Changes in Chloroplast Pigments of Olive Varieties during Fruit Ripening

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Changes in chlorophyll and carotenoid pigments of five olive (*Olea europaea* L.) varieties destined for milling were investigated at six consecutive ripening stages. There was a manifest dependence between olive variety, moment of picking, and chloroplast pigment composition of the fruits. Although the content of chlorophylls and carotenoids differed with fruit variety, ripening always involved their gradual loss, which becames more pronounced with increased presence of anthocyanin compounds. The relative rates of disappearance of chlorophylls and carotenoids were markedly different between varieties, implying that the catabolism of these pigments takes place at a relative rate inherent to each variety. The varieties less rich in pigments showed the most extreme behavior. The highest relative rate of disappearance was observed in fruits of the Blanqueta variety, and the lowest was observed in those of Arbequina. The chlorophyll *a*/chlorophyll *b* ratio remained practically constant during ripening, with a value very similar for Hojiblanca, Picual, Cornicabra, and Blanqueta, but much higher for Arbequina, implying that the structure of the photosynthetic apparatus is different in the latter variety. In the five varieties studied, lutein was the slowest carotenoid to be degraded, so that its percentage in the fruits increased with ripening, whereas β-carotene was the fastest to disappear. In ripe fruits covered with anthocyanins, chloroplast pigments were retained in both skin and pulp, with the rate of disappearance being much higher in the latter.

Keywords: *Chlorophylls; carotenoids; choroplastic pigments; ripening; olive varieties; olive oil*

INTRODUCTION

The commercial interest in color as a major factor affecting consumer acceptance of products has led to the study of chlorophyll and carotenoids in crops and vegetables.

Different varieties of a single species must be characterized by selecting those that provide the most appropriate chlorophyll and carotenoid pigment composition for the end product. The high content in chlorophylls and carotenoids of oil seeds is sometimes a problem because of the cost of removal during the technological processing to extract the oil. Many attempts have been made to reduce the green color of the canola seed (*1*−*3*). Changes in green color compounds during ripening of canola and mustard have been attributed to the different rates of synthesis and catabolism of these pigments depending on varieties (*4*). The same aspect was studied in green seeds of soybean (*5*) and in rapeseed (*6*), and with carotenoids in palm seeds (*7*).

In other green vegetables and fruits, the aim is precisely to prevent chlorophyll loss and the consequent yellowing, with the works setting out to investigate the physiological basis of senescence. Thus, Toivonen and Sweeney (*8*) studied the different loss of chlorophylls during ripening in two varieties of broccoli (*Brassica oleracea* L.): the Greenbelt variety retains chlorophylls in a stable form, whereas the Emperor variety shows a continuous degradation. Similar observations have been made in mango (*9*) and kiwi (*10*). Chung and Youn (*11*) assayed the levels of lycopene and α- and β-carotene, in ripe fruits of eight varieties of Korean native squash (*Cucurbita moschata*), and found differences of up to 10-fold between varieties.

Chlorophylls and carotenoids are the pigments responsible for the color of virgin olive oil. Their study began with the adaptation and application of specific analysis methodologies (*12*, *13*), the correlation of color and pigment concentration (*14*), the relationship with stability (*15*), and the pigment composition in monovariety oils (*16*). The latest achievements in this field demonstrate that the content, proportion, and class of pigments present in a virgin olive oil enable the authenticity of the product and the suitability of the process employed to be established (*17*).

Bearing in mind that the chlorophyll (*18*) and carotenoid (*19*, *20*) compounds contained in the oil also have biological utility, such as antioxidant action and provitamin A value, for the organisms that ingest it, their presence in virgin olive oil confers added value, more so when ingested in a fatty medium that aids their absorption and bio-availability (*21*).

Because about 80% and 40% of chlorophylls and carotenoids, repectively, are lost during oil extraction (*12*) that consists simply of mechanical trituration, beating, and centrifugation, our investigation focused on the changes ocurring in these pigments during ripening. For this purpose, five olive varieties destined for milling were chosen to represent the Spanish virgin

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olive oil. Information about the content and class of fruit pigments, together with other quality parameters, is critical in choosing the proper variety at the desired stage of ripeness of olives.

MATERIALS AND METHODS

Starting Material. The study was carried out with fruits of the olive, *Olea europaea* (L.), of five varieties, all destined for oil production. They were chosen both for being representative of different growing regions of Spain and for the large size of the areas under cultivation and volume of oil produced. The Hojiblanca and Picual varieties, representative of Andalucia, were from Cabra (Córdoba); the Arbequina variety is native to the region of Lérida, but in recent years has been grown in Andalucia (in our study, the fruits were from Cabra (Córdoba)); the Blanqueta variety is native to the region of Valencia; and the Cornicabra variety is from the region of Toledo. Sampling was done during fruit ripening in the season 1998−99, beginning when the fruit was developing but still green, and finishing when ripening had covered it with anthocyanins. The fruits (1 kg of sample) were picked from all around the perimeter of the tree. From each picking, 100 fruits were chosen at random to assess the main color at that moment, and to analyze the fruits. The changes, in sequence, were green, light green, mottled, reddish, purple, and black (*22*). The light green stage of ripeness is not homogeneous for all the varieties: Arbequina shows an overall yellow coloration, and Blanqueta is whitish. For the study of chlorophyll and carotenoid composition in skin and pulp, two varieties − Arbequina and Picual − were chosen in three specific stages of ripening: green, reddish, and black. The skin and pulp of each fruit were separated with a knife and those from several fruits were combined to provide a sufficiently representative sample.

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 from 4 to 15 g for each analysis according to the degree of ripeness of the fruits. Pigment extraction was performed with *N*,*N*-dimethylformamide (DMF) according to the method of Mínguez-Mosquera and Garrido-Fernández (*23*).The technique is based on the selective separation of components between *N*,*N*-dimethylformamide and hexane. The hexane phase (HP) carried over lipids and the carotene fraction and that corresponding to N,N,-dimethylformamide (DMFP) retained chlorophylls and xanthophylls. This system yields a solution of pigments free from the fatty matter which is characteristic of these fruits and which interferes with subsequent separation and quantification of pigments. All analyses were performed in triplicate under a green light. Details about the pigment identification have been described in previous papers (*13*, *16*, *24*).

Pigment Separation and Quantification. This was carried out by HPLC using an HP 1100 Hewlett-Packard liquid chromatograph fitted with an HP 1100 automatic injector and diode array detector. Detection was simultaneously performed at 410 nm to measure pheophytins, at 430 nm to measure chlorophylls *a* and neoxanthin, at 450 nm to measure chlorophylls *b*, violaxanthin, anteraxanthin, lutein, β-carotene, and β-cryptoxanthin, and at 666 nm to measure chlorophyllide. 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 of 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 13 000*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, Buchs, Switzerland) and 1 M ammonium acetate (Fluka) in water. The gradient scheme has been described in detail in a previous work (*13*). 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) (*13*). β-Cryptoxanthin was obtained from red peppers (*25*). All standards were purified by TLC using different eluents described in a previous publication (*13*). Duncan's tests were calculated using the program STATISTICA for Windows v. 5.0 (Statsoft Inc. 1984−1996).

RESULTS AND DISCUSSION

Pigment Profile. The chlorophyll and carotenoid composition (mg/kg dry weight) of fruits from five olive varieties (Hojiblanca, Picual, Cornicabra, Blanqueta, and Arbequina) in six consecutive ripening stages (green, light green, mottled, reddish, purple, and black) are shown in Figure 1. From the analytical point of view, in late stages of ripeness all the fruits showed low levels of pheophytin *a*, and in the case of the Arbequina and Blanqueta varieties, the presence of chlorophyllide *a* was detected systematically. As the quantitative contribution of both pigments was small, their values were been included with that of chlorophyll *a* for data treatment. No chlorophyll *b* derivative was detected. The carotenoid fraction comprised β-carotene, lutein, and the xanthophylls neoxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, and lutein epoxide. As the latter components were minor ones, for the quantitative study they were treated together as minor xanthophylls. In the case of Arbequina, esterified violaxanthin and neoxanthin were also determined.

Independent of the initial net concentration of each pigment, all tended to decrease with ripening. In all the ripe fruits, whose coloration is due to anthocyanin compounds, both chlorophylls and carotenoids continued to be present. There was a marked quantitative difference in pigment presence with variety, enabling the varieties to be ranked by their content in chlorophylls and carotenoids. The differences found, both between consecutive stages of ripeness and between varieties, were statistically significant (Duncan's test, $p < 0.05$). It is therefore obvious that the moment when the olives are picked and their variety directly affect the chlorophyll and carotenoid content of the fruits. Consequently, the concentrations of these compounds in the oil will depend on the variety and stage of ripeness of the fruit. These are important factors, bearing in mind that the presence of these pigments in the oil may contribute to increasing its stability, apart from any potential biological function.

Next, we studied in detail how the two pigment fractions disappear during fruit ripening in the different olive varieties.

Chlorophyll Fraction. The highest values for concentration of chlorophyll *a* and chlorophyll *b* were found in fruits of the Hojiblanca variety at all the ripening stages studied. The Picual variety followed closely, and then Cornicabra. Fruits of Blanqueta and Arbequina varieties showed a much lower level: Arbequina being the variety with the lowest chlorophyll pigments level during the first maturity stage and Blanqueta having the lowest levels during the next ones.

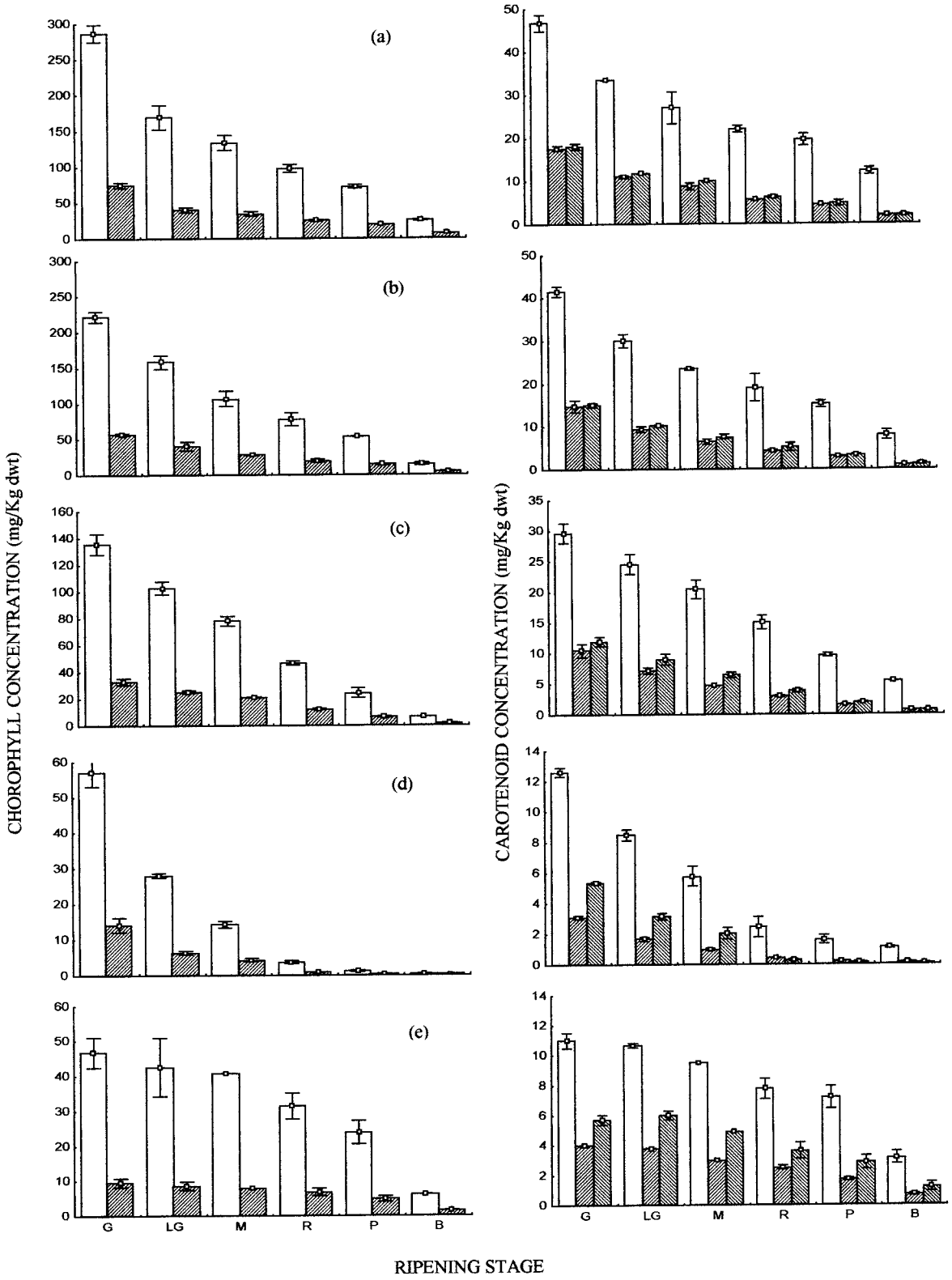

Figure 1. Evolution of the chlorophyll and carotenoid fractions (mg/Kg dwt) in six consecutive ripening stages of five *Olea europaea* varieties: Hojiblanca (a), Picual (b), Cornicabra (c), Blanqueta (d) and Arbequina (e). G, green; LG, light green; M, mottled; R, reddish; P, purple; and B, black. In chlorophylls figures: chlorophyll *a* (□) and chlorophyll *b* (□ with cross-hatching). In carotenoids figures: lutein (□), *β*-carotene (middle □ with cross-hatching), and minor xanthophylls (right □ with cross-hatching).

In general terms, chlorophyll *a* comprised some 80% of the chlorophyll fraction in all the olive varieties, and chlorophyll *b* comprised 20%. Differences in cholorophylls between varieties diminished or increased with fruit ripening. A notable example was the difference between the extremes of the varieties: in Hojiblanca and Arbequina in the green stage, the concentration of chlorophyll *a* and chlorophyll *b* in Hojiblanca was 6-fold that in Arbequina.

During ripening, the concentrations of both chlorophylls decreased continuously in all varieties, concomitant with the increase in anthocyanin pigmentation.

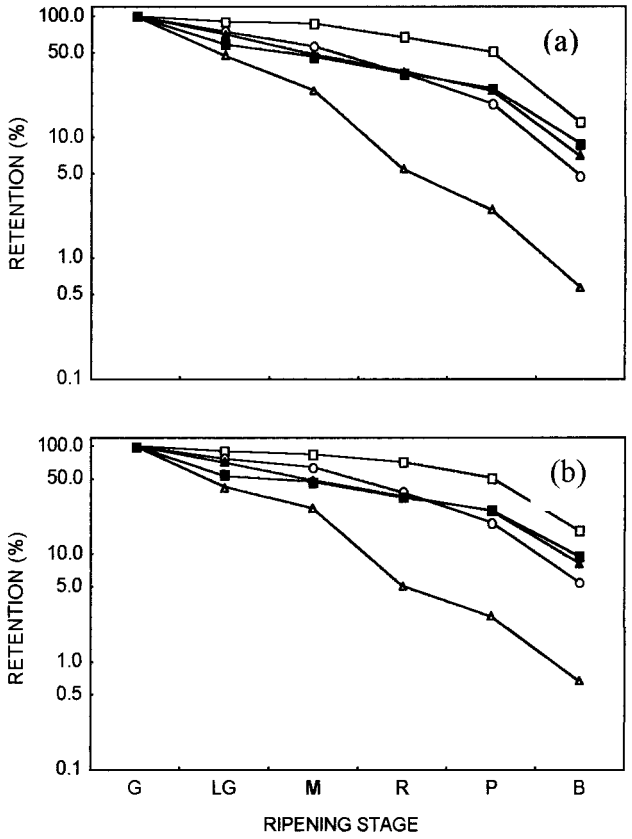

Figure 2. Changes in the retention of (a) chlorophyll *a* and (b) chlorophyll *b* during ripening of five *Olea europaea* varieties: Arbequina (□), Blanqueta (△), Cornicabra (○), Hojiblanca (■), and Picual (▲). Symbols same as Figure 1.

Figure 2 shows the percentage of retention of chlorophyll *a* and chlorophyll *b* for each variety in the stages of ripeness assayed. When this percentage is plotted semilogarithmically, the slope of the line joining contiguous stages of ripening can be considered a measurement of the relative rate of pigment disappearance. This enables varieties to be ranked by rate of retention, showing for each variety whether there is parallelism in the disappearance of the two chlorophylls. The ranking of varieties was similar for the two chlorophylls.

For chlorophyll *a* and *b*, the most extreme cases were the varieties with least pigmentation. Arbequina, with the lowest slope, was the variety retaining most chlorophylls, while Blanqueta, with the highest slope, was the variety with greatest chlorophyll degradation. Between these were the varieties with high pigmentation, Hojiblanca, Picual, and Cornicabra, which showed intermediate rates of retention. Arbequina and Blanqueta, apart from their extreme behavior, are the only varieties in which dephytylated derivatives have been detected, showing the presence of chlorophyllase (*26*). It could be assumed that the pattern of change, with three steps, is similar in all the varieties. The first change of relative rate was when the synthesis of anthocyanins begins in the fruits (mottled stage). This change was sharpest in the Blanqueta variety. The second change, much sharper in all varieties, tooks place when the fruits had a cover of anthocyanin pigmentation (purple stage). In the black stage, pigment degradation accelerated in all the varieties, being sharpest again in Blanqueta.

Therefore, graphs of Figure 2 show that there were three key moments in the disappearance of chlorophylls from olive fruits, directly related with the different

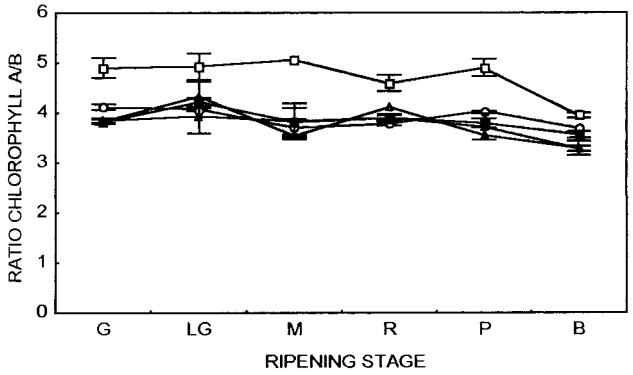

Figure 3. Chlorophyll *a*/chlorophyll *b* ratio evolution in five varieties during the ripening stages assayed. Arbequina (□), Blanqueta (△), Cornicabra (○), Hojiblanca (■), and Picual (▲). Symbols same as Figure 1.

presence of anthocyanin compounds. The pattern of change was observed in all varieties, but with marked differences in the rate of chlorophyll disappearance that seemed to be inherent to each variety. The levels of enzyme activity involved in chlorophyll catabolism must be very different from variety to variety.

Despite the inter-varietal differences, the behavior of chlorophyll *a* and chlorophyll *b* fitted the same pattern in each variety, both in the changes and in the similar slopes they present. This implied that the ratio between the two chlorophylls remained constant during the ripening phases studied, showing that chlorophyll *a* and chlorophyll *b* disappeared together in all the varieties. Figure 3 shows the chlorophyll *a*/*b* ratio for the five varieties in the stages of ripeness assayed. For all varieties, the ratio was more or less constant until the purple stage, and then sharply decreased in the last step of ripening. The least constant was that of the Blanqueta variety, which fluctuated within a narrow range (3.83 ± 0.27) in which were included Hojiblanca, Picual, and Cornicabra. The ratio for the Arbequina variety was practically constant at 4.8 ± 0.40. It was also higher than the other varieties, implying a low level of chlorophyll *b* in this variety. The latest studies at molecular level (*27*) on both light-harvesting complexes and centers of reaction confirm the notion that the light-harvesting complexes are rich in *b*-series while the centers of reaction are rich in *a*-series. From the results obtained, we should therefore assume that Arbequina presents a lower proportion of light-harvesting complexes and that the structure of the photosynthetic apparatus of this variety is not comparable with the rest. Research carried out with mature leaves (*28*−*30*) shows varying *a*/*b* ratio depending on the amount of light received. If mature structures present this adaptation, we can appreciate that there are genetic differences in the distribution of the protein−pigment complexes, differentiating varieties.

As reviewed by Gross (*31*), the chlorophyll contents, and in many fruits the chlorophyll *a*/*b* ratio, vary with the genus, species, variety, environmental factors, and ripeness stage. In the case of the olive, these differences found are at the inter-varietal level, which means that in different varieties chlorophyll degradation occurs at very different relative rates, and that even, as proposed by Johnson-Flanagan and Spencer (*4*) and Toivonen and Sweeney (*8*), the various enzymatic systems are not involved at the same level.

Carotenoid Fraction. The ranking of varieties by concentration of each carotenoid pigment, at whatever

stage of fruit ripeness, coincided with the ranking by chlorophylls. Hojiblanca and Picual presented the highest concentrations, closely followed by Cornicabra and, with lower concentrations, Blanqueta and Arbequina. The differences between extreme varieties were weaker than those shown for the chlorophyll fraction, but even so, in the green stage the carotenoid concentration of Hojiblanca was 4-fold that of Arbequina. These differences could increase or diminish during ripening.

For all the varieties and ripeness stages, lutein was the major carotenoid, always exceeding 50% of the carotenoid fraction. The Blanqueta variety always showed the highest percentage, while Arbequina showed the lowest, with Cornicabra, Picual, and Hojiblanca between the two. As fruit ripening proceeded, the concentration of lutein decreased, but its percentage increased from 55% as mean value in the green fruit (53% and 60% for Arbequina and Blanqueta, respectively) to 75% in the black fruit (70% and 82% for Arbequina and Blanqueta, respectively).

β-Carotene, the only carotenoid with provitamin A value present in the fruit, was the next major carotenoid in all ripeness stages and varieties. Its concentration decreased in both absolute value and percentage with ripening: from 15−20% in a green fruit to 10−15% in a black fruit. Blanqueta fruits always showed the lowest percentage of β-carotene.

The third group of carotenoids studied was that comprising the minor xanthophylls. In the highly pigmented varieties, the concentration of this fraction equaled or exceeded that of β-carotene, whereas in varieties of low pigmentation, its concentration was considerably greater (30%): in all stages of ripeness for Arbequina, and only in the first three for Blanqueta. Furthermore, in the Arbequina variety there was a net increase in the concentration of minor xanthophylls in the second ripeness stage assayed (30%). This is the result of a carotenogenic process that includes esterification of xanthophylls (*32*), which up to now has been found exclusively in this variety. The percentage of minor xanthophylls in Arbequina was higher (28−24%) than in the other varieties (22−15%) for all the ripeness stages assayed. Blanqueta, with an initial percentage of some 25% in the purple stage, had the lowest values (9%).

Consequently, of the eight general patterns of fruit carotenoid distribution reviewed by Gross (31), the olive fitted pattern 2. This group was widely distributed, and generally found in fruits whose color at ripeness is due to anthocyanin compounds and in which there is no chloroplast-chromoplast transformation during this process. Moreover, it was confirmed that in the variety Arbequina, as stated in a previous work (*32*), the distribution pattern was different from those of the other varieties: besides fitting that of a typical chloroplast, it fitted pattern 5, characterized by an unusual synthesis of epoxidized xanthophylls.

Although in general the carotenoid chloroplast pattern was very similar in all varieties of olive, there were small, but none the less important, differences between them. In fact, the traits that distinguished varieties intensify or diminish during ripening, as a consequence of the greater or lesser retention of pigments. To demonstrate this point, Figure 4 displays for each variety the percentage of retention of lutein, β-carotene, and minor xanthophylls between the ripeness stages assayed.

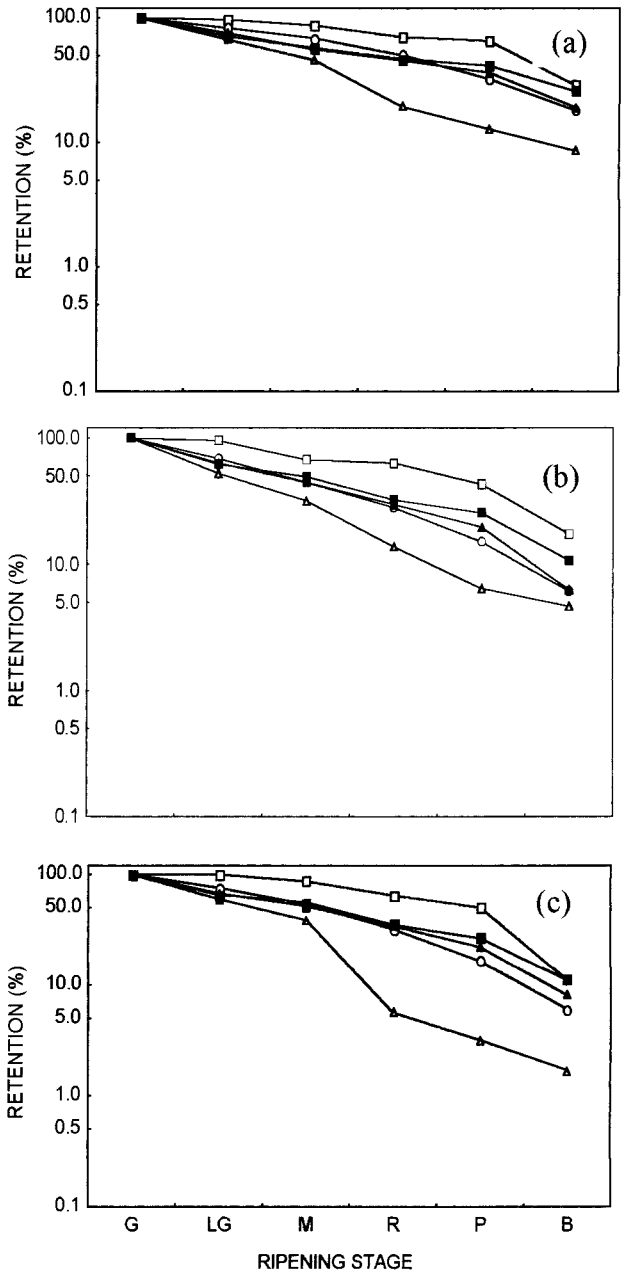

Figure 4. Changes in (a) lutein, (b) β-carotene, and (c) minor xanthophylls during the ripening stages studied of five olive varieties. Arbequina (□), Blanqueta (△), Cornicabra (○), Hojiblanca (■), and Picual (▲). Symbols same as Figure 1.

The ranking of varieties according to the retention of carotenoid pigments was, in the three cases, the same as that shown for chlorophylls. Arbequina retained the most carotenoids, but in Blanqueta they were degraded most rapidly. Again, the varieties of least pigmentation showed the most extreme behavior. Between the two were the other varieties, which reproduced for lutein the pattern shown for chlorophylls. Cornicabra retained more than Hojiblanca and Picual up to the reddish stage, when the order was inverted. For β-carotene and minor xanthophylls, this inversion tooks place in the light green stage.

In all the varieties, lutein showed the lowest slope of change, demonstrating that it was the slowest carotenoid to be degraded. This lower rate of degradation explained the increase in percentage of lutein with ripening, reaching 75% of the total. Gross established

in 1987 (*31*) that lutein is destroyed more slowly than β-carotene and expresses more than half of the residual carotenoids of the chloroplast.

Recent studies on the antioxidant action of carotenoids demonstrates that this capacity in lutein is greater than that of β-carotene, due to the lesser propagation of radical species resulting from the molecular structure of lutein. This structure impedes certain autoxidative mechanisms that in the case of β-carotene reduce its potential antioxidant action (*19*). The major presence of lutein in the fruit, and consequently in the oil, can confer even greater stability and more-beneficial and functional properties than does β-carotene.

The degradation of β-carotene was similar to that of the minor xanthophylls, although slightly lesser, except for the varieties of low pigmentation in certain stages of ripeness. It was established that β-carotene, presenting the steepest slope in all varieties, was the chloroplast carotenoid most rapidly destroyed, followed by the minor xanthophylls, and last, lutein.

Nevertheless, despite the differences in retention of the various carotenoids, the general change in its behavior was very similar to that of the chlorophylls. All the varieties underwent a change in relative rate in the mottled stage, causing a change in the slope of the line, and another when the fruit passed from purple to black, when there was a marked increase in the rate of degradation for each individual carotenoid in all the varieties.

Distribution of Chlorophylls and Carotenoids in the Skin and Pulp of the Olive. Botanically, the olive is a drupe, with a single seed comprising three main tissues: endocarp (pit), mesocarp (pulp or flesh), and exocarp (skin or outer layer). The assemblage of these three tissues, or pericarp, originates in the ovary wall by processes of cell division, expansion, and differentiation. In contrast to the endocarp, whose growth stops after some two months, the mesocarp continues growing through ripening. Its cells are parenchymatous and little differentiated, increasing in size from the outside of the fruit toward the center. Oil is stored inside the vacuoles of these cells. The exocarp is the thinnest layer of the fruit, and comprises the epidermis with its cuticle. Some stomas are formed in the epidermis, and are later converted into lenticels, which possibly act in gas exchange (*25*).

Fruit growth depends on what is assimilated by the leaves. Green fruits contain chlorophylls, and thus chloroplasts, and are able to carry out photosynthesis, although the amount of chlorophylls per unit area is very small in fruits, and the corresponding photosynthetic activity is low. These fruits contain greater amounts of chloroplasts in skin than in pulp. It has been found in the apple that, like the chloroplasts of the skin, the inner tissues are also able to carry out photosynthesis, although to a lesser degree than the skin (*31*).

As described above, during olive ripening the fruits become covered with anthocyanins, until the skin turns black. At the same time, the chlorophyll and carotenoid pigments tend to disappear, although they are still detected in the ripe fruits. The olive is a fruit that retains chloroplast pigments even in the presence of an evident dominance of anthocyanin compounds. To discover which part of the fruit retained this pigmentation, the skin and pulp were analyzed in olives of two representative varieties: one of low pigmentation, Arbequina (Table 1), and the other of high pigmentation,

Table 1. Chlorophyll and Carotenoid Pigments in Pulp and Peel of Arbequina Variety

	peel (mg/kg)					pulp (mg/kg)				peel/pulp		
	G	% loss[a]	R	% loss	B	G	% loss	R	% loss	B	G	R	B
						Chlorophyll Fraction							
chlorophyll *a*	11.94 ± 0.46	33	7.99 ± 0.29	84	1.26 ± 0.07	2.26 ± 0.60	85	0.33 ± 0.09	82	0.06 ± 0.03	5.93 ± 0.25	24.77 ± 5.6	23.63 ± 0.54
chlorophyll *b*	2.24 ± 0.18	37	1.40 ± 0.15	81	0.26 ± 0.18	0.41 ± 0.40	85	0.06 ± 0.01	83	0.01 ± 0.005	6.53 ± 0.53	22.28 ± 0.14	19.50 ± 0.6
total chlorophylls	14.17 ± 0.28	33	9.39 ± 0.14	83	1.52 ± 0.11	2.67 ± 0.40	85	0.39 ± 0.10	82	0.08 ± 0.03	5.78 ± 0.25	24.38 ± 5.06	22.04 ± 0.84
						Carotenoid Fraction							
lutein	2.97 ± 1.40	9	2.70 ± 0.15	60	1.07 ± 0.34	0.49 ± 0.34	85	0.24 ± 0.00	71	0.07 ± 0.02	6.33 ± 0.49	12.90 ± 0.51	14.86 ± 0.51
β-carotene	1.03 ± 0.25	31	0.71 ± 0.01	72	0.20 ± 0.01	0.15 ± 0.08	73	0.04 ± 0.01	0	0.04 ± 0.01	6.81 ± 0.36	20.85 ± 3.44	5.98 ± 1.00
minor xanthophylls	1.18 ± 0.06	15	1.00 ± 0.03	84	0.16 ± 0.01	0.26 ± 0.06	65	0.09 ± 0.00	89	0.01 ± 0.00	4.55 ± 0.06	6.44 ± 0.03	16.00 ± 0.01
total carotenoids	5.20 ± 1.50	15	4.41 ± 0.05	67	1.43 ± 0.38	0.90 ± 0.49	60	0.36 ± 0.02	67	0.12 ± 0.03	5.94 ± 0.62	12.03 ± 0.58	12.65 ± 1.04

[a] Percentage of loss for each pigment between consecutive ripeness stage.

Table 2. Chlorophyll and Carotenoid Pigments in Pulp and Peel of Picual Variety

	peel (mg/kg)				pulp (mg/kg)				peel/pulp		
	G	% loss[a]	R	% loss	B	G	% loss	R	% loss	B	G	R	B
						Chlorophyll Fraction							
chlorophyll *a*	60.12 ± 9.00	40	35.92 ± 3.58	90	3.48 ± 0.90	44.00 ± 12.00	62	16.87 ± 0.99	96	0.67 ± 0.35	1.43 ± 0.11	2.13 ± 0.20	5.03 ± 0.99
chlorophyll *b*	15.85 ± 3.87	36	10.14 ± 1.10	92	0.85 ± 0.14	11.13 ± 3.15	61	4.28 ± 0.25	97	0.14 ± 0.06	1.48 ± 0.06	2.37 ± 0.24	6.08 ± 1.19
total chlorophylls	75.97 ± 12.37	39	46.06 ± 4.68	91	4.34 ± 0.90	55.13 ± 15.83	62	21.15 ± 1.24	96	0.81 ± 0.41	1.44 ± 0.10	2.18 ± 0.21	5.21 ± 1.03
						Carotenoid Fraction							
lutein	14.06 ± 2.96	14	12.13 ± 1.57	82	2.16 ± 0.28	7.81 ± 2.65	49	3.94 ± 0.07	78	0.87 ± 0.16	1.91 ± 0.25	3.08 ± 0.33	2.91 ± 0.07
β-carotene	4.10 ± 0.59	43	2.33 ± 0.18	85	0.5 ± 0.08	1.77 ± 0.05	51	0.87 ± 0.07	91	0.08 ± 0.02	2.31 ± 0.27	2.70 ± 0.24	4.75 ± 0.59
minor xanthophylls	3.74 ± 0.25	26	2.77 ± 0.05	91	0.24 ± 0.01	2.32 ± 0.02	62	0.88 ± 0.00	96	0.04 ± 0.00	1.61 ± 0.12	3.15 ± 0.04	6.00 ± 0.01
total carotenoids	21.97 ± 4.72	22	17.22 ± 2.09	84	2.76 ± 0.36	11.89 ± 3.29	52	5.68 ± 0.01	83	0.98 ± 0.18	1.92 ± 0.12	3.03 ± 0.30	3.20 ± 0.06

[a] Percentage of loss for each pigment between consecutive ripeness stages.

Picual (Table 2), in three specific ripeness stages: green, reddish, and black. The percentage of loss for each pigment between consecutive ripeness stages is shown, and the ratio between the two zones was calculated for each.

The results obtained showed that both skin and pulp of the fruit retained chlorophylls and carotenoids. The chlorophyll and carotenoid profile in the two zones was very similar, and coincides with what we have stated above regarding the analysis of the whole fruit. It was observed that systematically, and notwithstanding the decrease in absolute value of the pigment concentration with ripening, the content of both chlorophylls and carotenoids was much higher in skin than in pulp. This accords with the observation that there are more chloroplasts per unit area in the skin than in the pulp, which obviously continues to be so even when the fruit becomes completely covered with anthocyanins (*31*).

In both varieties, chlorophylls always disappeared more rapidly than carotenoids, in both skin and pulp in the three stages of ripeness. At the same time, the loss of both pigment fractions was always more marked in the pulp than in the skin, so that although the chlorophyll/carotenoid ratio in the skin was greater than unity, in the pulp it becames lower.

The differences between skin and pulp were always greater in Arbequina than in Picual, and the residual pigmentation in the pulp of the ripe fruits of Arbequina was negligible. Moreover, the changes in pigments of skin and pulp were different. Whereas in Arbequina the differences between skin and pulp increased markedly from green stage to mottled, and then remained constant, in the Picual variety the increase was much slighter, but progressive. This means that in Arbequina the degradation was initially very different between skin and pulp, but equalized by the end of ripening. In contrast, in Picual the differences − although small − remained throughout ripening.

Despite the differences or similarities between skin and pulp of Arbequina and Picual fruits, it was evident that chlorophylls and carotenoids were retained in both parts, although the degradative process was greater in the pulp. Overall, the percentages of loss between ripeness stages were always higher in pulp than in skin. It is logical to assume that the loss in photosynthetic activity concomitant with ripening is preferentially in the fruit pulp, where the amount of light received is obviously less than in the skin.

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