Chlorophyll and Carotenoid Patterns in Olive Fruits, *Olea europaea* Cv. Arbequina

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In olive fruits of the cultivar Arbequina, the chlorophyll pigments decrease significantly throughout ripening, while the carotenoids decrease more gradually and discontinuously. There is no degradation of the carotenoid fraction in stages before complete ripeness. The presence of esterified xanthophylls exclusively in this variety suggests that the chloroplast pigment metabolism is different from that in other olive varieties studied previously. There are increases of specific carotenoids, violaxanthin, neoxanthin, antheraxanthin, lutein epoxide, and esterified xanthophylls between the light green and yellowish green ripening stages. Such increases are related to the detection of precursor carotenoids (phytofluene and ξ-carotene) in the yellowish green stage. Chlorophyllides (*a* and *b*) and α-carotene have also been detected exclusively in this variety. Quantitatively, the drastic change in color between light green and yellowish green ripening stages characteristic of this variety can be explained by the considerable reduction found in the chlorophylls/carotenoids ratio. The study of the pigments present in skin and pulp has shown that the pattern of carotenoid distribution differs depending on the fruit part concerned.

Keywords: *Olea europaea; Oleaceae; olive; ripening; biosynthesis; esterification; pigments; chlorophylls; carotenoids*

INTRODUCTION

Carotenoids are associated with chlorophylls in all photosynthetic tissues. Most fruits, when still immature, are green. As ripening progresses, photosynthetic activity decreases and the chlorophylls disappear. The carotenoids associated with these compounds may disappear at the same time. Alternatively, the concentration of carotenoids may be maintained or, as a result of the synthesis of new carotenoids, even increase. This is the case of the so-called carotenogenic fruits in which the typical pattern of chloroplast carotenoids, comprising β-carotene, lutein, violaxanthin, and neoxanthin, is transformed gradually into the much more complex, typical chromoplast pattern. Chromoplasts are plastids that accumulate carotenoids. In many cases, the pigments are accumulated with the aid of proteins that associate carotenoids located within a single structure. Vishnevetsky et al. (1996) have isolated and characterized a cDNA that encodes the chromoplast-specific carotenoid-associated protein (CHRC). A series of proteins take part in the process transforming chloroplast to chromoplast, some of which have already been identified. Lawrence et al. (1997) identified two cDNAs that encode proteins involved in chromoplast development.

The carotenogenic fruits are differentiated from those in which ripening is associated with the synthesis of anthocyanins and betalains (Gross, 1987). In such cases, the typical pigment pattern of the chloroplast does not change during ripening. However, the rates of disappearance of individual pigments may be very different, so that the relative amounts of the latter in the ripe fruit are changed. The qualitative distribution of the chloroplast pigment pattern and the rate of movement or interchange are what distinguish genera and varieties. For example, in olives of Manzanilla, Gordal, and Hojiblanca varieties (Mínguez-Mosquera and Garrido-Fernández, 1989; Mínguez-Mosquera and Gallardo-Guerrero, 1995), the qualitative pattern of chloroplast pigments does not vary with ripening, but in general matches that of the fruits, the final coloration of which is due to the synthesis of compounds of a different nature, such as the anthocyanins, which may even mask the presence of chlorophylls and carotenoids. At the same time, and despite the ripe fruit's high content in fatty matter (Mínguez-Mosquera and Garrido-Fernández, 1989), the xanthophylls are not esterified, indicating that the chloroplast remains intact (Goodwin, 1976). The concentrations of both chlorophylls and carotenoids decrease progressively as the date of fruit picking becomes later, giving way to the anthocyanin components, which begin to show themselves as small reddish spots that progressively cover the entire skin, followed by a violet-red coloration of the skin and pulp (Mínguez-Mosquera and Gallardo-Guerrero, 1995, 1996; Vázquez-Roncero, 1963).

Recent studies carried out on virgin olive oil obtained from olives of the variety Arbequina (an oil very much appreciated for its extremely high quality) have shown the presence of esterified xanthophylls (Gandul-Rojas and Mínguez-Mosquera, 1997). This is surprising, because in the other olive fruit varieties studied previously, carotenoid esterification does not occur, as is typical of noncarotenogenic fruits.

The aim of the present work is to confirm whether, during the ripening of olive fruits of the variety Arbequina, the hydroxylated xanthophylls have been esteri-

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10.1021/jf981158u CCC: $18.00
Published on Web 05/20/1999

fied and whether possible changes in the carotenoid pigment pattern, that do not fit the typical one of the chloroplast, have taken place.

EXPERIMENTAL PROCEDURES

Plant Material. The study was carried out on olives, *Olea europaea* (L.), of the Arbequina variety. The fruits were picked from trees in Cabra (Córdoba, Spain). Sampling was carried out at intervals of ~8 days from October to January during two consecutive harvests: 1995−1996 and 1996−1997. The fruits were picked from all around the four olive trees selected for the study, until a sample of ~1 kg was collected. On each occasion, 100 olive fruits were randomly selected to evaluate the most representative color at that moment. These were, in order of sequence of changes, as follows: intense green, light green, yellowish green, small reddish spots, turning color, purple, and black (Loudiyi et al., 1984).

Pigment Extraction. Samples were taken from a triturate, homogenized from 50 destoned fruits (~40 g) of the most representative color by accurately weighing from 4 to 15 g for each analysis depending on the degree of ripeness of the fruits. Pigments were extracted with *N*,*N*-dimethylformamide (DMF) according to the method of Mínguez-Mosquera and Garrido-Fernández (1989). The technique is based on the selective separation of components between DMF and hexane. The hexane phase removes the lipids, the carotene fraction, and diesterified xanthophylls (Gandul-Rojas and Mínguez-Mosquera, 1997), whereas DMF retains chlorophylls and free and monoesterified xanthophylls. In addition, the carotenoid pigments were saponified individually to identify the esterified xanthophylls. Each pigment isolated by TLC 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 and stored in the dark in a freezer at −30 °C until used. All analyses were performed in duplicate under a green light.

Pigment Isolation and Identification. The methods used for pigment identification are described in previous works (Gandul-Rojas and Mínguez-Mosquera, 1997; Mínguez-Mosquera et al., 1991). Pigments were identified from their spectral absorption maxima and peak ratios and by TLC and HPLC cochromatography with authentic samples. The on-line UV−vis spectra were recorded from 325 to 800 nm with a photodiode array detector. To calculate peak ratio, the height of the longest wavelength absorption band (III) is expressed as a percentage of the middle absorption band (II), the baseline in each case being the minimum between the two maxima (I00III/II) (Davies, 1975). For the esterified xanthophylls, the change in their retention times but not their spectroscopic characteristic was checked after alkaline hydrolysis.

Pigment Separation and Quantification. This was carried out by HPLC using a Waters model 600E liquid chromatograph fitted with an injection valve (Rheodyne model 7125) and a Waters model 994 photodiode array detector. Chromatograms were obtained on a recording integrator (Waters model 5200). A stainless steel column (25 × 0.4 cm i.d.), packed with 5 mm C_{18} Spherisorb ODS-2, was used. The column was protected by a cartridge (5 × 0.4 cm i.d.) packed with the same material. The solution of pigments in acetone was centrifuged at 13000*g* (MSE Micro Centaur model) prior to its 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) methanol/acetone (1:1 v/v). The ion pair reagent was 0.05 M tetrabutylammonium acetate and 1 M ammonium acetate in water (Mínguez-Mosquera et al., 1992). Detection was at 430 nm using a Waters 994 programmable photodiode array detector.

Table 1. Chromatographic and Spectroscopic Characteristics of Pigments from Olive Fruits Separated by HPLC

peak	pigment	k^a BS	k^a AS	position of peak[b] (nm) I	II	III	peak height relationship[c]
1	chlorophyllide *b*	0.17		466	600	650	3.3
2	chlorophyllide *a*	0.96		432	616	664	1.3
3	neoxanthin	3.70	3.70	414	438	466	90
4	violaxanthin	4.56	4.56	417	440	471	94
5	lutein epoxide (tentative)	5.12	5.12	418	440	468	85
6	antheraxanthin	5.20	5.20	(425)	446	474	22
7	lutein	6.25	6.25	424	446	474	60
8	chlorophyll *b*	8.63		466	602	650	3.3
9	β-cryptoxanthin	9.71	9.71	(431)	452	479	25
10	chlorophyll *a*	10.11		432	619	666	1.3
11	violaxanthin monoesterified	11.82	4.56	417	440	471	94
12	antheraxanthin esterified	12.97	5.20	(425)	446	474	22
13	neoxanthin esterified	13.19	3.70	414	438	466	90
14	α-carotene	13.11	13.11	426	446	474	59
15	β-carotene	13.41	13.41	(432)	454	481	26
16	ξ-carotene	13.63	13.63	330	348	366	70
17	phytofluene	13.88	13.88	378	398	422	94
18	violaxanthin diesterified	17.83	4.56	417	440	471	94

[a] Retention factor (k) = $t_r - t_m/t_m$, where t_r is the retention time of the pigment peak and t_m is the retention time of an unretained component; BS, before saponification; AS, after saponification. [b] The values in parentheses indicate inflection points. [c] Peak ratio I/III for chlorophylls and 100III/II for carotenoids.

Standards. Chlorophylls *a* and *b* and α-and β-carotenes 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, antheraxanthin, violaxanthin, and neoxanthin were obtained from a pigment extract of fresh spinach and separated by TLC on silica gel GF_{254} (0.7 mm) on 20 × 20 cm plates using petroleum ether (65−95 °C)/acetone/diethylamine (10:4:1) (Mínguez-Mosquera et al., 1992). β-Cryptoxanthin, phytofluene, and α-carotene were obtained from red peppers (Mínguez-Mosquera and Hornero-Méndez, 1993). All standards were purified by TLC using different eluents as described Mínguez-Mosquera et al. (1992).

Reagents. Solvents included acetic anhydride, acetone, diethyl ether, hexane, *N*,*N*-dimethylformamide, petroleum ether (65−95 °C), and pyridine (all of ACS grade). For HPLC, acetone, methanol (LC grade), and deionized water were used. Chemicals (all of ACS grade) used were NaCl, KOH, and Na_2SO_4.

RESULTS AND DISCUSSION

Pigment Identification. Chlorophyll and carotenoid pigments were isolated and purified using TLC and identified according to their spectroscopic and chromatographic characteristics, before and after saponification of each one separately. Table 1 shows the results obtained. The qualitative pigment profile from the fruit comprises chlorophyll *a*, chlorophyll *b*, and the carotenoids that typically accompany the chlorophylls in the chloroplast (Mínguez-Mosquera and Garrido-Fernández, 1989; Mínguez-Mosquera et al., 1991): lutein, β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin. Besides these pigments, new components are present, some of which were detected previously in virgin olive oil from Arbequina variety olives (Gandul-Rojas and Mínguez-Mosquera, 1997): chlorophyllides, esterified antheraxanthin, esterified neoxanthin, mono- and diesterified violaxanthin, lutein epoxide (tentative), α-carotene, ξ-carotene, and phytofluene.

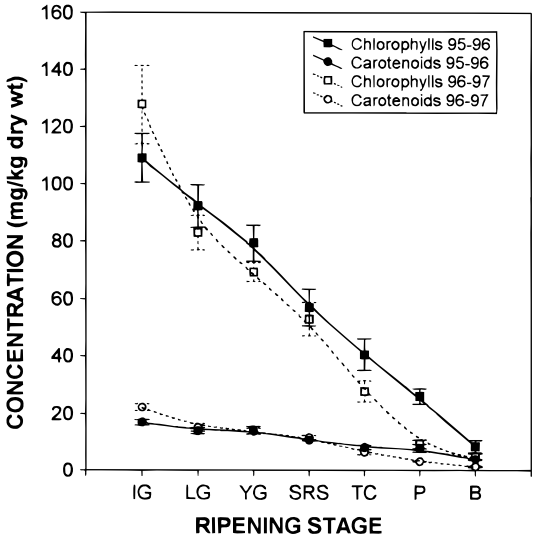

Figure 1. Changes in chlorophyll and carotenoid pigment content during ripening of cv. Arbequina olives, mean values ± SD ($n = 4$). IG, intense green; LG, light green; YG, yellowish green; SRS, small red spots; TC, turning color; P, purple; and B, black.

Derivative pigments associated with acidic medium in the oil extraction process are obviously absent in the fruit (pheophorbides, pheophytins, and the 5,8-furanoid carotenoids mutatoxanthin and luteoxanthin).

Chlorophyllides, esterified xanthophylls, α-carotene, ξ-carotene, and phytofluene are exclusive to this variety; these compounds have not been found in other varieties studied previously (Mínguez-Mosquera and Garrido-Fernández, 1989; Mínguez-Mosquera and Gallardo-Guerrero, 1995). This result is extremely important, as it can be considered a chemotaxonomic differentiating parameter for Arbequina variety olives.

Pigment Changes during Ripening. Figure 1 shows the changes in concentration of the chlorophyll and carotenoid pigment fractions in fruits of the olive (cv. Arbequina) during the different stages of ripeness checked according to the color changes in the fruits. The sequence of changes is parallel in the two harvests studied. The concentration of both pigment fractions falls progressively with fruit ripening, but the decrease is much more marked in the chlorophylls than in the carotenoids. In chlorophylls, this decrease is significant in all stages of ripeness up to purple color (Duncan test $p < 0.05$), whereas that decrease in carotenoids is slower and is not significant in some stages in which its concentration is practically constant.

This result is studied in detail by analyzing the change in concentration of the individual carotenoids (Table 2). The pattern of change in this fraction is set by the major pigment, lutein, which comprises >40% of the fraction. In the 1995−1996 harvest, when the fruit color changes from light green to yellowish green, the decrease in lutein, β-carotene, and violaxanthin does not exceed 9% and is not statistically significant, so that the concentration of these pigments can be considered constant between those two stages. In contrast, the concentration of the remaining carotenoids, including neoxanthin, antheraxanthin, lutein epoxide, β-cryptoxanthin, and esterified xanthophylls, increases in this ripening phase and is significant for antheraxanthin and the group of esterified xanthophylls, which increase 3- and 11-fold, respectively. A balance of the reaction shows the increase in esterified compounds to be the result not only of the transformation of the corresponding xanthophyll precursor but also of a net synthesis. During the change from turning color to purple, the carotenoid fraction is again constant, with even a significant ($p < 0.05$) synthesis of antheraxanthin and neoxanthin.

In the second harvest studied, the pattern of change in the individual carotenoids between the ripeness stages of light green and yellowish green is similar. Lutein decreases by 10.5%, β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin remain statistically constant, and there is a (nonsignificant) increase in lutein epoxide (32%) and esterified xanthophylls (11%). During the change from turning color to purple, all of the carotenoids decreased.

The increase in carotenoids during the yellowish green stage and the presence of esterified xanthophylls may indicate that, in this variety, a part of the chloroplasts is transformed into chromoplasts and a synthesis of β,β series carotenoids occurs.

Therefore, in the variety Arbequina, the pattern of carotenoid distribution is different from that of the other varieties. According to the classification of Gross (1987), this carotenoid distribution fits group 2, typical of the chloroplast, and group 5, characterized by an unusual synthesis of epoxide xanthophylls.

To confirm this, the pigment content was determined independently in both the yellow and still-green zones of fruits classified as yellowish green. The results (Table 3) show that between the green and yellow zones, there is a slight decrease in β-carotene, lutein, and neoxanthin, while violaxanthin, lutein epoxide, and β-cryptoxanthin remain statistically constant and only esterified xanthophylls increase significantly. Therefore, the different color between green and yellow zones cannot be explained by this small xanthophyll synthesis, as it contributes only 1% to the total carotenoid fraction. However, between these differentiated zones in the fruit, the chlorophyll fraction diminishes more (48%) than the carotenoid fraction (20%) and results in a very marked reduction in the chlorophyll/carotenoid ratio, which changes from 4.45 ± 0.18 to 2.87 ± 0.53. Physically, this decrease confers a more intense yellow color on the fruit.

Reports for other fruits, such as avocado, green fleshed melon, and Chinese gooseberry (which retain chlorophylls until full ripening), show differences in the plastid structure of the different fruit parts: the chloroplast structure is maintained only in the outer part, with some carotenogenesis being detected in the inner part (Gross, 1987). Consequently, we differentiated the inner part of the fruit (flesh) from the outer (skin) in the cv. Arbequina and studied the carotenoid composition independently. The pigment concentration in the pulp was $\sim 1/10$ that in the skin. Differences were established by expressing the results as a percentage of the total carotenoid fraction (Table 3). The results are noteworthy, as the proportion of the major carotenoid (lutein) is considerably lower in the pulp, whereas the proportions of β-carotene, violaxanthin, antheraxanthin, lutein epoxide, and the esterified xanthophylls are significantly higher. The proportions of neoxanthin and β-cryptoxanthin do not differ between the skin and pulp.

These results show that the pattern of carotenoid pigment distribution in olives of the cv. Arbequina varies depending on the fruit part concerned, probably

Table 2. Carotenoid Pigment Composition of Fruits during Ripening of Cv. Arbequina Olives[a,b]

	concentration[c] at ripening stage						
pigment	IG	LG	YG	SRS	TC	P	B
				Harvest 1995−1996			
carotenes	3.42 ± 0.25	3.02 ± 0.40	2.94 ± 0.41	2.28 ± 0.10	1.89 ± 0.29	1.67 ± 0.44	0.73 ± 0.07
lutein	7.71 ± 0.48	6.50 ± 0.47	5.92 ± 0.87	4.65 ± 0.19	3.73 ± 0.36	3.08 ± 0.46	2.30 ± 0.21
violaxanthin	2.58 ± 0.22	2.23 ± 0.34	2.20 ± 0.06	1.80 ± 0.32	1.19 ± 0.16	1.19 ± 0.25	0.38 ± 0.12
antheraxanthin	0.21 ± 0.07	0.14 ± 0.02	0.39 ± 0.17	0.33 ± 0.11	0.19 ± 0.02	0.38 ± 0.11	0.12 ± 0.06
lutein epoxide	0.54 ± 0.15	0.46 ± 0.04	0.50 ± 0.19	0.23 ± 0.21	0.26 ± 0.07	0.35 ± 0.07	0.07 ± 0.04
neoxanthin	2.40 ± 0.74	1.46 ± 0.15	2.01 ± 0.30	1.19 ± 0.06	0.73 ± 0.05	0.93 ± 0.16	0.24 ± 0.08
β-cryptoxanthin	0.06 ± 0.04	0.15 ± 0.12	0.20 ± 0.24	0.08 ± 0.03	0.04 ± 0.04	0.08 ± 0.08	0.02 ± 0.00
esterified	0.02 ± 0.03	0.01 ± 0.03	0.11 ± 0.11	0.11 ± 0.10	0.13 ± 0.02	0.15 ± 0.05	0.04 ± 0.02
				Harvest 1996−1997			
carotenes	5.81 ± 1.58	3.43 ± 0.86	3.25 ± 0.16	2.45 ± 0.49	1.59 ± 0.27	0.67 ± 0.29	0.50 ± 0.18
lutein	9.72 ± 0.53	6.20 ± 0.65	5.54 ± 0.48	4.55 ± 0.34	2.79 ± 0.42	1.67 ± 0.29	0.66 ± 0.14
violaxanthin	3.61 ± 0.62	2.14 ± 0.11	1.93 ± 0.45	1.76 ± 0.15	0.82 ± 0.24	0.34 ± 0.12	0.11 ± 0.03
antheraxanthin	0.77 ± 0.09	0.82 ± 0.17	0.78 ± 0.12	0.64 ± 0.05	0.31 ± 0.10	0.13 ± 0.07	0.04 ± 0.02
lutein epoxide	0.68 ± 0.07	0.28 ± 0.20	0.37 ± 0.06	0.38 ± 0.20	0.14 ± 0.06	0.08±0.04	0.02±0.01
neoxanthin	1.64 ± 1.09	2.16 ± 0.40	1.83 ± 0.10	1.52 ± 0.26	0.75 ± 0.19	0.30 ± 0.07	0.05 ± 0.03
β-cryptoxanthin	nd	0.06 ± 0.03	0.04 ± 0.01	0.06 ± 0.03	0.02 ± 0.02	0.01 ± 0.01	nd
esterified	0.02 ± 0.04	0.09 ± 0.06	0.10 ± 0.07	0.11 ± 0.03	0.06 ± 0.04	0.02 ± 0.02	nd

[a] Destoned olive basis, mean values ± SD ($n = 4$). [b] Key as in Figure 1: carotenes = β-carotene + α-carotene + phytofluene + ξ-carotene; esterified = total esterified xantophylls; nd, not detected. [c] Milligrams per kilogram of dry weight.

Table 3. Carotenoid Pigment Composition in Different Fruit Parts of Cv. Arbequina Olives[a,b]

	zones of the fruit			
	concentration[c]		percentage	
pigment	green zone	yellow zone	skin	pulp
total chlorophylls	57.62 ± 3.38	29.75 ± 5.52	76.40 ± 2.42	68.78 ± 4.34
total carotenoids	12.97 ± 0.94	10.41 ± 0.98	23.60 ± 2.42	31.22 ± 4.34
chls/carots	4.45 ± 0.18	2.87 ± 0.53	3.27 ± 0.45	2.26 ± 0.52
carotenes	3.15 ± 0.15	2.34 ± 0.21	5.69 ± 0.56	9.87 ± 0.81
lutein	4.99 ± 0.50	3.73 ± 0.23	9.55 ± 1.23	6.12 ± 1.09
violaxanthin	1.89 ± 0.27	1.90 ± 0.52	3.29 ± 0.28	7.21 ± 0.95
antheraxanthin	0.33 ± 0.07	0.31 ± 0.02	1.17 ± 0.23	2.14 ± 0.36
lutein epoxide	0.57 ± 0.14	0.66 ± 0.36	1.08 ± 0.17	2.26 ± 0.28
neoxanthin	1.99 ± 0.26	1.24 ± 0.29	2.09 ± 0.47	2.28 ± 0.30
β-cryptoxanthin	0.06 ± 0.01	0.06 ± 0.02	0.48 ± 0.15	0.77 ± 0.18
esterified	0.04 ± 0.01	0.11 ± 0.02	0.25 ± 0.03	1.62 ± 0.43

[a] Destoned olive basis, mean values ± SD ($n = 6$). [b] Key: chls/carots = total chlorophylls/total carotenoids; carotenes = β-carotene + α-carotene + phytofluene + ξ-carotene; esterified = total esterified xanthophylls. [c] Milligrams per kilogram of dry weight.

as the result of a carotenogenic process in the pulp. The detection of carotenoid precursors such as phytofluene and ξ-carotene in the yellowish ripening stage shows that there is a de novo synthesis of some carotenoids. However, there is no net synthesis of the carotenoid fraction in the fruit, because the genesis is in the inner part, where the pigment concentration is $\sim 1/10$ that in the outer. Such small increases are diluted when the whole fruit is analyzed and are unnoticeable within the standard deviation normal for natural products.

A contributory factor is that lutein, which is the major pigment and sets the pattern for the carotenoid fraction, does not take part in this biosynthetic process. Although the presence of lutein's precursor, α-carotene, has been detected in this olive variety, it seems that the biosynthetic pathway of the β,ε series is less favored than that originating in the β,β series. The most significant increases in concentration are detected in antheraxanthin and the esterified xanthophylls that include monoesterified antheraxanthin and neoxanthin and mono- and diesterified violaxanthin, all of which are of the β,β series.

The results are novel, as none of the olive varieties previously studied had esterified xanthophylls, nor has any increase been reported in the carotenoid pigments during the fruit's yellow stage. For comparative purposes, Table 4 includes the change in chlorophylls and carotenoids during the different ripeness stages in olives of the varieties Gordal, Hojiblanca, and Manzanilla (Mínguez-Mosquera and Garrido-Fernández, 1989; Mínguez-Mosquera and Gallardo Guerrero, 1995). The data have been recalculated by taking into account the

Table 4. Chlorophyll and Carotenoid Pigment Content in Different Varieties of Olive Fruits[a,b]

	concentration[c]		
ripening stage	Gordal	Hojiblanca	Manzanilla
	Chlorophylls		
intense green	920.62 ± 121.04	704.28 ± 26.79	412.74 ± 22.56
light green	601.99 ± 88.39	563.91 ± 21.52	308.94 ± 22.93
yellowish green	350.40 ± 24.55	454.21 ± 37.70	231.81 ± 21.75
small red spots	214.13 ± 10.23	344.62 ± 21.22	154.92 ± 9.37
turning color	110.84 ± 7.81	244.19 ± 30.31	100.21 ± 14.30
purple	58.74 ± 8.89	82.15 ± 26.26	39.98 ± 5.86
black	22.45 ± 7.05	34.91 ± 2.68	16.51 ± 5.95
	Carotenoids		
intense green	136.94 ± 18.49	139.04 ± 3.95	80.57 ± 8.68
light green	84.34 ± 13.35	107.99 ± 7.10	54.20 ± 3.50
yellowish green	60.41 ± 5.99	81.27 ± 7.03	40.36 ± 4.95
small red spots	30.08 ± 3.88	68.38 ± 5.51	28.24 ± 2.88
turning color	21.25 ± 3.84	56.66 ± 5.52	20.04 ± 1.98
purple	16.52 ± 2.77	31.96 ± 3.62	14.47 ± 1.75
black	5.24 ± 1.61	25.64 ± 1.04	6.74 ± 2.47

[a] Destoned olive basis, mean values ± SD. [b] Values from Gordal Hojiblanca and Manzanilla supplied by Mínguez-Mosquera and Garrido-Fernández (1989) and Mínguez-Mosquera and Gallardo-Guerrero (1995). [c] Milligrams per kilogram of dry weight.

differences in moisture between the varieties, to express them as milligrams per kilogram of dry weight. During ripening, olive fruits pass through a series of colors, including intense green, light green, yellowish green, mottled, purple, and black. Specifically, in the cv. Arbequina the yellowish green stage is followed by a completely yellow one, which is not seen in the other varieties.

As observed in Table 4, during olive ripening in all varieties, chlorophyll and carotenoid pigments gradually decrease in concentration and tend to disappear, giving way to anthocyanins. Apart from differences in fruit size and moisture between varieties, there are differences in composition (and specifically that of chloroplast pigments) among the varieties Gordal, Hojiblanca, and Manzanilla. Such differences are only quantitative. These are varieties of fruits with the same pattern of chloroplast pigment distribution, which does not change during ripening. This indicates that the chloroplast remains intact and is typical of noncarotenogenic fruits (Goodwin, 1976). Differences among varieties are only in concentration of the pigments and in their rates of interchange and movement.

If the percentage of retention of chlorophyll and carotenoid fractions during ripening is represented semilogarithmically (Figure 2), the slope of the line joining two contiguous states can be considered a measurement of the relative rate, enabling the order of pigment degradation to be established so that varieties can be compared. Gordal begins ripening with the greatest pigmentation concentration in the intense green stage (1058 mg/kg of dry wt), followed by Hojiblanca (844 mg/kg), Manzanilla (493 mg/kg), and Arbequina, with almost 90% less pigmentation than Gordal. In Figure 2a, the line showing the change of percent of chlorophyll retention is steepest in Gordal, followed by Manzanilla, Hojiblanca, and Arbequina. This means that the rate of pigment degradation during ripening is highest in Gordal. The latter's pigmentation level is exceeded by that of Hojiblanca in the yellowish green stage, and this difference is maintained until the end of ripening (Table 4). The pigmentation level in Manzanilla is always lower, although its relative rate of degradation is slower than that of Gordal, and differences become smaller with ripening.

The cv. Arbequina shows a greater retention of pigments despite its levels of pigmentation being very much lower than those of the other varieties. In the intense green stage, the fruits have a total concentration of ~150 mg/kg (Figure 1), which is not reached by the other varieties until the turning color stage.

As Figure 2a shows, the relative rate of chlorophyll degradation for each olive variety is practically constant in the first stages and increases considerably toward the end of the ripening. In Hojiblanca and Manzanilla varieties this event begins with the turning color stage, whereas in Gordal and Arbequina varieties that increase takes place in the next stage (purple color).

Apart from the presence of α-carotene, phytofluene, and ξ-carotene, and the esterification of xanthophylls mentioned above, the main quantitative difference between Arbequina and the other varieties studied lies in the slow degradation of the carotenoid fraction (Figure 2b). Whereas in Arbequina there is no degradation of carotenoids on passing from the light green stage to the yellowish one (slope 0) (and in some specific cases positive increases were detected), in the other varieties

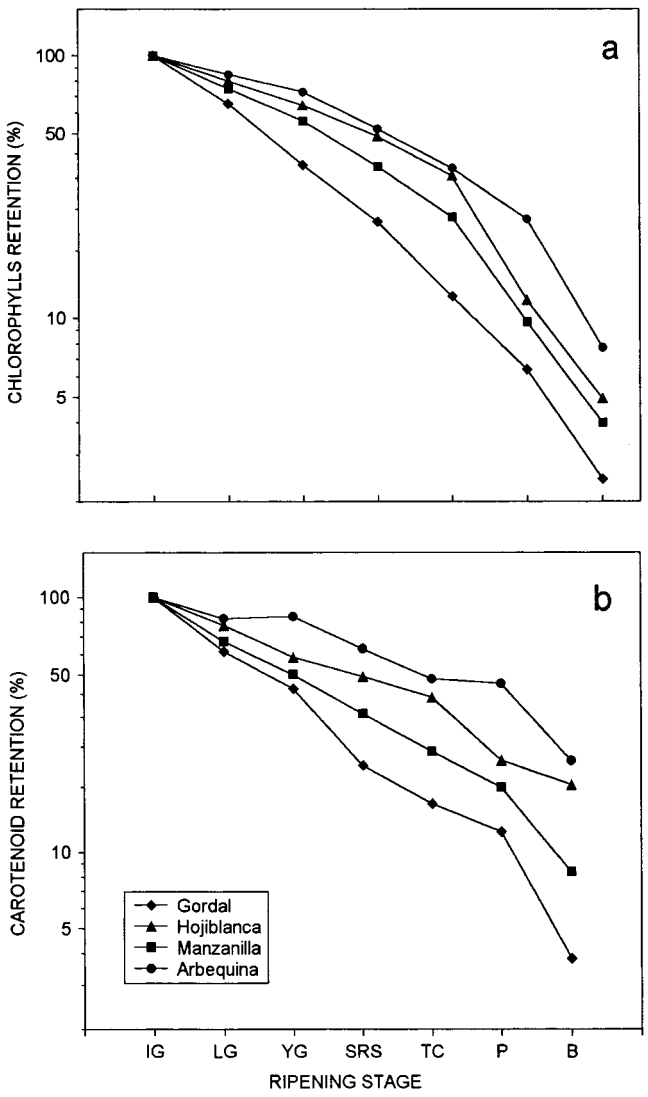

Figure 2. Changes in (a) chlorophyll and (b) carotenoid pigment retention during ripening of different olive varieties.

the passing from one ripeness stage to the next is accompanied by a decrease in concentration of both chlorophylls and carotenoids. This decrease is statistically significant in all cases, even during the green-yellow stage (Duncan test $p < 0.05$).

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Received for review October 19, 1998. Revised manuscript received March 26, 1999. Accepted March 31, 1999. We express our sincere gratitude to the Comision Interministerial de Ciencia y Tecnología of the Spanish Government (CICYT) for supporting this research project, OLI 96-2151.

JF981158U