Unusual Carotenogenesis in Fruits with Pronounced Anthocyanic Ripening (*Olea europaea* Var. *Arbequina*)

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Fruits of the *Olea europaea* Arbequina variety showed a carotenogenic stage overlapping the biosynthesis of anthocyanins. At the start of ripening, the carotenoid concentration increases (20%) or is maintained (besides the esterification of xanthophylls found in this variety), compared with a decrease (30%) found in the same period in the Picual variety (used as a control that was representative of the rest of the varieties). Both the β,β and β,ϵ carotenoid series were found to be implicated in this carotenogenic process. Differences in both varieties for the chlorophyll *a*/*b* ratio, chlorophylls/carotenoids ratio, and relative carotenoid composition may indicate the existence of a different metabolism of chloroplast pigments, but also indicates a different structure and function of the photosynthetic apparatus. This fact shows that the photosynthetic behavior in the Picual variety is similar to that of shaded leaves, and in the Arbequina variety is similar to that of sun leaves.

Keywords: *Carotenogenesis; carotenoids; chlorophylls; chloroplastic pigments; metabolism; olive*

INTRODUCTION

Work on the chlorophyll and carotenoid composition of edible products from the olive is relatively recent. Analysis of chlorophylls in a lipid matrix has required the adaptation of existing methodologies (*1*). Since the solution of the initial problem, significant advances have been made in understanding the metabolism of these compounds during olive ripening (*2*) and of the effect of the different systems of industrial processing on their structures (*3*, *4*), and the degradation mechanisms and kinetics have been established (*5*, *6*).

Most unripe fruits are green; their color is due to the presence of chloroplast pigments. With ripening, photosynthetic activity diminishes and the chlorophylls disappear. At the same time, the concentration of carotenoids associated with these compounds decreases continuously. However, when the fruit becomes ripe, this fraction may disappear, or its concentration may remain constant or increase.

Qualitatively, the chloroplast pigment profile in fruits of the Manzanilla, Gordal, Picual, Blanqueta, Cornicabra, and Hojiblanca olive varieties does not vary with ripening. Instead, it matches the profile generally found in fruits whose final color is due to the synthesis of other compounds, such as the anthocyanins, which can even mask the presence of chlorophylls and carotenoids (*7*).

In olive fruits, independently of the ripe fruit's high content in fatty matter, the xanthophylls are not esterified (*1*). This indicates that the chloroplast remains intact during ripening (*8*). However, the study of the chlorophyll and carotenoid composition of different single-variety virgin olive oils has provided the first contradictory evidence: esterified xanthophylls are present in the oil extracted from fruits of the olive Arbequina variety. The case is an exception, as they have not been detected in other single-variety virgin olive oils (*9*). This exclusivity, together with the fact that Arbequina was the only variety known whose fruits become markedly yellow at the start of ripening, when the other varieties are pale green, led us to study the chlorophyll and carotenoid profile of the fruits of this variety (*10*). That study confirmed the presence of esterified xanthophylls in the fruit, and noted the possibility of a certain carotenogenesis with the start of ripening, specifically between the ripeness states of pale green and yellow. The partial esterification of the carotenoids in this variety has two important repercussions for the oils extracted from its fruits. On one hand, esterification increases the stability of the carotenoids (*11*), making the color of the respective oils stable and ensuring a determined level of substances able to show antioxidant activity (*12*). On the other hand, the esterification of the carotenoid pigments helps to improve their bioavailability, as the presence of fatty matter is a determinant factor for increasing their absorption and transport (*13*, *14*). The carotenoids esterified and solubilized in olive oil have a lipid environment that impregnates all the pigments; at molecular level, the carotenoid has its own lipid environment, thereby increasing the bioavailability when these micronutrients are ingested (*15*).

The characterization and study of the carotenogenic process has been very productive in the past few years (*16*, *17*). Research has focused, above all, on genetic mechanisms controlling the process, mainly in the tomato and pepper, which show the highest degree of carotenogenesis. In contrast, it has not been easy to study, and less so to demonstrate, a carotenogenic process in a fruit with anthocyanic ripening (such as a variety of olive). That is the aim of the present study.

The confirmation of this point would explain for the first time the differences found between the oil extracted from the Arbequina fruits (with excellent organoleptic

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Table 1. Changes in Content of Chloroplastic Pigments during the Initial Phase of Ripening of Fruits of the Arbequina and Picual Varieties[a]

		chlorophylls		carotenoids	
ripeness state	total pigments	concentration[b]	% loss	concentration	% loss
		Arbequina variety			
green	76.99	56.28 ± 5.62	-	20.71 ± 0.78	-
yellow					
1	78.50	53.67 ± 3.03	4.64[c]	24.83 ± 0.56	+19.89[d]
2	62.12	41.37 ± 0.36	26.49	20.75 ± 0.02	+0.19
3	57.69	38.89 ± 2.53	30.90	18.79 ± 1.40	9.27
mean value	66.10	44.64	20.68	21.46	+3.62
mottled	50.66	33.70 ± 1.27	40.12	16.96 ± 2.45	18.11
		Picual variety			
green	350.62	279.12 ± 5.76	-	71.5 ± 0.73	-
pale green					
1	295.4	232.87 ± 4.32	16.57	62.53 ± 3.05	12.54
2	218.0	170.13 ± 3.43	39.05	47.87 ± 0.40	33.05
3	185.39	144.00 ± 1.17	48.41	41.39 ± 0.99	42.11
mean value	232.93	182.33	34.67	50.60	29.23
mottled	165.66	129.20 ± 7.21	53.71	36.46 ± 0.05	49.01

[a] Mean value ± SD ($n = 3$). [b] Milligrams per kilogram of dry weight. [c] Loss accumulated. [d] Gain accumulated.

characteristics and peculiar chemical properties) and the rest of the olive varieties. For this purpose in the present study, we carried out a detailed study of the beginning of ripening of Arbequina fruits and compared the results with those of Picual fruits, which we used as a control that was representative of the rest of the varieties.

MATERIALS AND METHODS

Starting Material. The study was carried out with fruits of the olive, *Olea europaea* (L.), of two varieties: Arbequina and Picual. The olive trees, three per variety, were situated at the Agricultural Experimental Station at Cabra (Córdoba). Sampling was done during fruit ripening in the season 1998−99. Olive ripening is not synchronous over the tree, but takes place in stages. Thus, after the beginning of anthocyanin synthesis, the tree bears green fruits, spotted ones (with anthocyanin compounds appearing as small red spots on the fruit surface), and purple ones (fruits having the surface completely covered with anthocyanins). Their proportion varies with ripening: the less-ripe fruits gradually disappear, giving way to those that are riper. For our study, sampling was done at the start of ripening, when the first spotted fruits appeared on the tree, and before the purple ones appeared. The sample consisted of 3 kg of developed fruits with coloration from green to spotted, collected from around the whole perimeter of each tree.

Extraction and Identification of Pigments. Samples were made from a triturate homogenized from 50 de-stoned fruits (ca. 40 g) of the most representative color by accurately weighing 4 g for each analysis according to the degree of ripeness of the fruits. Pigment extraction was performed with *N*,*N*-dimethylformamide according to the method of Mínguez-Mosquera and Garrido-Fernández (*1*). The technique is based on the selective separation of components between *N*,*N*-dimethylformamide and hexane. The hexane phase carried over lipids, di-esterified xanthophylls, and the carotene fraction, while the phase corresponding to *N*,*N*-dimethylformamide retained chlorophylls and xanthophylls (free and mono-esterified). This system yields a solution of pigments that is free from the fatty matter characteristic of these fruits and which interferes with subsequent separation and quantification of pigments. The hexane phase was dissolved in diethyl ether (100 mL) and treated in a decanting funnel with 100 mL of a 20% solution of KOH in methanol. Distilled water was added to break the phases after 1 h, with the pigments passing to the ether phase and the soaps formed to the aqueous phase. The ether phase was washed three times with water and another three times with an aqueous solution of Na_2SO_4 (2%) to neutrality. It was concentrated to dryness under vacuum at a temperature <30 °C. The final residue was dissolved in a small volume of acetone. All analyses were performed in triplicate under a green light. Details about the pigment identification have been described in previous papers (*9*, *18*, *19*).

Pigment Separation and Quantification. This was carried out by HPLC using a HP 1100 Hewlett-Packard liquid chromatograph fitted with an HP 1100 automatic injector and diode array detector. Detection was simultaneously performed at 410, 430, 450, and 666 nm. External standard calibration was used for quantitation. Data were collected and processed with an LC HP ChemStation (Rev. A.05.04). A stainless steel column (25 × 0.46 cm), packed with 5-μm C_{18} Spherisorb ODS-2 (Teknokroma, Barcelona, Spain) was used. The column was protected with a precolumn (1 × 0.4 cm i.d.) packed with the same material. The solution of pigments in acetone was centrifuged at 13000*g* (MSE model micro centaur) prior to injection into the chromatograph (20 μL). Separation was performed using an elution gradient (flow rate 2 mL min^{-1}) with the mobile phases (A) water/ion pair reagent/methanol (1:1:8, v/v/v) and (B) acetone/methanol (1:1, v/v). The ion pair reagent was 0.05 M tetrabutylammonium acetate (Fluka, Chemie AG Switzerland) and 1 M ammonium acetate (Fluka) in water. The gradient scheme has been described in detail in a previous work (*19*). External standard calibration was used for quantitation. Chlorophylls *a* and *b*, α-carotene, and β-carotene were supplied by Sigma Chemical Co. (St. Louis, MO). Reference samples of 9-cis and 13-cis β-carotene were supplied by Hofmann-La Roche (Basle, Switzerland). Lutein, anteraxanthin, violaxanthin, and neoxanthin were obtained from a pigment extract of fresh spinach and separated by TLC with silicagel GF_{254} (0.7 mm) on 20 × 20 cm plates using petroleum ether (65−95 C)/acetone/diethylamine (10:4:1) (*19*). β-Cryptoxanthin was obtained from red peppers (*20*). All standards were purified by TLC using different eluents described in a previous publication (*19*).

RESULTS AND DISCUSSION

Changes in Chlorophylls and Carotenoids. Table 1 shows the changes in content of total pigments and of the isochromic chlorophyll and carotenoid fractions of fruits of the Arbequina and Picual varieties during the initial phase of ripening. It also shows the percentage loss or gain accumulated during the ripeness states studied for each pigment fraction considered. In the case of Arbequina, the color changes from green to yellow. It is at first weak, but becomes more and more intense, until the anthocyanin compounds appear as small reddish spots (mottled fruit). In contrast, the Picual

Table 2. Carotenoid Composition of Fruits of the Arbequina and Picual Variety[a]

	lutein	β-carotene	neoxanthin	n. esterified	violaxanthin	v. esterified	anteraxanthin	β-criptoxanthin
				Arbequina variety				
green	11.20 ± 0.52	3.98 ± 0.04	1.37 ± 0.22	0.14 ± 0.01	2.31 ± 0.09	0.14 ± 0.00	1.24 ± 0.00	0.33 ± 0.02
yellow 1	12.99 ± 0.63	4.80 ± 0.27	1.28 ± 0.02	0.26 ± 0.02	3.17 ± 0.11	0.17 ± 0.01	1.74 ± 0.10	0.45 ± 0.02
yellow 2	11.48 ± 0.50	3.60 ± 0.25	0.96 ± 0.03	0.33 ± 0.01	2.22 ± 0.26	0.19 ± 0.01	1.68 ± 0.09	0.30 ± 0.04
yellow 3	9.88 ± 0.52	3.49 ± 0.46	0.99 ± 0.05	0.33 ± 0.02	2.28 ± 0.19	0.19 ± 0.01	1.34 ± 0.08	0.30 ± 0.10
mottled	9.46 ± 1.58	3.01 ± 0.01	0.75 ± 0.31	0.33 ± 0.02	1.69 ± 0.28	0.22 ± 0.02	1.28 ± 0.19	0.21 ± 0.03
				Picual variety				
green	41.54 ± 0.57	14.39 ± 0.24	6.25 ± 0.17	-	5.73 ± 0.01	-	2.62 ± 0.04	0.17 ± 0.06
pale green 1	39.75 ± 0.36	11.21 ± 1.05	5.16 ± 0.54	-	4.16 ± 0.36	-	1.95 ± 0.13	0.29 ± 0.03
pale green 2	29.22 ± 0.25	7.96 ± 0.16	4.24 ± 0.22	-	3.48 ± 0.51	-	1.68 ± 0.07	0.21 ± 0.05
pale green 3	27.17 ± 0.59	6.77 ± 0.09	2.42 ± 0.06	-	3.19 ± 0.12	-	1.40 ± 0.08	0.22 ± 0.00
mottled	23.56 ± 0.06	6.11 ± 0.20	2.40 ± 0.26	-	2.78 ± 0.03	-	1.27 ± 0.28	0.21 ± 0.02

[a] Mean value ± SD ($n = 3$). Abbreviations: n. esterified, neoxanthin monoesterified; v. esterified, violaxanthin mono and di-esterified. Units are milligrams per kilogram of dry weight.

fruit, like the other olive varieties, during this stage shows only a diminishing intensity of the green coloration until it becomes spotted. In previous studies, the phase of yellow coloration in the Arbequina variety and that of pale green in the other varieties has been considered a determined ripeness state within the color sequence during ripening of olive fruits. In the present work, that stage was broken down into three individual stages, depending on the degree of color of the fruit. The results were studied both for these individualized stages and as an average for the overall stage, so that they could be contrasted with results from earlier research.

Except for the presence of esterified xanthophylls in the Arbequina variety, the chlorophyll and carotenoid profiles of the fruits of the two varieties are qualitatively the same, as already reported in detail in an earlier work (*7*). Quantitatively, however, there are interesting differences. The total pigment content is between three- and 4-fold larger in Picual than in Arbequina, and in both varieties these compounds are degraded with ripening, as in all the varieties of olive studied. However, when the content and changes of each isochromic pigment fraction are considered separately, there are marked differences between the presence and behavior of these fractions, depending on variety. The chlorophyll fraction concentration in Picual is 4-fold that in Arbequina, whereas, in general, that of carotenoids is only 3-fold. Moreover, whereas the chlorophyll fraction concentration gradually falls in both varieties as the fruits ripen, that of the carotenoid pigments differs, differentiating the two olive varieties. In Arbequina, the yellow stage involves an increase in the mean carotenoid fraction concentration of 3.62% as noted in an earlier work reporting for this stage an increase in carotenoids of 2.15% (*10*). In the same period, this fraction fell by 29.23% in the Picual variety.

Following step-by-step the carotenoid changes in yellow fruits of the Arbequina variety, it is observed that in state 1, the carotenoid concentration increases by approximately 20%, in state 2 it increases by 0.2%, and in state 3 it falls by 9%, confirming the possible existence in these stages (and for this variety) of a certain net synthesis of carotenoids. Separation of the yellow state into three correlative stages according to apparent fruit color has shown that the carotenoid fraction concentration increases significantly (Duncan test $p < 0.05$). During the same period, the fruits of Picual lose some 12, 33, and 42% of the carotenoid concentration. Thus, the pattern of this pigment fraction differs during this ripening phase depending on olive variety. At the same time, in both varieties there is a gradual loss of chlorophylls, which in state 3 is 30.9% for the Arbequina variety and 48.41% for the Picual variety.

Last, when the fruits reach the mottled state, the Picual variety has lost some 53.71% of chlorophylls, compared to a loss of 40.12% in Arbequina, while the carotenoid losses are 49.01% for Picual and 18.11% for Arbequina. The total percentage loss of pigmentation in this stage of ripening is higher for both chlorophylls and carotenoids in Picual than in Arbequina, and in both varieties it is the chlorophyll fraction that is most affected. The difference in the presence and behavior of these isochromic pigment fractions depending on variety explains both the different surface color of the fruits and the disparity between varieties in the carotenoid catabolic stage.

Table 2 shows the carotenoid compositions of the fruits of the two varieties during the studied ripening stage. In the Picual variety, there is a gradual decrease in the concentration of each carotenoid pigment with ripening, typical of this phase in the life cycle of fruits with anthocyanic ripening. In contrast, in the Arbequina variety, there are significant increases (Duncan test $p < 0.05$) in almost all the carotenoid pigments during the same ripeness states. In particular, in the first yellow state, there is a specific net increase in the concentration of lutein, β-carotene, violaxanthin, antheraxanthin, and β-cryptoxanthin. In contrast, the concentration of esterified xanthophylls increases gradually and continuously.

During the development of a noncarotenogenic fruit, the chlorophylls gradually disintegrate and the chloroplast carotenoid concentration tends to fall. In a carotenogenic fruit, the chloroplast pattern is different: while chlorophylls disappear, carotenoids begin to be synthesized, either those already present or new ones. Gross (*21*) describes increases in the concentration of carotenoids, including lutein, during ripening of the Dancy Tangerine (*Citrus reticulata*). The same author, in a later recompilation (*22*), generalizes on the fact that during the carotenogenic process, the predominant β,ε carotenoid series is usually replaced in part by β,β carotenoids. In the Arbequina variety, there are increases in both the β,β carotenoid series (β-carotene, violaxanthin, antheraxanthin, and β-cryptoxanthin) and in the β,ε series (lutein).

Research on the carotenogenic process taking place in fruits has always been in those where the biosynthetic activity is very high: tomato (*17*) and pepper (*16*, *20*). It has been established that, in contrast to the

Table 3. Main Relationships between the Chloroplastic Pigments Fractions of Fruits of the Arbequina and Picual Variety[a]

	chlorophylls/carotenoids	chlorophyll *a*/*b*
	Arbequina variety	
green	2.71 ± 0.17	4.91 ± 0.20
yellow 1	2.16 ± 0.14	5.33 ± 0.30
yellow 2	2.00 ± 0.06	6.62 ± 0.51
yellow 3	2.06 ± 0.03	6.69 ±0.05
mottled	1.99 ± 0.08	6.81 ± 0.32
	Picual variety	
green	3.90 ± 0.15	3.86 ± 0.15
pale green 1	3.72 ± 0.07	3.99 ± 0.11
pale green 2	3.62 ± 0.05	3.97 ± 0.12
pale green 3	3.49 ± 0.11	4.04 ± 0.09
mottled	3.54 ± 0.09	4.06 ± 0.07

[a] Mean value ± SD ($n = 3$).

chloroplast, which contains a limited pool of carotenoids varying little throughout the plant kingdom, the chromoplast presents a great profusion of carotenoids in all varieties (*23*). In fruits of the Arbequina variety, the carotenogenic process is expressed at a lower level, and although the increase in carotenoids is not spectacular, it is statistically significant (Duncan test $p < 0.05$), and sufficient to show that during the initial stage of ripening there is a carotenogenic process. This is supported, exclusively for this olive variety, by the presence of esterified xanthophylls, which appear progressively during ripening in carotenogenic fruits. Xanthophyll esterification takes place at the level of newly formed carotenoids in the chromoplasts and not at the level of chloroplasts. This suggests that as the chromoplasts are formed at the cost of the chloroplasts, the carotenoids enter the plastoglobules and are esterified by the fatty acids present there. The process is physiologically important, as it increases the lipophilic nature of the xanthophylls, helping their accumulation within the plastoglobules, which is the main characteristic of this organelle (*24*, *25*). Consequently, in these fruits, the chloroplast does not remain intact during ripening, but degenerates to chromoplast.

Relationship between Pigments and Structure/Function. Chlorophylls and carotenoids form part of the photosynthetic apparatus: either in reaction centers or in antenna complexes. Thus, the different composition in chloroplast pigments shown by the Arbequina and Picual varieties (the latter representing the many other olive varieties studied; *1*, *7*) is a reflection of the difference in the structures of the photosynthetic apparatus in the two varieties.

Table 3 shows the main relationships between the chloroplast pigment fractions. It can be observed that the ratio of chlorophylls to carotenoids is clearly higher in Picual (3.65 ± 0.16) than in Arbequina (2.18 ± 0.31) in all states of ripeness studied. It can be established that although both varieties contain the same pigment profile, the relative amount of each great group is different.

The same table shows explicitly that between the two varieties there are systematic differences in the ratio of chlorophyll *a* and chlorophyll *b* throughout ripening. The mean value in the Arbequina variety is 6.07 ± 0.58, and in Picual the mean value is 3.98 ± 0.08. The difference between the two varieties (2.0 units) is large enough to postulate differences in the photosynthetic apparatus. As Anderson et al., (*26*) established, there is an inverse ratio between the chlorophyll *a*/*b* ratio and the degree of thylakoid stacking. Consequently, the different relative presence of pigments in the two varieties is a reflection of the structural differences in the photosynthetic apparatus.

The chlorophyll *a*/*b* ratio is an indirect measurement of the reaction center/antenna complex distribution in the thylakoid. The antenna complexes are relatively rich in chlorophyll *b*, whereas the reaction centers are rich in chlorophyll *a* (*27*). Studies on modifications in the *a*/*b* series ratio have been carried out mainly in leaves subjected to different conditions of light. Under shade conditions, the *a*/*b* series ratio decreased (*28*). That is, the light-collecting centers increased more than the reaction centers.

The chlorophyll *a*/*b* ratio in fruits has usually been described as remaining around 2.5−4.0 when studied in peppers (*29*), gherkins (*30*), tomatoes (*31*), and olives (*1*). The Picual variety could be considered within the average, but not the variety Arbequina, which structurally must possess fewer antenna complexes than the other varieties.

Table 4 shows the percentage contribution of each carotenoid pigment in the fruits of the two varieties. In general, in the ripeness states studied, the percentage of lutein and neoxanthin is higher in the fruits of Picual, whereas those of the other individual carotenoids are higher in fruits of Arbequina. In a work carried out in leaves of different plant genera (*32*) subjected to conditions of light and darkness, it was found that in shaded leaves, the percentage of lutein and neoxanthin is higher than in sun leaves, whereas the latter exhibited higher percentages of β-carotene and the violaxanthin cycle, in accord with earlier evidence (*33*). In adittion, the same authors also found that chlorophyll *a*/*b* ratios are higher in sun leaves than in shaded leaves, and, from the data shown, we have calculated the chlorophyll/carotenoid ratio, which is higher in shaded leaves than

Table 4. Percentage of Individual Carotenoids of Fruits of the Arbequina and Picual Varieties[a]

	lutein	β-carotene	neoxanthin	n. esterified	violaxanthin	v. esterified	anteraxanthin	β-criptoxanthin
				Arbequina variety				
green	54.15	19.25	6.62	0.68	11.17	0.53	6.00	1.60
yellow 1	52.24	19.36	5.16	1.05	12.79	0.52	7.02	1.81
yellow 2	55.40	17.37	4.63	1.59	10.71	0.77	8.11	1.45
yellow 3	52.69	18.61	5.28	1.76	12.16	0.80	7.15	1.60
mottled	55.94	17.80	4.43	1.95	10.00	1.00	7.57	1.24
				Picual variety				
green	58.67	20.68	8.74	-	8.01	-	3.66	0.23
pale green 1	63.57	17.93	8.25	-	6.65	-	3.12	0.46
pale green 2	62.58	16.95	9.93	-	7.41	-	3.58	0.45
pale green 3	65.64	16.36	5.85	-	7.71	-	3.38	0.53
mottled	64.62	16.76	6.58	-	7.62	-	3.48	0.57

[a] Abbreviations: n. esterified, neoxanthin monoesterified; v. esterified, violaxanthin mono and di-esterified.

in sun leaves. Taking into account the chlorophyll/carotenoid ratio, the chlorophyll *a*/*b* ratio, and the percentage composition of the individual carotenoids, the photosynthetic behavior in the Picual variety is similar to that of shaded leaves, and in the Arbequina variety is similar to that of sun leaves.

The photosynthetic apparatus has mechanisms to dissipate the excess energy during saturation of light, thereby protecting pigments and chloroplast membrane proteins from damage (caused by the formation of active oxygen species). The degree of energy dissipation is measured by chlorophyll fluorescence quenching, which has been found to be correlated with the degree of deepoxidation of the xanthophyll cycle (*34*). In conclusion, we might postulate that the high percentages of violaxanthin and antheraxanthin in the Arbequina variety, compared with those in Picual, constitute an adaptive response to excess light: an attempt to dissipate all the excess energy.

Moreover, Brugnoli et al. (*32*) found a significant direct relationship between the amount of photoconvertible violaxanthin (measured as % violaxanthin + antheraxanthin + zeaxanthin) and the *a*/*b* series ratio in the four species studied in shaded and sun leaves. Similar results were reported by Demming-Adamms (*35*), in leaves of 22 species exposed to both sun and shaded habitats. Again, excepting the differences between studies carried out in leaves and ours in fruits, there is a parallelism between the two parameters in the olive varieties: Arbequina shows a high *a*/*b* series ratio and high violaxanthin + anteraxanthin percentage, whereas the Picual variety shows very low values for these two parameters. Up to now, no functional explanation has been found from the ratio between the two parameters (*36*).

Considering that the fruits of Arbequina and Picual came from the same plot and were picked at the same time, the illumination conditions to which they were subjected can be considered the same. The response (related with the chloroplast pigment composition) of the photosynthetic apparatus to a particular intensity of light in the Picual variety is like that of a leaf acclimatized to shade, and that in the Arbequina variety is like that of a leaf adapted to sunlight. In the same situation, the responses of the two varieties are very different, and are a reflection of a difference at genetic level between the variety Arbequina and the other olive varieties.

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