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Pigment Profile in Non-Spanish Olive Varieties (*Olea europaea* L. Var. Coratina, Frantoio, and Koroneiki)

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The analysis, for the first time, of the chlorophyll and carotenoid profile of olive fruits of the varieties Coratina, Frantoio, and Koroneiki has revealed important differences with Spanish varieties. First, a high chlorophylls/carotenoids ratio and a low chlorophyll *a*/*b* ratio imply that the photosynthetic apparatus has structural differences with respect to other olive varieties; second, in the carotenoid fraction, a low percentage of lutein, a high percentage of β-carotene, and a high content in neoxanthin are signs that in these three olive varieties the carotenoid biosynthetic pathway is displaced, favoring the β,β series over the α,β series. These differences in the chlorophyll and carotenoid profiles of the fruit are reflected in the corresponding virgin olive oils. It is proposed that the limits of the pigmentary parameters of authenticity of virgin olive oil previously established for the Spanish varieties be extended to obtain markers at a general level, independent of the geographical origin.

KEYWORDS: Carotenoid; chlorophyll; Coratina; Frantoio; Koroneiki; olive fruit; varieties; virgin olive oil

INTRODUCTION

The color of virgin olive oil is determined by its composition in chlorophylls and carotenoids. Although historically the importance of these two pigment fractions has been mainly for the sensation they instill in consumers, today's new emphasis considers them to be functional ingredients (*1*). Furthermore, due essentially to their function in the photosynthetic membranes of the fruit, these pigments present a series of stoichiometric relationships that can be used as quality indices.

In this context, a study carried out in 2000 (*2*) with 50 recently extracted Spanish single-variety virgin olive oils established for the chloroplast pigment fraction two authenticity indices for all virgin olive oils, independent of the fruits' variety and ripeness stage. The first index is the chlorophylls/carotenoids ratio, at around 1.14 ± 0.04, with a margin of variation of between 1.40 ± 0.03 (for the most-pigmented oils, from the beginning of the season) and 0.53 ± 0.02 (for those from the end of the season, with less coloring, as the chlorophyll fraction is degraded more quickly than the carotenoid) (*3*).

The second authenticity index for Spanish virgin olive oils is that the ratio of minor carotenoids to lutein is around 0.47 ± 0.03. The upper limit of variation for this parameter is established as 1.23 ± 0.04 and is due mainly to the oils of the variety Arbequina, characterized by a lower content in lutein (*4*). The lower value for this ratio, 0.23 ± 0.05, comes basically from the oils of the end of the season. This is because as the fruit ripens, lutein is the carotenoid that, proportionally, is the most slowly degraded (*4*), becoming by far the major carotenoid in the chloroplast and, consequently, in the oils extracted from these fruits.

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A more exhaustive study of the data defined pigment parameters able to differentiate between recently extracted Spanish single-variety virgin olive oils. A discriminatory analysis, using three variables (percent violaxanthin, percent lutein, and total pigments), enabled virgin olive oils obtained directly in the mills to be classified by their variety and identified with 100% success by considering a subcategory denominated "end of season" (*2*). The percentage of lutein enabled differentiating oils of the variety Arbequina ($46.3\% \pm 3.8$) from those of "end of season" (always having values above 81.00%). The rest of the oils from other varieties showed a lutein content of between 57 and 77%. The percentage of violaxanthin and its isomers statistically differentiated the oils of the varieties Arbequina ($13.1 \pm 1.4\%$), Blanqueta ($8.2 \pm 1.7\%$), and Hojiblanca ($5.3 \pm 0.4\%$). The oils of the varieties Picual and Cornicabra formed a distinct group, with a violaxanthin content between 2.0 and 4.0%. However, those two varieties are clearly differentiated by their total pigment content. The canonical discriminant function explained perfectly 83.9, 95.1, and 100% of the accumulated variance.

Although Spain accounted for 46.40% of the world production of olive oil in the season 2007/2008 (International Olive Oil Council, IOOC), other olive varieties of non-Spanish origin are found on the international market. After Spain, the next largest producers of virgin olive oil at the international level are Italy (17.85% of world production in the season 2007/2008) and Greece (11.66%).

Virgin olive oils from the varieties Frantoio (*5*), Coratina (*6*), and Koroneiki (*7*), the main Italian and Greek varieties, have been partially characterized at the level of carotenoids (lutein and β-carotene); however, the chloroplast pigment profile of the fruit is not known. Other Italian varieties have also been characterized (*8*). The varieties of olives best described at the pigmentary

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level are Spanish ones—in particular, Hojiblanca, Picual, Arbequina, Cornicabra, and Blanqueta (*3*, *9*).

The aim of the present work is to characterize both the chlorophyll and the carotenoid fractions in the main olive varieties of non-Spanish origin. First, given the present lack of knowledge on this topic, the two pigment fractions in the fruits of these varieties are characterized. The second stage examines whether the pigmentary indices of authenticity proposed for the virgin olive oils of Spanish varieties are equally valid for the non-Spanish varieties.

MATERIALS AND METHODS

Chemicals. Tetrabutylammonium acetate and ammonium acetate were supplied by Fluka (Zwijndrecht, The Netherlands), HPLC reagent grade solvents were purchased at Teknokroma (Barcelona, Spain), and analysis grade solvents were supplied by Panreac (Barcelona, Spain). The deionized water used was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA).

Plant Material. The main non-Spanish varieties (*Olea europaea* L.) from the most representative olive-oil-producing countries, according to the IOOC, were selected: from Italy, Coratina and Frantoio, and from Greece, Koroneiki (50−60% Greek production). Olives and virgin olive oils were provided by Aceites del Sur-Coosur in Dos Hermanas (Sevilla, Spain). Sampling was done during fruit ripening in the season 2007/2008, 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, mottled, and purple during the ripening of the fruits. The oils requested from industry had recently been extracted to avoid any kind of contamination. Oil was extracted from fruits picked during two consecutive harvests, 2007/2008 and 2008/2009, to obtain the greatest possible variability.

Pigment Extraction. For fruit, samples were taken from a homogenized triturate, prepared from 100 destoned fruits (ca. 40 g) of the most representative size 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) saturated with $MgCO_3$ (*10*). The solid residue was collected by vacuum filtration and the extraction repeated until filtrates were colorless. For olive oil, samples of 15 g of virgin olive oil were dissolved with DMF saturated with $MgCO_3$ (*11*). The extracts combined in a funnel were repeatedly treated with hexane. In both materials, chlorophylls, chlorophyll derivatives, and xanthophylls were retained in the DMF phase. The hexane phase contained lipids and carotenes. The DMF phase was treated with 10% (w/v) NaCl solution at 0 °C, and the chlorophylls and xanthophylls were transferred to 100 mL of a mixture of diethyl ether/hexane (1:1 v/v). The aqueous layer was washed with diethyl ether and finally discarded, eliminating polyphenols and other water-soluble compounds. The combined organic phases were filtered through anhydrous Na_2SO_4 and evaporated to dryness under vacuum at a temperature below 30 °C. The dry residue was dissolved in 1.5 mL of acetone prior to HPLC. Analysis was immediate or followed storage at −20 °C for not more 18 h. All analyses were performed under green light.

Standard Pigments for HPLC. Chlorophyll (chl) *a* and chl *b* were purchased from Sigma. Chlorophyllide was formed by enzymatic de-esterification of chlorophyll. The reaction mixture contained 100 mM Tris-HCl (pH 8.5) containing 0.24% (w/v) Triton X-100, chl *a* dissolved in acetone, and crude enzymatic extract from *Ailanthus altissima* (Mill.) leaves in a 5:1:5 ratio (*12*). The C-13 epimer of chl *a* was prepared by treatment with chloroform (*13*). 13^2-OH-chlorophylls *a* and *b* were obtained by selenium dioxide oxidation of chlorophyll *a* at reflux heating for 4 h in pyridine solution under argon (*14*). 15^1-OH-lactone chlorophylls *a* and *b* were obtained by alkaline oxidation in aqueous medium. For this purpose, solid and chromatographically pure chlorophyll (*a* and *b*) was dissolved in acetone, mixed with 0.5% NaOH, and exposed to atmospheric oxygen at room temperature for 10 min. The resulting oxidation products were transferred to diethyl ether by addition of water saturated with NaCl, and 15^1-OH-lactone chlorophylls were isolated by NP-TLC and semipreparative HPLC (*15*). All Mg-free derivatives were obtained from the corresponding chl parent dissolved in diethyl ether by acidification with two to three drops of 5 M HCl (*16*). β-Carotene, lutein, violaxanthin, neoxanthin, and antheraxanthin were obtained from a pigment extract of green olives saponified with 3.5 M KOH in methanol (*17*) and isolated by TLC on silica gel GF_{254} (0.7 mm thickness) using petroleum ether (65−95 °C)/acetone/diethylamine (10:4:1, v/v/v). Luteoxanthin, auroxanthin, neochrome, and mutatoxanthin were obtained by acidification with 1 M HCl in ethanol (*18*). All standards were purified by NP- and RP-TLC (*17*).

Analysis of Chlorophylls and Carotenoids by HPLC. The separation and quantification of pigment products were carried out by HPLC using a HP 1100 Hewlett-Packard liquid chromatograph fitted with a HP 1100 automatic injector HPLC. A stainless steel column (20 × 0.46 cm i.d.), packed with 3 μm C_{18} Mediterranea Sea (Teknokroma, Barcelona, Spain) was used. The column was protected by a precolumn (1 × 0.4 cm i.d.) packed with the same material. Separation was performed using an elution gradient (flow rate = 1.25 mL min^{-1}) with the mobiles phases water/ion pair reagent/methanol (1:1:8, v/v/v) and methanol/acetone (1:1, v/v). The ion pair reagent was 0.05 M tetrabutylammonium and 1 M ammonium acetate in water. The column was stored in methanol/water (1:1, v/v). The gradient scheme (*17*) is a modification and, briefly, is initially 75% A and 25% B, changing to 25% A in 8 min, isocratic for 2 min, changing to 10% A in 8 min and then to 100% B in 5 min, isocratic 7 min, and returning to initial conditions in 5 min. Sequential detection was performed with a photodiode array detector at 410, 430, 450, and 666 nm. Data were collected and processed with a LC HP ChemStation (Rev. A.05.04). Pigments were identified by cochromatography with authentic samples and from their spectral characteristics. The online UV−vis spectra were recorded from 350 to 800 nm with the photodiode array detector.

Statistical Analysis of Data. All experiments were carried out in triplicate. Data were expressed as means ± SD. The SD was always < 10%. The data were analyzed for differences between means using one-way analysis of variance (ANOVA). Duncan's multiple-range test was used as a post hoc comparison of statistical significance (p values < 0.05). All statistical analyses were performed using Statistica for Windows (version 5.1, StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Pigment Profile in Non-Spanish Olive Fruits. Analysis of the chlorophyll and carotenoid profile of the fruits of the varieties Frantoio, Coratina, and Koroneiki, in various ripeness stages, identified the previously reported typical pigments of the olive fruit (*3*). Thus, chlorophylls *a* and *b* were identified as the mayor components of the chlorophyll fraction, and—at approximately 1/100 of that level—pheophytins and de-esterified and allomerized chlorophylls. The carotenoid fraction was represented by lutein, β-carotene, violaxanthin, antheraxanthin, neoxanthin, and lesser amounts of β-cryptoxanthin and some esterified xanthophylls. The results obtained are the first on the topic, as no bibliographic references have yet been found on the chlorophyll and carotenoid composition of the fruits of these varieties.

Given the total pigment content (sum of the chlorophylls and carotenoids), and in accord with the classification established (*3*), the three varieties can be considered to be highly pigmented (**Table 1**)—at the same level as the Spanish varieties Hojiblanca and Picual, which contain > 300 mg/kg of dry weight (dw) of total pigments at the green stage (*3*). In comparison, those varieties designated as having low pigmentation, such as Arbequina and Blanqueta, present < 100 mg/kg of dw of total pigments at the green stage. The high value of the ratio between the two pigment fractions is noteworthy. At the green stage, the chlorophylls/carotenoids ratio fluctuates between 5.80 and 7.20, values very much higher (significant differences, $p > 0.05$) than those for the Spanish varieties (4.40−2.70 at the green stage) (*4*). This is more marked for the varieties Coratina and Koroneiki. As the chlorophylls and carotenoids form part of the photosynthetic apparatus, either in reaction centers or in antenna complexes, any

Table 1. Pigment Composition of the Main Non-Spanish Olive Fruit Varieties (Milligrams per Kilogram of Dry Weight)[a]

variety	ripening stage (RI)[b]	chlorophylls	carotenoids	chl/carot[c]	chl *a*/*b*[d]
Frantoio	green (1)	258.26 ± 27.68	44.30 ± 1.50	5.87 ± 0.37	3.81 ± 0.15e
	mottled (2)	136.89 ± 12.51	28.35 ± 1.46	4.82 ± 0.17	3.19 ± 0.23e
	mottled (3)	123.67 ± 12.74	24.55 ± 2.16	5.02 ± 0.33	3.23 ± 0.16e
	purple (5)	56.07 ± 4.95	13.80 ± 1.20	4.07 ± 0.10	3.23 ± 0.19e
Koroneiki	green (1)	290.76 ± 19.01	40.68 ± 4.03	7.20 ± 0.79	5.00 ± 0.51f
	mottled (2)	199.60 ± 11.51	23.32 ± 1.44	8.59 ± 0.82	4.86 ± 0.11f
	mottled (3)	123.64 ± 10.11	17.61 ± 1.11	7.02 ± 0.31	4.53 ± 0.22f
Coratina	green (1)	302.89 ± 31.96	48.82 ± 0.41	6.21 ± 0.57	3.92 ± 0.24e
	mottled (2)	119.21 ± 1.26	19.94 ± 0.19	6.20 ± 0.12	3.70 ± 0.25e
	mottled (3)	107.61 ± 6.53	21.13 ± 1.20	5.09 ± 0.28	3.58 ± 0.24e
	purple (4)	96.55 ± 3.66	15.37 ± 0.13	6.28 ± 0.22	4.27 ± 0.13e
	purple (6)	33.73 ± 2.26	6.54 ± 5.22	5.19 ± 0.34	4.29 ± 0.18e

[a] Mean values ± SD ($n = 3$). [b] RI, ripening index (*28*), where 0 is dark green, 1 is light green, 2−3 are mottled fruit, and 4−7 are black epidermis with pulp ranging from white to black. [c] Chls/carot, ratio of total chlorophylls/total carotenoids. [d] Chl *a*/*b*, ratio of chlorophyll *a*/chlorophyll *b*. Different letters following values indicate significant differences ($p < 0.05$): e, no differences with Hojiblanca, Picual, Cornicabra, and Blanqueta varieties; f, no differences with Arbequina variety (*3*).

Table 2. Percentage Composition of Individual Carotenoids of the Main Non-Spanish Olive Fruit Varieties [a]

variety	ripening stage (RI)[b]	% lutein	% β-carotene	% violaxanthin	% neoxanthin	% antheraxanthin
Frantoio	green (1)	52.18 ± 1.64c	14.65 ± 1.79d	11.00 ± 0.29f	17.48 ± 0.44g	4.56 ± 0.49h
	mottled (2)	52.60 ± 2.13c	19.49 ± 1.00d	8.88 ± 0.66f	13.65 ± 1.91g	5.29 ± 0.41h
	mottled (3)	54.18 ± 0.95c	12.89 ± 1.19d	12.28 ± 0.36f	16.82 ± 0.66g	3.79 ± 0.41h
	purple (5)	59.53 ± 3.86c	13.02 ± 0.73d	9.24 ± 0.40f	14.58 ± 0.33g	3.63 ± 0.21h
Koroneiki	green (1)	53.33 ± 2.28c	15.68 ± 1.12d	11.70 ± 0.58f	16.64 ± 0.98g	2.55 ± 0.09i
	mottled (2)	57.78 ± 2.12c	8.33 ± 0.31d	14.36 ± 0.11f	16.26 ± 0.40g	3.23 ± 0.13i
	mottled (3)	59.27 ± 2.73c	10.27 ± 0.54d	13.24 ± 0.17f	15.08 ± 0.28g	2.13 ± 0.20i
Coratina	green (1)	45.36 ± 1.41c	20.44 ± 1.33e	13.75 ± 0.55f	17.53 ± 0.73g	2.90 ± 0.17i
	mottled (2)	44.50 ± 1.29c	31.14 ± 1.94e	9.08 ± 0.54f	12.98 ± 0.32g	2.29 ± 0.14i
	mottled (3)	54.02 ± 0.74c	18.24 ± 1.20e	9.08 ± 0.59f	16.07 ± 0.84g	2.58 ± 0.29i
	purple (4)	49.29 ± 1.99c	17.07 ± 1.76e	13.4 3 ± 0.39f	16.78 ± 0.71g	3.42 ± 0.06i
	purple (6)	53.51 ± 3.64c	17.26 ± 1.29e	10.55 ± 1.40f	15.13 ± 1.50g	3.22 ± 0.24i

[a] Mean values ± SD ($n = 3$). Different letters in the percentages of each carotenoid indicate significant differences ($p < 0.05$) for each carotenoid between varieties: c, e, no significant differences with Arbequina variety (*23*); d, no significant differences with Hojiblanca and Picual varieties (*23*); g, significant differences with Arbequina, Hojiblanca, and Picual varieties (*23*); h, i, no significant differences with Hojiblanca and Picual varieties (*23*). [b] RI, as in **Table 1**.

differences in the balance between these two fractions imply differences in the structure of the photosynthetic machinery. Thus, there are structural differences between the varieties examined and the Spanish ones.

The chl *a*/*b* ratio is an indirect measurement of the distribution of the reaction centers/antenna complexes in the thylakoid. The antenna complexes are relatively rich in chlorophyll *b*, whereas the reaction centers are rich in chlorophyll *a* (*19*). The chl *a*/*b* ratio in fruits has been determined as 2.5−4.0 in citrus (*20*), pistachio nuts (*21*), and peppers (*22*). In olives, the chl *a*/*b* ratio in general (varieties Hojiblanca, Picual, Blanqueta, and Cornicabra) has been determined as 3.84 ± 0.28 throughout fruit ripening (*3*)—the exception being fruits of the variety Arbequina (4.71 ± 0.40), which has a photosynthetic structure that is different from that of the other the Spanish varieties. The data of **Table 1** demonstrate that the varieties Frantoio and Coratina (3.66 ± 036) are very similar (no significant differences, $p < 0.05$) to the average for the Spanish varieties. The fruits of the variety Koroneiki present values significantly higher for the chl *a*/*b* ratio (4.93 ± 0.10), indicating that they have fewer antenna complexes, similar (no significant differences, $p < 0.05$) to the fruits of the variety Arbequina.

In the three non-Spanish varieties, the major component in the carotenoid fraction is lutein (**Table 2**), with a percentage of 52.48 ± 4.60, similar (no significant differences, $p < 0.05$) to the mean value of 52.10% for fruits of the variety Arbequina (*23*). These contents are relatively low, compared with representative Spanish varieties such as Hojiblanca (69.64%) or Picual (72.64%). The low lutein content in the fruits of the variety Arbequina is explained partly by the fact that its fruits (exceptionally) go through a carotenogenic process during ripening, favoring primarily the β,β synthetic pathway (**Figure 1**, mainly violaxanthin and antheraxanthin, in the variety Arbequina) over the α,β series, which synthesizes lutein. Although the definitive origin is unknown, it is evident that the α,β synthetic pathway is impeded in the fruits of the varieties Frantoio, Koroneiki, and Coratina.

In this context, the mean percentage content of β-carotene in the fruits of the variety Coratina (21.72%) is similar to that for the variety Arbequina (19.53%), whereas the values for the varieties Koroneiki and Frantoio are significantly lower (significant differences, $p > 0.05$) (12.01% and 15.01%, respectively) and closer to those for the varieties Picual (13.44%) and Hojiblanca (15.34%) (*23*). The mean percentage content of antheraxanthin for the three varieties examined (3.41%) is similar to the mean for the Spanish varieties Picual (2.97%) and Hojiblanca (3.01%). In contrast, the violaxanthin content (10.09%) is intermediate between the mean for the Spanish varieties Hojiblanca (5.17%) and Picual (4.90%) and the exception (Arbequina, 13.42%).

Most striking in the fruits of the three varieties studied is the high content of neoxanthin, the last carotenoid of the β,β series

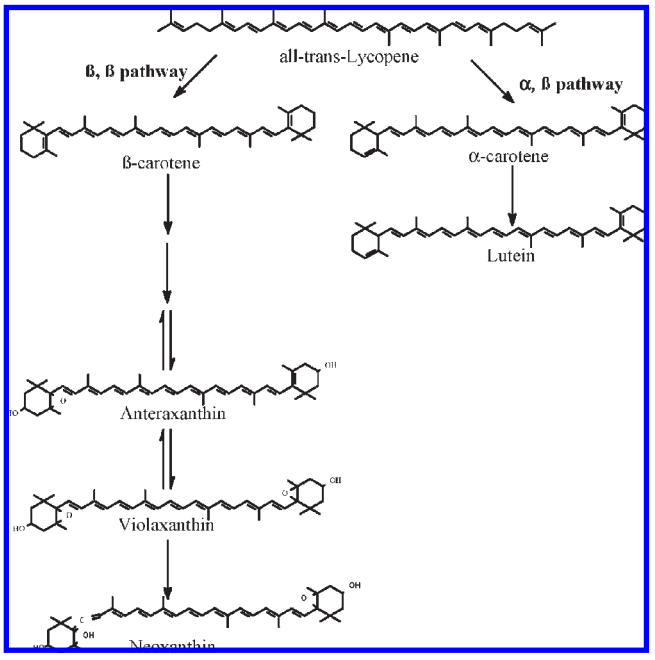

Figure 1. Carotenoid biosynthetic pathway: α,β and β,β pathways. NSY, neoxanthin synthase.

(15.81 ± 1.53%), in comparison with contents for the Spanish varieties (Hojiblanca, 6.68%; Picual, 5.73%; Arbequina, 6.71%) (*23*). This result suggests that in the fruits of these three varieties, not only is the carotenoid synthetic pathway displaced toward the β,β series, but, in addition, the enzyme neoxanthin synthase (NSY), the last enzyme of the pathway, is very potentiated, generating a higher content of neoxanthin, in detriment to the carotenoids synthesized in the α,β pathway (**Figure 1**).

In conclusion, there are great differences—both structural and metabolic—at pigment level between the fruits of the three varieties studied and the Spanish ones, differences that are reflected as the different proportional content of these chloroplast pigments and which could be used as parameters of varietal identification.

Pigment Profile Inherent to Virgin Olive Oil. *Variety-Independent Parameters*. The pigmentary indices of authenticity of virgin olive oil (*2*) were established in virgin olive oils obtained exclusively from varieties of Spanish origin. For comparison with varieties of non-Spanish olives, virgin olive oils from the main varieties of the most representative olive-oil-producing countries, according to the IOOC, were analyzed.

Qualitatively, the pigment profile of the virgin olive oils from the varieties Coratina, Frantoio, and Koroneiki is the same as that from the Spanish varieties. The profile is defined and established as inherent to olive oil in general (*2*); in brief, it comprises the pigments present in the fruit plus those that might be formed during the extraction process.

Table 3 shows the total chlorophyll and carotenoid content for the non-Spanish olive varieties during two consecutive harvests, 2007/2008 and 2008/2009. The differences in the pigment content between harvests are due to the ripening stage of the olives used for olive oil extraction. Oils obtained from green fruits have more pigmentation than oils obtained from purple fruits (**Table 1**).

The first authenticity index is that the chlorophylls/carotenoids ratio is balanced around unity and within fixed limits (1.40−0.53); in general, the ratio is higher for the more-pigmented oils. The results obtained for this parameter in the oils from the non-Spanish varieties are shown in **Table 3**, where it can be seen that only the variety Frantoio presents the same values as the Spanish varieties (no significant differences, $p < 0.05$). The oils from the varieties Coratina and Koroneiki present a chlorophylls/carotenoids ratio higher than the 1.4 set as maximum for the Spanish varieties. Data on the chlorophyll and carotenoid pigment content have been published for the varieties Coratina (*6*) and Koroneiki (*7*, *24*), from which we have calculated the chlorophylls/carotenoids ratio—the values obtained were much higher than 1.40 and therefore coincide with those shown in **Table 3**. These higher values are due to the higher proportion of the chlorophyll fraction in the source fruits (as we have demonstrated and as mentioned above), so that the olive oils extracted from them present a higher chlorophylls/carotenoids ratio. The fruits of the variety Frantoio present lower values for this ratio, and in the corresponding oils this parameter is within the limits set for the Spanish varieties.

The second parameter of authenticity is the ratio between the minor carotenoids (β-carotene, violaxanthin, antheraxanthin, neoxanthin, and β-cryptoxanthin) and lutein. For this ratio, the variety Frantoio presents the same mean values set for Spanish oils (no significant differences, $p < 0.05$). However, the varieties Coratina and Koroneiki present values above the 1.23 set as maximum for the Spanish varieties. These results are confirmed from the data published in the literature, which we have used to calculate the ratio for the varieties Coratina (*6*, *25*) and Koroneiki (*7*).

With these data we conclude that the parameters of authenticity established for the Spanish varieties (*2*) are not equally valid for the varieties Frantoio, Coratina, and Koroneiki. It is necessary to broaden the limits of variation of these pigment-related parameters to establish authenticity indices for international use.

Variety-Dependent Parameters. The pigmentary parameters able to differentiate between single-variety virgin olive oils (*2*) are three variables: percent violaxanthin, percent lutein, and total pigments. They were established by analyzing 50 Spanish single-variety virgin olive oils.

With regard to the percentage of lutein (**Table 3**), the variety Frantoio presents values similar to the Spanish ones, taking 57−81% as the Spanish mean value (no significant differences, $p < 0.05$). Exceptionally low values are presented by the varieties Coratina (mean = 38.72%) and Koroneiki (mean = 36.84%). For the Spanish varieties, lutein comprises more that half the carotenoid fraction, so the values of these two varieties are striking. These results are endorsed by data in the literature, from which we have calculated the percentages for both Coratina (*6*,*25*) and Koroneiki (*7*). The low lutein content in the fruits of the varieties Coratina and Koroneiki (**Table 2** explains why the corresponding virgin olive oils are characterized by a low content of this carotenoid. However, the data found for the variety Frantoio do not explain the results obtained for its oils. A comparison is not possible because the literature lacks data on the carotenoid composition of the fruit, but presumably these oils had been extracted from riper fruits, in which the percentage of lutein is higher (**Table 2**, Frantoio purple). It is possible that during fruit ripening in the variety Frantoio the degradation of lutein is much slower than in the other varieties, so that its percentage content is higher in riper fruits and, consequently, in the corresponding virgin olive oils.

The percentage of violaxanthin also offers outstanding possibilities as a parameter to differentiate between non-Spanish varieties. Thus, Frantoio and Coratina present the highest violaxanthin values, similar to those reported for the variety Arbequina (13.1%) (no significant differences, $p < 0.05$). However, there are hardly any values published for non-Spanish varieties with regard to this parameter—only for the variety Coratina (*6*), for which we have calculated 8.04% of violaxanthin,

Table 3. Pigment Composition, Authenticity Pigment Index, and Variety-Inherent Parameters of Non-Spanish Virgin Olive Oil (Milligrams per Kilogram of Virgin Olive Oil)[a]

variety	harvest	chlorophylls	carotenoids	chls/carot[b]	carot min/lutein[c]	% lutein	% violaxanthin
Coratina 1	2007	11.82 ± 0.10	5.41 ± 0.03	2.18 ± 0.07d	0.82 ± 0.08d	47.91 ± 2.75d	7.38 ± 0.02f
Coratina 2	2008	7.76 ± 0.75	4.04 ± 0.44	1.92 ± 0.21d	3.48 ± 0.31d	22.70 ± 2.51d	11.6 ± 0.11f
Koroneiki 1	2007	16.88 ± 1.55	6.26 ± 0.55	2.70 ± 0.10d	1.64 ± 0.13d	35.67 ± 3.55d	6.46 ± 0.06g
Koroneiki 2	2008	3.32 ± 0.31	3.89 ± 0.33	0.85 ± 0.01d	1.81 ± 0.16d	38.02 ± 3.56d	6.50 ± 0.06g
Frantoio 1	2007	4.87 ± 0.71	4.03 ± 0.03	1.21 ± 0.17e	0.63 ± 0.01e	57.95 ± 0.56e	13.19 ± 0.01f
Frantoio 2	2008	2.48 ± 0.15	4.80 ± 0.28	0.52 ± 0.00e	0.73 ± 0.00e	61.40 ± 0.15e	8.90 ± 0.04f

[a] Mean values ± SD ($n = 3$). Different letters in each column indicate significant differences ($p < 0.05$) for each parameter between varieties: e, no significant differences with Spanish varieties (*2*); f, no significant differences with Arbequina variety (*2*). [b] Chls/carot: ratio of total chlorophylls/total carotenoids. [c] Carot min/lutein: ratio of minor carotenoids/lutein.

Table 4. Other Important Pigments Indices of Non-Spanish Virgin Olive Oils[a]

variety	harvest	% dephytylated chlorophylls	% β-carotene	% neoxanthin
Coratina 1	2007	0.47 ± 0.02b	37.14 ± 0.03c	4.83 ± 0.01b
Coratina 2	2008	1.10 ± 0.09b	58.70 ± 0.18c	4.50 ± 0.02b
Koroneiki 1	2007	1.60 ± 0.01b	47.70 ± 0.21c	5.30 ± 0.06b
Koroneiki 2	2008	1.17 ± 0.07b	45.81 ± 0.36c	3.44 ± 0.04b
Frantoio 1	2007	0.60 ± 0.03b	24.20 ± 0.05d	2.80 ± 0.01b
Frantoio 2	2008	1.50 ± 0.09b	16.46 ± 0.05d	7.86 ± 0.05b

[a] Mean values ± SD ($n = 3$). Different letters in each column indicate significant differences ($p < 0.05$) between varieties: d, no significant differences with Arbequina variety (*2*).

a value very similar to that obtained in the present work (**Table 3**). The variety Koroneiki presents intermediate values for violaxanthin—around 6−7% (closer to values for the variety Hojiblanca).

Thus, these two parameters—the percent of lutein and the percent of violaxanthin—continue to be varietal differentiators, not only between Spanish and non-Spanish varieties but also between non-Spanish varieties.

Other Important Pigmentary Indices in Non-Spanish Virgin Olive Oils. In the study on authenticity of oils from Spanish olive varieties, the relative content of β-carotene is a parameter that was not postulated as a varietal differentiator, as it was practically the same in all of the Spanish varieties. The percentage ranges between 5 and 20%, depending above all on the ripeness stage of the source fruit. That is, lutein is by far the major carotenoid in the oils obtained from Spanish varieties. Even in the oils of the variety Arbequina, with a higher percentage of β-carotene (16−30%), lutein is the major carotenoid (*2*, *9*). In contrast, the non-Spanish varieties presented a β-carotene content (**Table 4**) much higher than 30% (the maximum for the Spanish oils), and in some cases β-carotene was the major chloroplast carotenoid and, thus, an important differentiating factor. This has also been reported for certain Italian varieties, such as Biancolilla and Nocellara del Belice (*8*), with a β-carotene content exceeding that of lutein, reaching values of > 40% of the carotenoid fraction. On the other hand, the variety Frantoio presented a β-carotene content similar to that of the Spanish varieties, also coinciding with that reported for other Italian varieties (*8*). In conclusion, although the percentage of β-carotene provides no differentiating information in the main Spanish varieties, when the study is extended to non-Spanish varieties, it is found to be a parameter differentiating between the virgin olive oils of Italian varieties.

Within the chlorophyll fraction analyzed, the relative content of dephytylated derivatives (chlorophyllides and pheophorbides) is noteworthy. These compounds are formed in the source fruit by the action of chlorophyllase, the first enzyme implicated in the senescent degradation of chlorophylls. In highly pigmented varieties such as Hojiblanca and Picual, chlorophyllase activity is very low throughout the vegetative cycle of the fruit, so that the content in dephytylated derivatives is very low (below 1%) in the fruit and, consequently, in the oil (*12*). In contrast, the fruits of little-pigmented varieties (such as Arbequina and Blanqueta) exhibit a very high chlorophyllase activity during ripening (*12*,*26*); consequently, the content of dephytylated derivatives in the fruit and in the corresponding oils is higher (2.5−5.0%). Similar values have been reported even after 24 months of storage for the virgin olive oils of this variety (*9*). **Table 4** shows the percentage content of de-esterified chlorophyll derivatives in the three varieties analyzed. The values are around 1.5%, which allows postulating a marked chlorophyllase activity in the fruit.

It is striking that while the percentage of neoxanthin is high in the fruits of the three varieties analyzed, this carotenoid presents lower values in the corresponding virgin olive oils. It might be assumed that this carotenoid is destroyed to a greater extent during the olive oil extraction process; however, for other olive varieties this is not so (*27*). Given that virgin olive oils do not correspond exactly with the fruits analyzed, it is possible that those oils were obtained from fruits riper than those analyzed in the present work. During olive ripening there is a systematic degradation of the carotenoids; lutein is the carotenoid most slowly degraded (*3*), and its relative presence in the fruit increases in detriment to the rest of the carotenoids. The use of very ripe fruits (with a lower percentage of neoxanthin) would explain the values obtained for neoxanthin in the virgin olive oils of Coratina, Frantoio, and Koroneiki.

ABBREVIATIONS USED

DMF, *N*,*N*-dimethylformamide; IOOC, International Olive Oil Council; NSY, neoxanthin synthase.

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