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Relation between Rind Pigmentation and Internal Quality of Blood Orange 'Sanguinelli': Physicochemical and Sensory Studies

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Abstract: This study evaluated the relation between rind colour and the internal physicochemical and sensory qualities of 'Sanguinelli' blood oranges, one of the main blood orange cultivars grown in the Mediterranean region. To this end, 400 fruits were harvested in three different orchards and classified according to rind pigmentation intensity (slight, medium, intense, very intense). All fruits were individually evaluated by determining rind and pulp colour, total soluble solids, acidity, maturity index, juice yield, firmness, and size. Moreover, 71 consumers performed a triangle test to evaluate if fruit sensory properties depended on rind pigmentation. Our results revealed (for the first time) that pulp pigmentation and total soluble solid (TSS) content strongly depend on rind colouration. Among the fruit from the same orchard, the redder the pigmented fruit was (externally) the deeper the red pulp, and the higher the TSS became. This pattern was corroborated in the three orchards under study. Other characteristics, such as acidity, juice yield, firmness, and fruit size, did not depend on external pigmentation. Sensory studies showed that the more coloured the fruit, the higher the accumulated sugar content; consumers perceive these fruits as being sweeter than slightly pigmented ones. This information can be very useful for the citrus industry as external colour may become a quality index for blood oranges, as well as for consumers to make purchase decisions.

Keywords: total soluble solids; anthocyanin; colour; triangle test; consumers

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1. Introduction

Among the different groups of oranges, blood oranges (*Citrus sinensis* L. Osbeck) stand out for their unique, red-coloured flesh and rinds, due to their anthocyanin content. These soluble pigments belong to the flavonoid compounds family and are responsible for the characteristic colours of other fruits, such as pomegranates [1], grapes [2,3], or different kinds of berries [4,5].

The popularity of these oranges is growing worldwide [6], but they are cultivated mainly in the Mediterranean region. In Italy, blood oranges have a long-standing tradition and are well placed on the markets. The 'Sanguinello', 'Tarocco', and 'Moro' varieties have protected geographical indications (Rossa di Sicilia) [7]. In recent years, consumers in Spain have focused on blood oranges; production is increasing, with a focus mainly on the 'Sanguinelli' cultivar, which comes from spontaneous mutation of the blood orange 'Doble Fina'.

In addition to anthocyanin compounds being used to confer colour to fruit, these compounds are known for their high antioxidant capacity [8]. Awareness about the health benefits of phytonutrients is driving consumer purchases as well as fruit consumption. Thus, anthocyanin levels in the pulps of blood oranges represent important quality indices for fresh and processed products [9].

However, when growers harvest the fruit and when consumers purchase it in stores, they can only evaluate the external fruit appearance. Therefore, it is necessary to investigate if the rind colouring intensity of blood oranges is related to pulp pigmentation and, therefore, to the anthocyanin content of the edible part. Along these lines, two different studies carried out with Tarocco and Moro [9,10] found that exposing fruits to cold storage at 8 °C increased the anthocyanin content of both the pulp and rind. According to these studies, when fruits are exposed to low temperatures after harvest, they undergo an anthocyanin accumulation process and, therefore, colour enhancement, which happens in parallel to the pulp and rind. However, no studies have approached the relation between external and internal colourings at harvest.

Recently, Cebadera-Miranda et al. [11] described varieties with an intense yellow–orange colouring and the reddest pulp, and others with a more reddish peel colouring and a more yellow–orange pulp. However, we herein wished to examine the relation between rind and pulp colouration from a different perspective. Fruits from the same variety may present wide pigmentation variability, even if they come from the same orchard. The causes of such variability are unclear, but Lo Piero [12] suggested that it can be at least partially related to the position of fruits on the canopy. In this study, we wished to investigate if a relation exists between the intensity of rind pigmentation and pulp pigmentation when fruits of the same cultivar are evaluated. That is, if the external fruit colour is related to the internal colouration and if, therefore, the fruit appearance may act as an indicator of flesh anthocyanin content. Moreover, it was necessary to evaluate if internal characteristics, other than the pulp colour, depend on the rind colour. Furthermore, it was necessary to evaluate if sensory fruit properties, other than visual appearance, are linked to rind pigmentation.

In this context, this study aimed to answer two different questions about the relation between the external appearance and the internal quality of 'Sanguinelli' blood oranges: (1) is the physicochemical quality of pulp linked to rind pigmentation intensity? (2) Are sensory properties perceived by consumers linked to rind pigmentation intensity?

2. Materials and Methods

2.1. Plant Material

Blood oranges (*Citrus sinensis* L. Osbeck) cv. Sanguinelli were obtained from a packing house located in Valencia (Valencian Community, Spain). In the second half of February (the 2019 season), three different fruit batches corresponding to the fruit harvested from three commercial orchards were collected at the packing house, the day after harvest (Orchard 1, Orchard 2, and Orchard 3). The mean values of day and night temperatures from 1 January to harvest date for each orchard were as follows (mean ± SD): 12.8 ± 1.9 and 8.8 ± 2.5 for Orchard 1, 12.7 ± 2.4 and 7.8 ± 3.9 Orchard 2, and 12.9 ± 2.2 and 6.2 ± 3.3 for Orchard 3.

Then fruit were transferred to the Postharvest Department of the Valencian Institute for Agricultural Research (IVIA), where they were divided into lots according to rind pigmentation. The fruits from Orchards 1 and 2 were divided into three lots: slight (P1), medium (P2), and intense (P3) rind pigmentation. In Orchard 3, fruits were divided into two lots according to fruit appearance: medium (P2) and very intense (P4) pigmentation (Figure 1).

Three steps were followed to divide fruit into batches according to their pigmentation: (1) upon the arrival of fruit from each orchard to the laboratory, batches of 50 fruits with different rind pigmentations were initially made by one researcher with experience in blood oranges. (2) Then, the fruit previously selected for batches were mixed and another researcher was asked to group the fruit into 50-fruit batches based on the perceived colour. In case of doubts about any specific fruit, it could be removed and substituted for another one that did not cause the researchers to doubt. In general, a maximum of 1 or 2 fruits per lot of 50 fruits generated disagreement between both researchers and were substituted for consensus. (3) Finally, fruit were mixed again, and we asked a consumer to divide the fruit into 50-fruit batches based on the colour he/she perceived. For the three orchards,

consumers who made the final batches showed no difference with respect to the lots that were made by researchers in the previous steps. The three consumers who participated in this study were invited to do so based on their consumption of blood oranges (at least once every two weeks during the season) and their interest in participating. They were given a box of chocolates for participating.

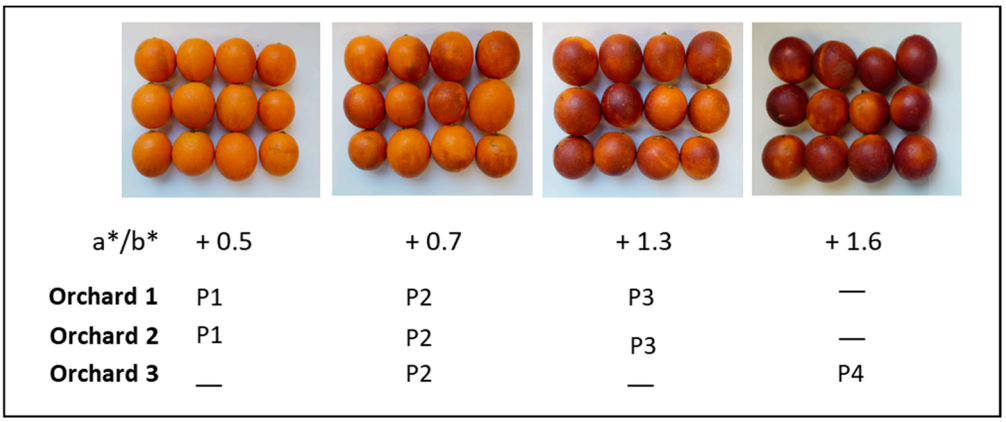


Figure 1. 'Sanguinelli' blood oranges grouped according to increased rind pigmentation intensity (P1—slight, P2—medium, P3—intense, P4—very intense).

The 50 fruits per lot were numbered and the following non-destructive measurements were individually taken on each one: rind colour, firmness, diameter, and weight. Then, juice was obtained individually from all 50 fruits to make the following determinations: juice yield (JY), juice colour, total soluble solids (TSS), titratable acidity (TA), and maturity index (MI).

Moreover, in view of the physicochemical results obtained when evaluating the fruit from Orchard 1, sensory studies were planned to be included when analysing the fruit from Orchard 2. Total anthocyanin content was determined in juice samples used for sensory evaluation.

2.2. Physicochemical Analysis

Firmness measurements were taken using an Instron Universal Testing Machine (model 3343, Instron, Ltd., Buckinghamshire, UK). The results are expressed as the percentage of millimetres of fruit deformation resulting from 10 N pressure, applied by a 3.5 cm plunger on the longitudinal axis at constant speed. Weight (in grams) was determined using an analytical balance while diameter (in millimetres) was measured using a calliper.

As reported in previous studies [9], among the several CIELAB parameters, the a*/b* ratio is the one that best displays changes in rind and pulp pigmentation in blood oranges [9,13,14]. Hence, in this study, the a*/b* ratio was also considered as the colour index (a*/b* < −0.5 correspond to dark green tones, a*/b* ≈ 0 indicates the colour break from green to yellow, a*/b* ≈ +0.5 correspond to orange tones, and a*/b* > +0.5 correspond to red tones).

Pigmentation of the blood orange rind is usually quite heterogeneous around the fruit surface. Previous studies have described the determination of the rind colour by taking measurements along the equatorial axis [9] or by taking three consecutive measurements in the darkest part of the peel, the clearest part, and on the base of the fruit [11]. These procedures may be valid to evaluate colour evolution during storage [9] or to compare samples from different varieties [11]. However, in order to study in-depth the relation between the external pigmentations and internal characteristics of fruit from the same variety, a method is needed to better reflect the rind pigmentation heterogeneity. Thus, in this study, a new method to measure rind colour was proposed. After visually evaluating fruit, an estimation of the percentage of surface displaying each pigmentation intensity (I1-absence—very light, I2-medium, I3-intense, and I4-very intense) was recorded. To this

end, the skin colour of each fruit was evaluated all round its surface; firstly, one 180° fruit face was evaluated before it was rotated to evaluate the opposite 180° fruit face. Two to three different pigmentation areas were generally detected on each fruit (Figure S1). Then measurements of the parameters L*, a*, b* of the CIELAB space were taken in all of these areas using a Minolta colorimeter (model CR-300; Minolta Co., Ltd., Osaka, Japan), and the ratio a*/b* was calculated for each area. Then, a unique a*/b* value was calculated per individual fruit according to the following formula:

a*/b* = [(% of surface with intensity 1 × a*/b*-value in this area) + (% of surface with intensity 2 × a*/b* value in this area) + (% of surface with intensity 3 × a*/b* value in this area) + (% of surface with intensity 4 × a*/b*value in this area)]/100.

Pulp colouration was determined by squeezing each fruit individually via an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). The a*/b* value was measured on juice samples, which were also used to determine JY, TA, and TSS. The juice yield was expressed as a percentage, calculated by dividing the volume of juice by the total fruit weight. The TA was determined by titration with a 0.1 N NaOH solution, using phenolphthalein as the indicator, expressed as g of citric acid per 1 L of juice. The TSS in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd., Tokyo, Japan) and data were expressed as %. The maturity index (MI) was calculated as TSS/TA.

2.3. Sensory Evaluation

Sensory studies were performed with the fruit from Orchard 2 to evaluate if rind pigmentation had an effect on the juice sensory properties. The triangle discrimination test technique [15,16] was used to determine whether there were any detectable differences in the sensory properties of the juice samples. A panel of 71 consumers evaluated the juice samples from fruit lots P1 (slight rind pigmentation) and P3 (intense rind pigmentation) (Figure 1). To this end, after separating the volume of juice needed to determine the aforementioned physicochemical characteristics, the remaining juice obtained from fruit lots P1 and P3 was used in the sensory analysis. For each lot, five juice samples were obtained by mixing the juice that remained from 10 fruits in each one. Before the sensory evaluation, colour, TA, TSS, MI, and total anthocyanin content were determined in these five juices of each type. Then consumers compared the juices obtained from fruits with low pigmented rinds (juice P1) versus very intense rind pigmentation (juice P3). Panellists were simultaneously presented with three samples—two from juice P1 and one from juice P3, or vice versa.

Consumers were between the ages of 22 and 61 years; the male/female ratio (%) was between 43/57. All consumers voluntarily agreed to participate in the evaluation session. Panellists were seated in partitioned booths and samples were randomly presented to avoid any positional bias as the middle sample was usually chosen as "odd". The possible combinations of samples from the triad were: AAB, ABA, BAA, BBA, BAB, and ABB. All samples were coded with three-digit random numbers. In order to avoid consumer responses being conditioned by juice colour, samples were served in opaque cardboard glasses with perforated lids and a red-coloured straws (Figure S2). In addition, red lights were used in the tasting booths; 30-mL juice samples were served at room temperature and panellists were provided with glasses of water for palate cleansing, which they used between samples. Then they were asked to taste samples from left to right and to indicate the odd sample. They were also asked to indicate the main reason why they found the odd sample different compared to the other two.

In addition to the physicochemical characterisations (colour, TA, TSS, and MI) of the juice samples used for the sensory evaluation, two 2-mL samples were frozen for the posterior total anthocyanin content (TAC) evaluation. The total anthocyanin content (TAC) was determined by the pH differential method [17]. Anthocyanin pigments undergo reversible structural transformations with changes in pH manifested by strikingly different absorbance spectra. The coloured oxonium form predominates at pH 1.0 and the colourless

hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurements of the total anthocyanins, expressed as mg/L.

2.4. Statistical Analysis

An ANOVA was applied to the physicochemical data to evaluate if they depended on external pigmentation. A multiple comparison between means was run by Duncan's multiple range test (p = 0.05). To determine if significant differences were perceived by consumers between the evaluated juice samples, the number of correct answers in the triangle test was calculated and the significance of differences according to binomial distribution was established. All statistical analyses were performed with the XL-stat programme (2019 version).

3. Results

3.1. Pulp Pigmentation Is Linked to Rind Pigmentation

In this study, the rind pigmentation of fruit depended on the orchard. The fruit from Orchards 1 and 2 showed similar pigmentation ranges and were divided into three lots according to pigmentation intensity (P1—slight, P2—medium, and P3—intense). However, the fruit from Orchard 3 were generally more coloured and homogenous, and only two lots were obtained (P2—medium and P4—very intense pigmentation). A representation of the different pigmentation intensities is shown in Figure 1.

One of the objectives of this study was to evaluate to what extent of information (i.e., about internal fruit properties) that the 'Sanguinelli' rind colour provides us with. Therefore, we needed to be sure that the colour measurements reflected, as much as possible, the human perception of fruit colour. To this end, the heterogeneous distribution of pigmentation around the rind surface was considered when measuring the external colour. The procedure is explained in detail in the Material and Methods section; it mainly took into account the intensity and area of pigmentation while measuring the rind colour. Our results show a gradual increase in the a*/b* index, parallel to the rind pigmentation intensity. Thus the colour index of samples shown in Figure 1 is as follows: P1= 0.51 $\pm$ 0.07; P2= 0.72 $\pm$ 0.16; P3 = 1.28 $\pm$ 0.3; P4 = 1.59 $\pm$ 0.37.

Figure 2 shows the mean values for the different physicochemical characteristics determined per pigmentation group and orchard. For all three orchards, significant differences in the a*/b* values were detected among the different sample groups created based on the visual perception of rind colour (Figure 2A, letters above bars). When the a*/b* values of the different lots (P1, P2, P3, and P4) were compared among orchards, no significant differences were detected among the lots with the same rind pigmentation intensity (Figure 2A, letters inside bars). This reveals that the visual fruit classification was consistent among the orchards.

The mean a*/b* values of the juice obtained from the 50 fruits belonging to each group are shown in Figure 2B. The ANOVA analysis, performed to explore if pulp colour depended on rind pigmentation, revealed a clear pattern that was detected in the three orchards. Thus, for each orchard, the more intense the rind pigmentation was, the more coloured the juice was. In all cases, except for P2 and P3 from Orchard 2, significant differences in juice pigmentations were detected according to the rind colour.

However, when taking into account the fruit from the three orchards to perform the statistical analysis, some differences in the juice colour were detected between fruits with the same rind pigmentation, but from different orchards. For example, the juice of the P1- fruit from Orchard 1 had a significantly lower a*/b* value than that of the juice from the P1-fruit from Orchard 2. Therefore, despite detecting a common pattern in all three orchards (the more intense the rind pigmentation, the more coloured the juice), significant variabilities existed among the orchards.

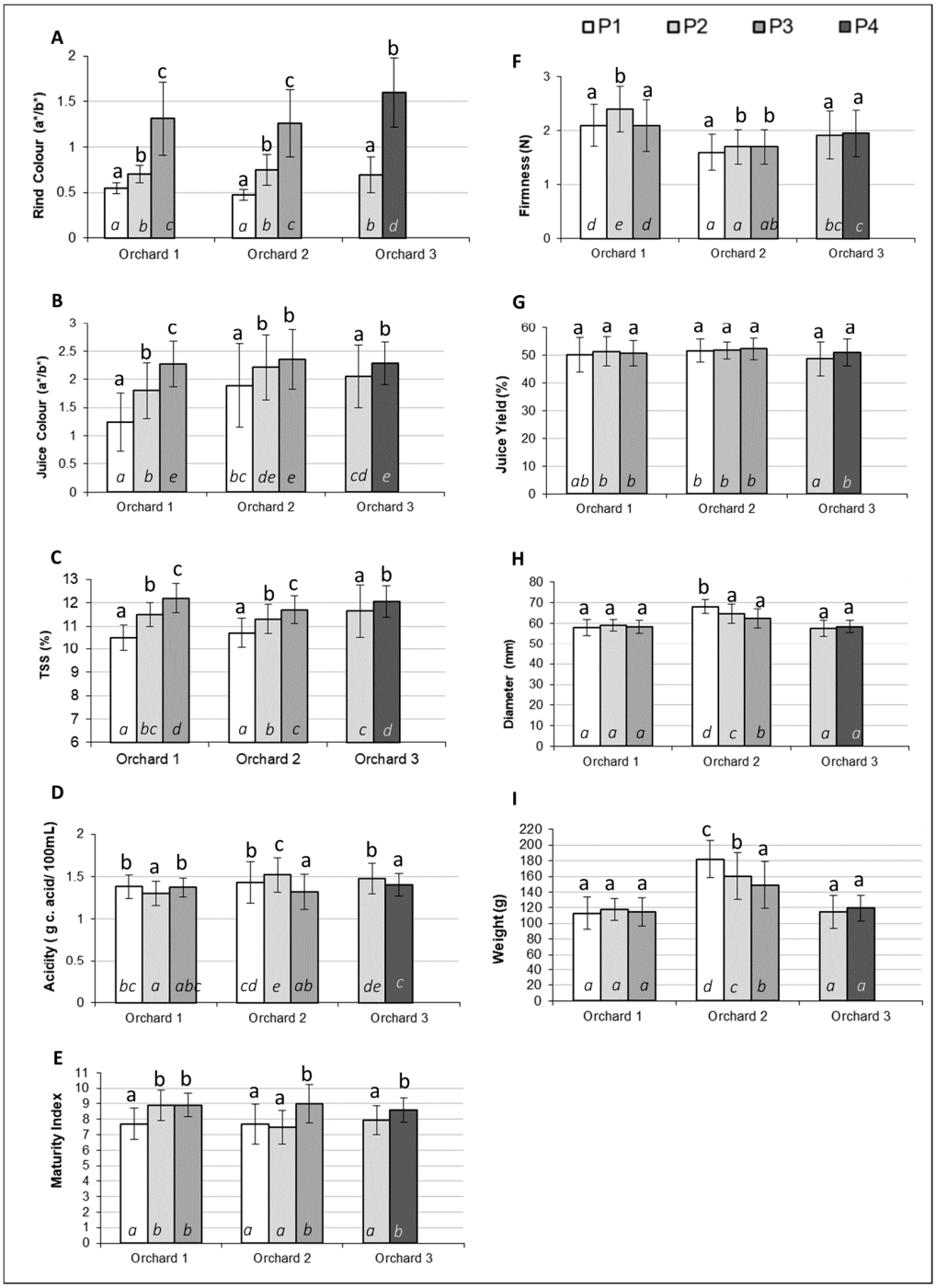


Figure 2. Effect of rind pigmentation intensity (P1—slight, P2—medium, P3—intense, P4—very intense) of 'Sanguinelli' on internal properties ((**A**)—rind colour, (**B**)—juice colour, (**C**)—TSS, (**D**)—titratable acidity, (**E**)—maturity index, (**F**)—firmness, (**G**)—juice yield, (**H**)—diameter, (**I**)—weight). For each orchard, different letters above the bars indicate significant differences among fruit lots. The italics at the bottom of the bars denote differences among fruit lots when comparing all the fruit lots; i.e., when comparing the lots from the three orchards by Duncan's multiple range test ($p = 0.05$).

A Pearson correlation analysis revealed that correlation between a*/b* values of rind and a*/b* values of juice was significant for Orchard 1 but not for Orchard 2 and 3. Despite the coefficient correlation being relatively low, r = 0.29, significance was detected when correlation was performed, taking into account data from the total dataset (Table S1).

To more profoundly study the relation between the rind and pulp colour, the frequency distribution histogram was obtained (Figure 3). To help interpretate these data, a juice colour scale was developed in this study (Figure 4). Bars in the histogram (Figure 3) represent the percentages of juice samples (y-axis) within a certain range of a*/b* values (X-axis). A similar distribution was detected in the three orchards under study. The fruit with slight rind pigmentation (P1) displayed wide pulp pigmentation variability, with a*/b* values ranging between 0.25 and 2.25 (Orchard 1) and between 0.25 and 3.75 (Orchard 2). As observed in Figure 4, these values correspond to the fruit with orange pulp and red-coloured pulp, respectively. In the fruit with rind colour P2, variability was narrower than in P1, as pulp colour was generally more pigmented, and most of the samples had a*/b* values that fell within the range of 1.25–2.50 (Figure 3). In the P3 group, no juice with a*/b* values lower than 1.25 was detected, and most samples concentrated a*/b* values between 2 and 2.75, which corresponded to deep red juice. Finally, the most rind-pigmented fruit (P4) obtained pulp a*/b* values between 1.50 and 3, which were once again related to deep pigmented pulp (Figure 4). Therefore, it can be stated that, although certain intra- and inter-orchard variabilities can be expected, the more pigmented the skin colour is, the higher the probability of finding fruit with a deep red pulp.

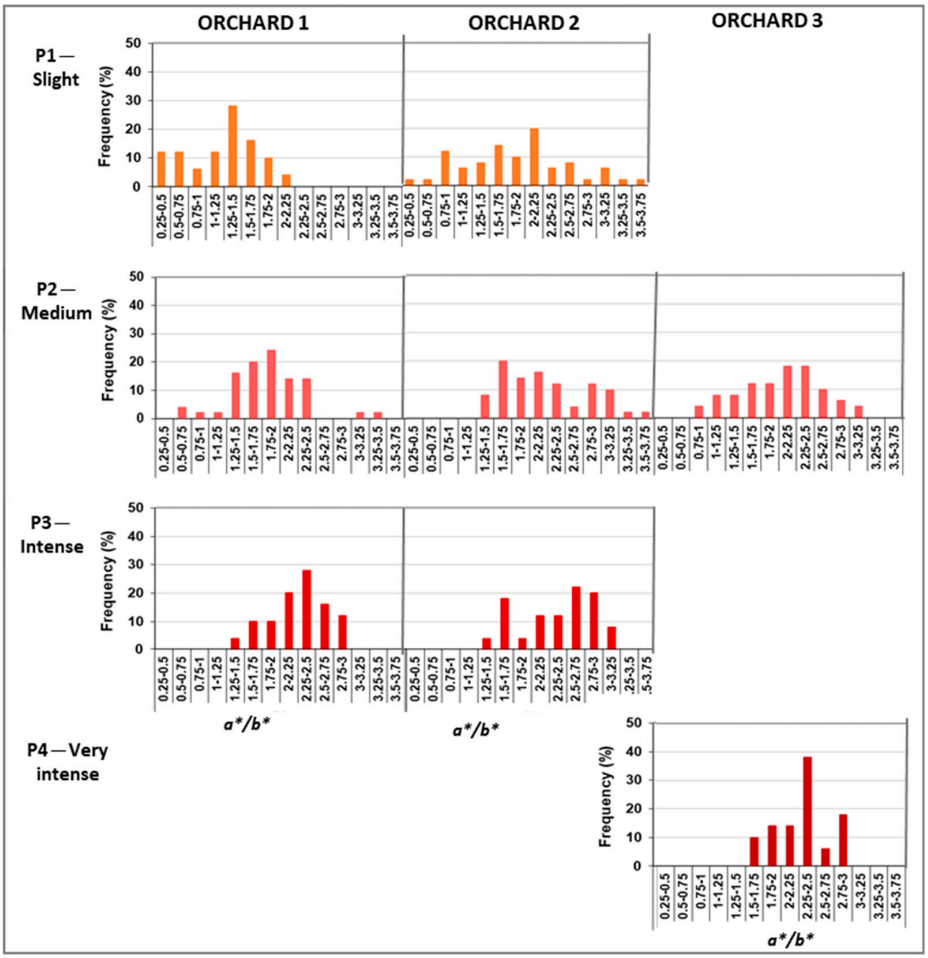


Figure 3. Frequency distribution of the pulp colour (a*/b* values of juice) of 'Sanguinelli' blood oranges grouped according to rind pigmentation (P1—slight, P2—medium, P3—intense, P4—very intense). Each group of intensity per orchard consisted of 50 individually analysed fruits.

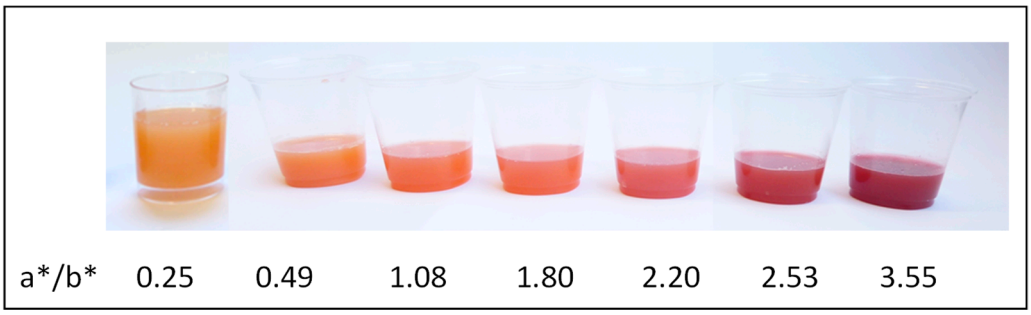


Figure 4. Relation between visual colour of juice and a*/b* index.

3.2. Concentration of Total Soluble Solids Depends on Rind and Pulp Pigmentation

Figure 2C shows the content of total soluble solids depending on external colouration. Interestingly, our results showed for the fruits of each orchard that the more intense the rind pigmentation was, the higher the content of soluble solids in the juice. This effect was corroborated in the fruits from the three orchards under study.

Similar to that reported for juice colouration, certain variabilities among orchards were observed, mainly in the more intensely pigmented fruit.

Once again, we looked closely at the relation between TSS and rind colour by obtaining the frequency distribution histogram, in which bars represent the percentage of juice samples with a certain range of TSS content (Figure 5). TSS ranged between 8.5 (P2-Orchard 3) and 13.5 (P3-Orchard 1). Similar to that described for pulp colouration, TSS showed certain intra- and inter-orchard variability but, in all cases, the more pigmented the rind, the higher the frequency of the samples with high TSS. This was clearly observed in Orchard 2, where the most frequent TSS content was 10.5–11% for P1-fruit, 11–11.5% for P2-fruit, and 11.5–12% in P3-fruit.

Moreover, as a close relation between rind pigmentation and pulp colouration was observed in this study, the relation between pulp colour and total soluble solids was also investigated. To this end, the Pearson correlation between the a*/b* values and the TSS of the juice samples were studied by taking into account the 400 fruits individually evaluated in this study. The correlation coefficient was r = 0.53 and the *p*-value was lower than 0.05, which indicates that TSS content was significantly related to pulp colouration. Significant correlations were also detected when data from each of the orchards were analysed separately (Table S1). As the a*/b values of juice increased, as colouration changed from orange to deep red, a positive correlation between them and TSS indicated that the more pigmented the pulp, the higher the TSS content.

Parallel to the increment in TSS associated with rind pigmentation, the MI was found to be linked to some extent to rind colouration (Figure 2E). Although this relation was not as clear as that of the rind colour and TSS, it was still evident that the most pigmented rind fruit had a higher MI than the less coloured fruit.

3.3. Physicochemical Characteristics Not Linked to Pigmentation

Apart from juice colour, TSS, and MI, the other physicochemical characteristics herein evaluated did not show a relation with rind colour. In the three orchards, firmness values came close to 2% of deformation with juice yield at around 50%, and no differences were found among the sample groups (Figure 2F,G).

Fruit size, determined as diameter and weight, was slightly bigger in the fruit from Orchard 2. In this orchard, an effect of pigmentation was observed, as the most pigmented fruits were smaller. However, this pattern was not detected in Orchards 1 or 3 (Figure 2H,I).

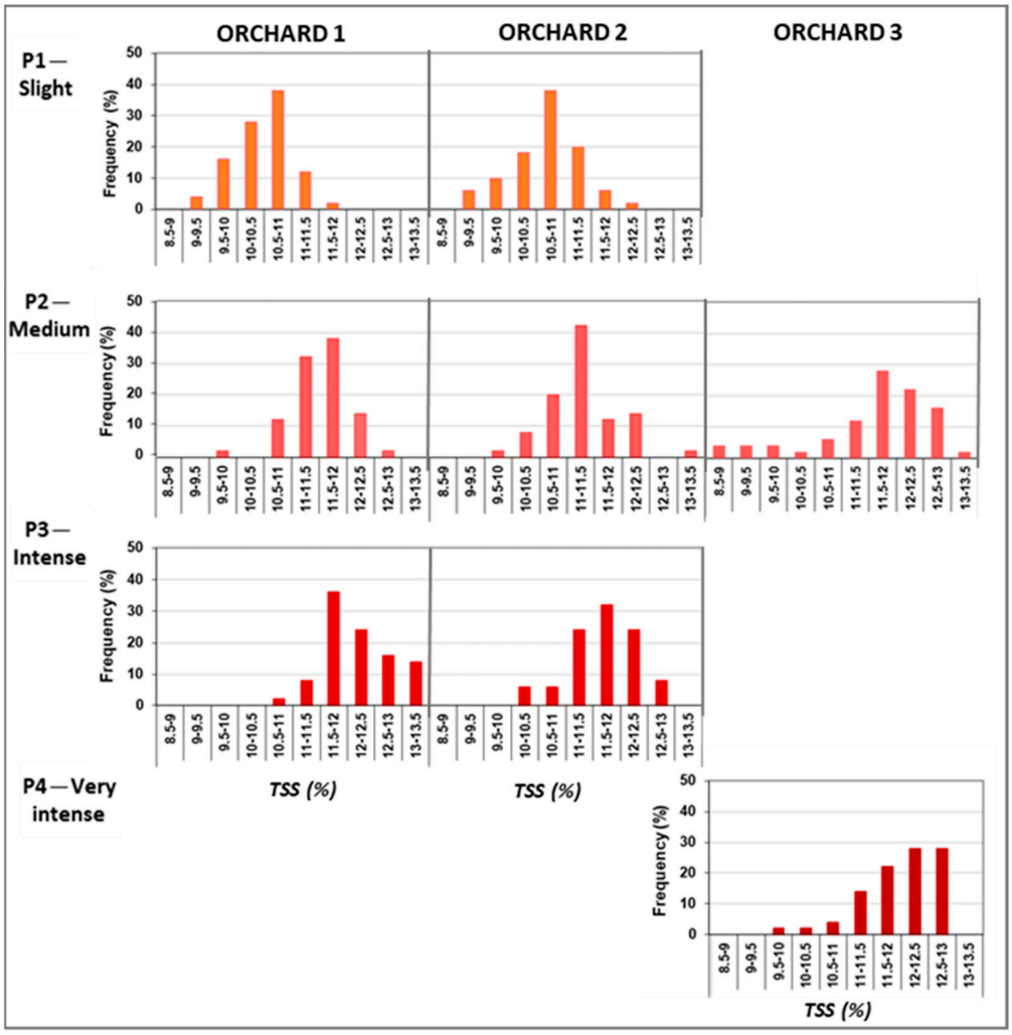


Figure 5. Frequency distribution of the total soluble solid content of 'Sanguinelli' blood oranges grouped according to rind pigmentation (P1—slight, P2—medium, P3—intense, P4—very intense). Each group of intensity for each orchard consisted of 50 individually analysed fruits.

3.4. Effect of Rind Pigmentation on Sensory Properties

The second main objective of this study was to investigate if the sensory properties perceived by consumers when tasting 'Sanguinelli' fruits depended to some extent on rind pigmentation. To this end, a triangle test was carried out.

The test was performed with fruit from Orchard 2, and juice samples were obtained from the fruits with slight (a*/b* = 0.55) and intense (a*/b* = 1.31) rind pigmentations (Table 1). The mean values of the TSS content, TA, MI, total anthocyanin content, and the colour of the juice samples obtained from these fruits are shown in Table 1. According to our previous results, the juice obtained from the most pigmented rind fruit (juice P3) was the most coloured and had a higher TSS than the juice obtained from the less pigmented rind fruit (juice P1). As no differences were detected in TA, the higher TSS of juice P3 resulted in a higher MI. In agreement with its more intense pigmentation, juice P3 showed a higher total anthocyanin content than juice P1. The results from the triangle test revealed significant differences in their sensory properties (p-value = 0.04). The main reasons given by consumers (94% of those who gave a correct answer) involved differences in the sweetness/acidity level.

Table 1. Physicochemical characteristics of juice samples with markedly different rind pigmentations (Orchard 2, samples P1—slight rind pigmentation and P3—intense rind pigmentation) and results from the triangular test performed by consumers. TA is expressed as g citric acid/100 mL and TAC as mg/L. For each parameter, * indicates significant differences between the two different juice samples.

Samples	**Physicochemical**					
Juice	a*/b* Rind	a*/b* Juice	TSS (%)	TA	MI	TAC
P1	0.55 *	1.12 *	11.2 *	1.5	7.4 *	40 *
P3	1.31	2.36	12.1	1.4	8.6	59
	Sensory					
Juice	Trials		Correct answers		*p*-value	
P1 vs. P3	71		51		0.04	

* Significant differences between juices according to the LSD test (p-value < 0.05).

4. Discussion

Rind pigmentation heterogeneity is a characteristic of most blood orange varieties. According to Kafkas et al. [18], the most coloured area usually corresponds to the part of the fruit with a north orientation.

An accurate procedure to measure rind colour, considering the rind pigmentation heterogeneity, was herein stablished. This method allows obtaining colour measurements that better reflect the human eye perception of fruit colouration. Accurate colour data were the basis to evaluate the relation between rind pigmentation and the internal fruit properties. From a practical point of view, when colour is determined by a colorimeter, this method can be slow, but useful, for researchers. Moreover, it could be implemented into colour determination with automatic calibrator machines.

The individual evaluation of 400 fruits revealed a clear relation between juice and rind pigmentation in one of the main varieties cultivated in the Mediterranean region. To date, the relation between rind and pulp colour has been mainly approached by comparing different varieties. Accordingly, Cebadera-Miranda et al. [11] reported that it is possible to find fruit with an intensely coloured rind, but a slight coloured pulp, and vice versa. However, we focused on variability within the same variety, and our results revealed that the more pigmented the rind, the higher the probability of an intensely coloured pulp.

As pulp pigmentation is linked to anthocyanin content in Sanguinelli fruits [11], our results imply that, to some extent, rind pigmentation is an indicator of pulp anthocyanin content. In this sense, as consumer interests in healthy foods increase, one reason to promote blood oranges is based on their high anthocyanin content. Hence, this information can be very useful for the citrus industry and consumers, as intensely pigmented oranges may offer added value due to their high content of antioxidant compounds.

However, it is worth noting that significant inter-orchard variability was detected. Thus the criterion that the more pigmented the rind, the higher probability of obtaining fruit with a high anthocyanin content, seems to apply mainly when comparing fruit from the same lot.

According to Lo Piero [12], anthocyanin accumulation in blood orange cultivars is affected by different factors, such as variety, maturity, cultivation region, cultural practices, and many other environmental factors. Therefore, some of these factors are likely to contribute to the inter-orchard variability herein observed. Among them, it is possible that temperature contributes to the most intense rind pigmentation of fruit from the Orchard 3. Thus, the highest temperatures at day and the lowest at night were recorded for this orchard. According to Butelli et al. [19], all blood orange varieties require strong day–night thermal clines for intense colour formation in fruit flesh, and varieties such as 'Moro', with the potential for high pigmentation, are strongly dependent on the prevailing climatic conditions during fruit ripening for full colour development.

However, we should note that, as herein reported for the first time, under the same conditions (same orchard), anthocyanin accumulation in 'Sanguinelli' blood orange pulp parallels to rind anthocyanin accumulation, to some extent.

Interestingly, our results also revealed that the TSS content was clearly related to rind and pulp pigmentation. Along these lines, in a study in which the development and maturation process of blond and blood oranges were compared, Muccilli et al. [20] found a higher accumulation of enzymes related to sugar in the pulp of blood oranges. These authors linked the higher sugar metabolism required in blood cultivars to the need for carbon skeletons required for anthocyanin biosynthesis [20]. More recently, Carmona et al. [21] carried out a protein analysis while storing 'Moro' at low temperatures and described that when fruits were stored at 9 °C, anthocyanin accumulation took place, which correlated with the promotion, among others, of the protein belonging to the metabolism of sugars. Therefore, some previous information has indirectly reported the accumulation of sugars during processes in which anthocyanins also accumulated, such as fruit development or storage at low temperatures. However, this study demonstrates for the first time that among the fruit of the same lot, the more pigmented the fruit is, the higher the TSS content. If we assume that the MI is an indicator of the fruit maturity stage, it can be stated that the most pigmented fruits were in the most advanced maturity stage. However, it is important to clarify that, at least partially, the accumulation of sugars in blood oranges seems to run parallel to anthocyanin accumulation, and is not directly related to the maturation process. It is well-known that increments in TSS associated with citrus fruit maturation are accompanied by a drop in the acidity level. However, our results showed no differences in the TA depending on the pigmentation for any of the three studied orchards. Similar to TA, other physicochemical characteristics, such as fruit size, firmness, or juice yield, were not related to pigmentation.

Finally, we conducted a sensory test to evaluate if fruit pigmentation could affect the sensory properties perceived by consumers. The initial hypothesis that led us to approach this question was that anthocyanin content was reported to affect the sensory properties of hibiscus drinks [22]. Moreover, in wine, anthocyanin content may affect the sensory profile by a reaction with other compounds [23,24]. Our results showed that consumers found differences between juice samples obtained from slight versus intense pigmented rind oranges. These samples showed significant differences in juice colour, which corroborated the stablished relation between external and internal pigmentation. Moreover, a physicochemical analysis showed that they differed in terms of MI, with an almost 1-point difference. Such a difference in MI was associated with a higher TSS content of the most pigmented fruit. The great majority (94%) of consumers that identified the odd sample, referred the sweetness/acidity perception as the main detected difference. Therefore, the differences in the MI index were identified as the main factors to affect the sensory properties perceived by consumers. Hence, our hypothesis in this regard is that the anthocyanin content itself does not affect sensory properties, but the TSS accumulation that occurs parallel to anthocyanin accumulation may result in sensory differences between highly pigmented fruit and slightly pigmented fruit.

To summarise, our results revealed that the internal properties of 'Sanguinelli' blood oranges are linked to rind pigmentation. For the fruit from the same orchard, the more intense the rind colour, the more pigmented the pulp and, therefore, the higher anthocyanin content. Moreover, TSS content also increases in parallel to anthocyanin accumulation. As the acidity level does not depend on fruit pigmentation, this increment in TSS results in fruit with a higher MI, which leads consumers to perceive that the most pigmented fruit is sweeter than the slightly pigmented fruit. Therefore, the intensity of rind pigmentation may act as a quality parameter linked to nutritional and sensory properties. Sorting fruit by rind colour would allow the industry to commercialize batches of fruit with homogenous properties and to give added value to the most pigmented ones.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8050448/s1, Figure S1: Visual estimation of rind colour areas where colour measurements were taken per fruit. After evaluating each side of the fruit (one 180° fruit face and the opposite 180° fruit face), the areas with the same intensity (I) were added and then divided by 2; Figure S2: Samples presentation in booths to consumers for the triangle test; Table S1: Pearson correlation between the colour index (a*/b*) of rind and juice, and between the colour index (a*/b*) of juice and total soluble solids.

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