on average, than those from the lower ones (12.53 ± 0.08 and 11.78 ± 0.06 °Brix, respectively). The difference observed in the TSS of fruit from both canopy layers was related to the percentage of blood oranges in the range of 10 to 11 °Brix and the percentage of fruits with more than 13 °Brix. In this sense, fruits with less than 11 °Brix represented 15% and 5% of the total for grouped and individual fruits in the lower canopy layer. Meanwhile, fruits with more than 13 °Brix were ca. 15% in the lower canopy layer and 25% in the upper canopy layer (Figure 4A,B). Normally, soluble solids are considered as photoassimilates translocated from the leaves to the fruit juice sacs. In this sense, light exposure strongly influences soluble solids content, the light distribution within the canopy being an important factor. Thus, fruits that receive more sunlight in the upper layers of the tree are expected to acquire more photoassimilates [24]. In addition, the activity of the sucrose-6-phosphatase in the later stages of the citrus development leads to active synthesis of sucrose within the sac cells [25]. The activity of this enzyme depends on the citrus species, since in mandarins the activity

increased during the maturation stage, while in grapefruits the activity increased from the cell division to the cell expansion and decreased during the fruit maturation [26]. This factor might explain the differences between different citrus fruit species in the sugar profile.

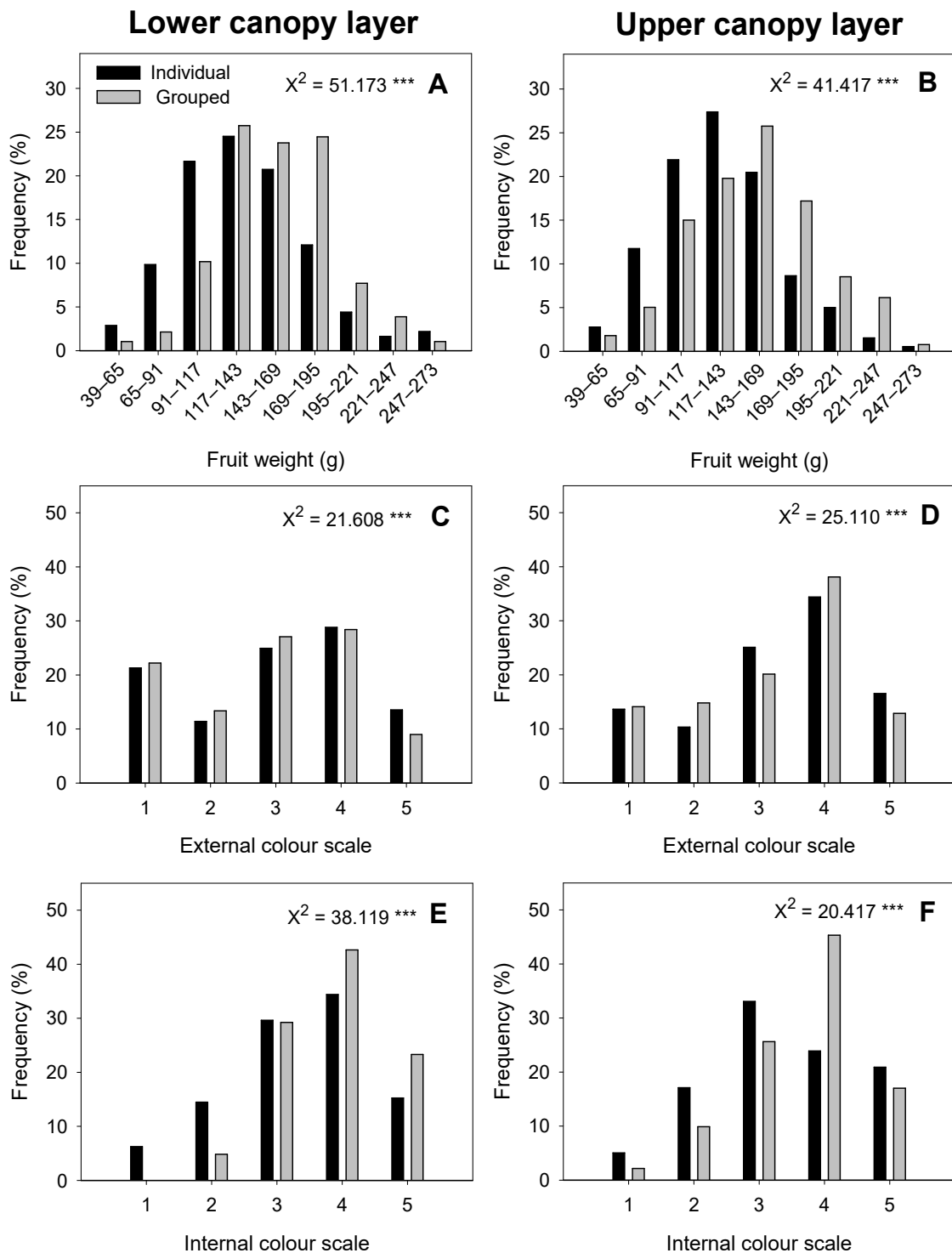


Figure 3. Frequency distribution of fruit weight (A,B), external colour (C,D) and internal colour (E,F) of 'Sanguinelli' blood oranges analysed from different canopy layers (upper vs. lower) and fruit shoot positions (individual vs. grouped). Significant differences are presented with chi-square (χ^2) value, and asterisks denote significant differences (* $p < 0.1$, ** $p < 0.05$ and *** $p < 0.01$).

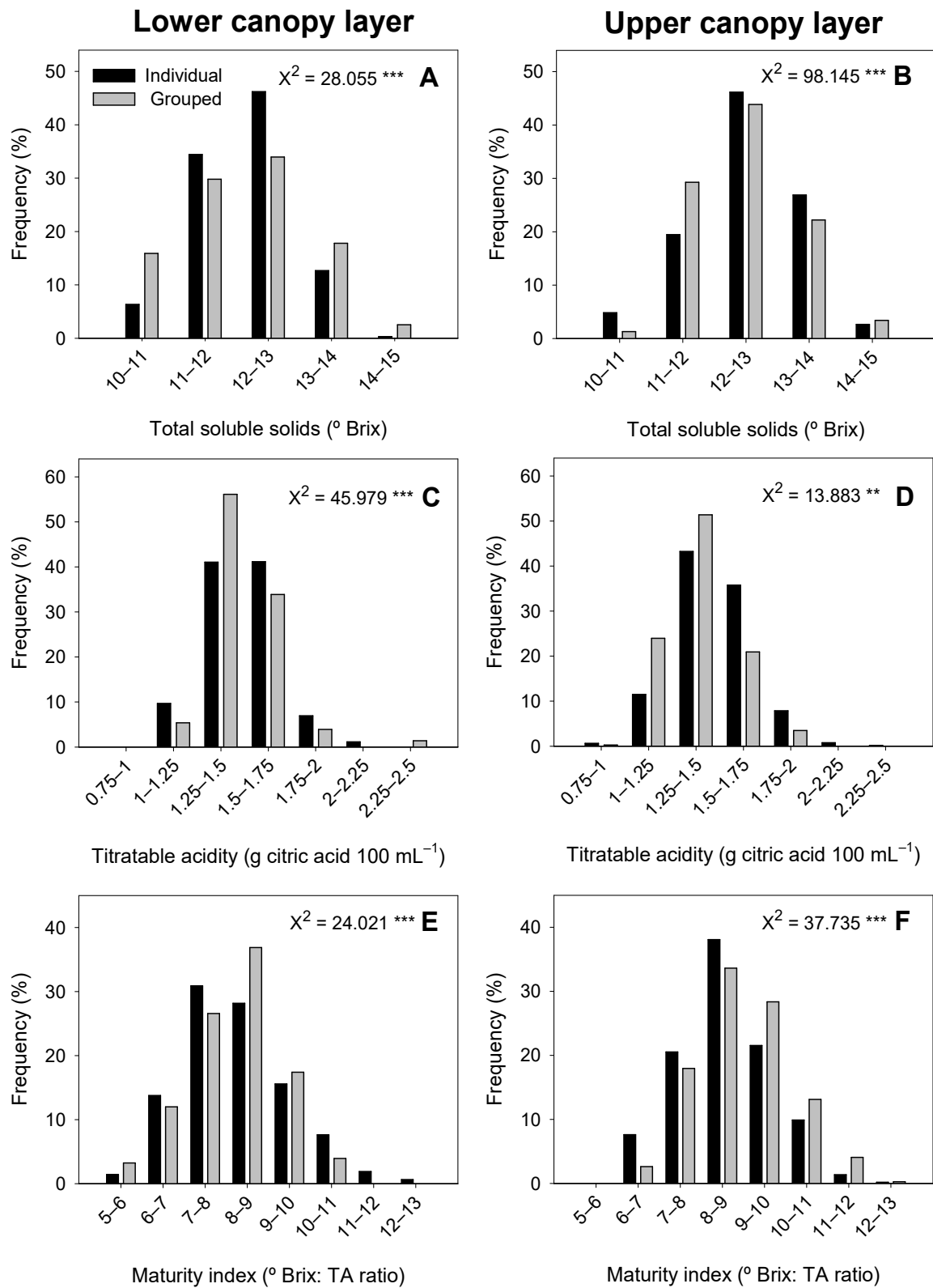


Figure 4. Frequency distribution of total soluble solids (A,B), titratable acidity (C,D) and maturity index (E,F) of ‘Sanguinelli’ blood oranges analysed from different canopy layers (upper vs. lower) and fruit shoot positions (individual vs. grouped). Significant differences are presented with chi-square (X²) value, and asterisks denote significant differences (* *p* < 0.1, ** *p* < 0.05 and *** *p* < 0.01).

On the other hand, TA was significantly affected by the shoot position of the fruit, being higher in the individual blood orange fruits compared to grouped ones (1.48 ± 0.02 and 1.42 ± 0.03 , respectively), while canopy layer and orientation had no significant ($p < 0.05$) influence in TA (Table 2). Those results were confirmed with the data shown in Figure 4C,D, where individual fruits with a TA higher than 1.5 g of citric acid equivalent per 100 mL^{-1} of juice were 50% of the total fruits analysed compared to the 35% in the grouped ones in both canopy layers. Titratable acidity of the 'Sanguinelli' blood oranges is determined by the citrate content in the vacuole of the juice sac cells and the vacuolar acidification. These two processes are coregulated, since the citrate accumulation during the first part of the fruit growth is accompanied by a proton influx that reduces the vacuolar pH, and during the second part of the fruit development, the transport of the vacuolar citrate through the cytoplasm, to be used as energy supply, is accompanied by a proton efflux due to the activity of citrate/ H^+ transporter [27]. Although citrate is the major organic acid in citrus fruit, the presence of ascorbic acid, oxalic acid and malic acid has also to be considered [28]. According to previous studies, TA is negatively regulated by the light, the fruits with the highest TA being in the inner parts of the canopy [14]. However, other results in apples reported no significant differences in terms of TA, comparing fruits from the upper and the lower layers of the canopy [7]. Moreover, ascorbic acid biosynthesis in apple fruit was increased by the light in the peel but not in the flesh [29]. Therefore, there is an important controversy in the effect of the canopy layer on the TA fruit levels, requiring further research. Finally, the MI of the blood oranges harvested in different canopy layers and shoot positions did not show significant differences ($p < 0.05$) among them (Table 2). In contrast, the study of the frequency of fruit with different MI showed that the grouped blood oranges had a higher percentage of fruits with a MI in the range of 8 to 13 compared to the individual ones, independently of the canopy layer (Figure 4).

Blood orange orientation in the tree did not show a significant effect on the quality traits assessed. However, a significant correlation was observed when the normalized data of the quality traits were represented beside the canopy layer and fruit shoot position in a heatmap (Figure 5).

The normalized heatmap matrix indicated that fruit weight was strongly correlated (1.41) with grouped fruits from the lower layer of the canopy. Meanwhile, this parameter was negatively correlated with individual fruits from the lower layer and all fruits harvested from the upper layer (-0.15 , -0.34 and -0.93 , respectively). The correlation of the IC was positive with the fruits harvested from the lower layer (0.67 and 1.00), and negative in the fruits harvested from the upper layer (-1.10 and -0.58). Regarding the TA, this parameter showed a higher positive correlation with the individual fruits (0.83 and 0.45) compared to the grouped ones (-1.44 and 0.15). The fruits harvested from the upper layer of the canopy had a positive correlation with the MI (0.90 and 0.83), TSS (0.84 and 0.86) and EC (0.86 and 0.74), without high differences between grouped and individual fruits. Contrarily, those parameters had a negative correlation with the fruits harvested in the lower layer of the canopy. In this study, the heatmap matrix showed that the quality traits are divided into two major groups depending on the correlation, fruit weight and IC being together, and TSS, EC and TA being in the other group. In citrus fruit, it has been reported that the external appearance is not necessarily related to the internal maturation process [19] (Figure 5). However, other results have revealed that the TSS of the juice is linked to the peel pigmentation [30]. In this sense, previous results in 'Moro' blood oranges reported an increase in enzymatic activity related to the sugar biosynthesis in the peel and the flesh during the development and maturation process, those compounds being used as carbon sources for the anthocyanin biosynthesis [31]. Those results were in accordance with our study, since the most correlated quality traits in the heatmap matrix were TSS, MI and EC.

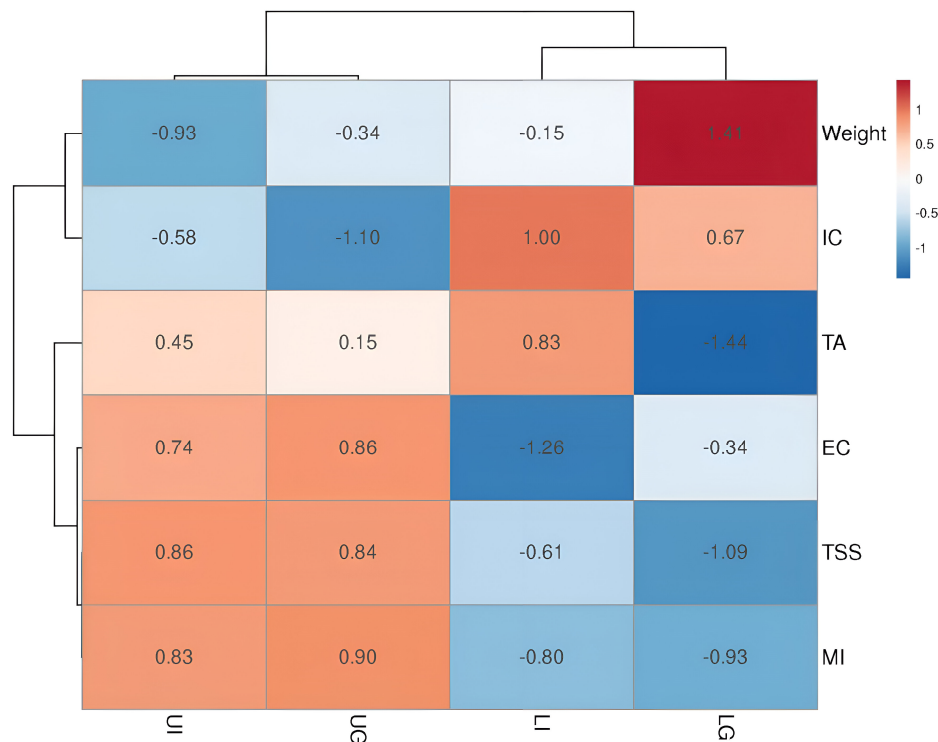


Figure 5. Heatmap based on the relation between quality parameters of ‘Sanguinelli’ blood oranges harvested in different canopy layers, shoot positions and the quality traits. The X axis corresponds to the samples from different canopy layers and shoot positions and are abbreviated as follows: lower canopy layer (L), upper canopy layer (U) and individual (I) or grouped (G), respectively. The Y axis corresponds to the most relevant quality traits measured: weight, internal colour (IC), external colour (EC), total soluble solids (TSS), titratable acidity (TA) and maturity index (MI). The heatmap shows the analysis of the normalized data for each sample.

A principal component analysis (PCA) was applied to the results about the quality traits of fruits harvested from different canopy layers and fruit shoot position, since the orientation had no relevant effect on the blood orange quality (Figure 6).

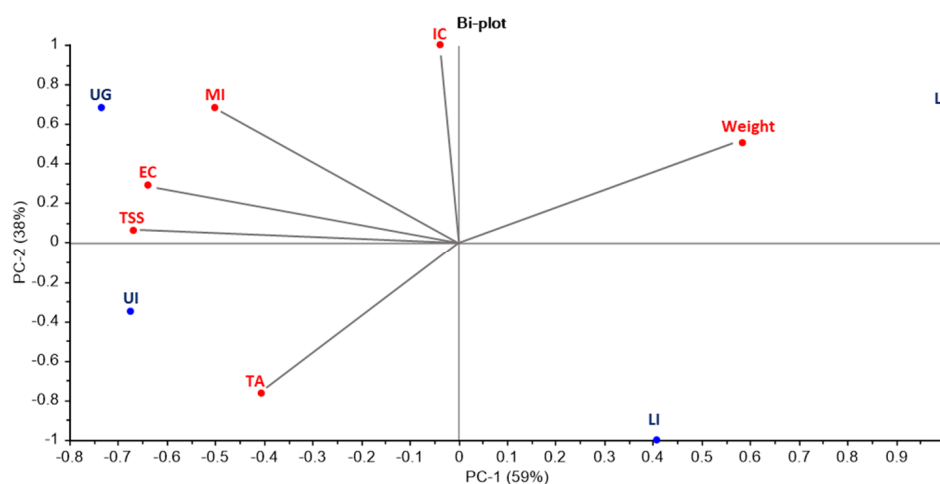


Figure 6. Principal component analysis (PCA) biplot showing the relationships among different samples and the quality traits measured. Samples harvested are shown in blue (●) and the vectors of the quality traits are shown in red (●). Samples are abbreviated as follows: lower canopy layer (L), upper canopy layer (U) and individual (I) or grouped (G), respectively. Titratable acidity (TA), total soluble solids (TSS), external colour (EC), internal colour (IC) and maturity index (MI).

The PC-1 and PC-2 accounted for the 59% and 38% of the total variance of the X and Y variables, respectively, the accumulative variance contribution being 97%. PC-1 is clearly identified with weight (0.46), EC (−0.50) and TSS (−0.52), while PC-2 is related to IC (0.64), TA (−0.49) and MI (0.44). Moreover, in the positive side of the PC-1, weight was the most relevant parameter and IC, MI, EC, TSS and TA contributed to the negative side. The most important parameters in the positive side of the PC-2 were TSS, EC, MI, IC and weight and TA in the negative side. The results showed that the PC-1 allowed three groups to be differentiated, as follows: one formed by fruits from the upper layer of the canopy and two more formed by the individual and grouped fruits from the lower layer of the tree, respectively. Regarding the PC-2, the results showed two major groups divided into individual and grouped fruits, independently of the canopy layer. The individual and grouped fruits harvested from the upper layers of the canopy were closely related to TSS, EC, MI and TA, while individual and grouped fruits from the lower layers were more dependent on the TA and weight, respectively. Those results are in line with previous research about the effect of the canopy position (upper vs. lower layer) on the fruit quality traits [10,12,30,32].

4. Conclusions

The present study showed the effect of the canopy layer (upper vs. lower layer), fruit shoot position (individual vs. grouped) and fruit orientation (west vs. east) on the main quality traits of ‘Sanguinelli’ blood oranges. Results revealed that the canopy layer determined the EC and TSS, while the fruit shoot position was strongly related to the fruit weight and slightly related to the IC, TA and MI of the ‘Sanguinelli’ blood oranges. However, canopy orientation had no influence on the fruit quality traits. Therefore, the number of fruits per shoot is an important variable in controlling fruit size, this study being the first that describes the influence of fruit shoot position (individual or grouped) in the quality traits of blood oranges. In this sense, designing crop patterns according to the canopy layer and the fruit shoot position would lead the growers to obtain fruits with better quality properties and increase their incomes.

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