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Control of Olive Oil Adulteration with Copper−Chlorophyll Derivatives

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The present work proposes an analytical method able to detect in an adulterated olive oil sample the addition of the copper complexes of chlorophylls (E 141i). The method consists of a pigment extraction in liquid phase and subsequent analysis by HPLC−DAD. The profile of chlorophyll pigments of an olive oil is determined essentially by its content in pheophytins (*a* and *b*), but in no case any copper derivative. Different samples of colorant E 141i have been analyzed, the natural coloring additives used to adulterate vegetable oils. The 99.59 ± 0.52% of the chlorophyll pigments present in the different samples of E 141i colorant are not those of an olive oil (more than 75% are cupro-derivatives). Thus, the simple detection of one of the compounds in an olive oil indicates adulteration. The major chlorophyll derivative in all the E 141i colorants samples is Cu−pyropheophytin *a* and its limit of detection (LOD) defined at a signal-to-noise ratio of about 3 was 6.58 ng/g.

KEYWORDS: Adulteration; colorants; copper−chlorophylls; E 141i; HPLC; olive oil

INTRODUCTION

Olive oil is defined as a mixture of virgin olive oil and refined olive oil (*1*). The latter is colorless, as it has undergone a refining process that includes, among other operations, one of decolourising. If the virgin olive oil intended for the mixture comes from an olive variety with little pigmentation or from ripe fruits (*2*), the resulting olive oil will be very pale. In general, the consumer identifies colored oil with virgin olive oil, while olive oils with little color recall refined seed oils. Moreover, each market has its own preferences — for example, Japan demands highly colored olive oils. All this has led some firms to boost the color of their olive oils with green colorant additives. Nevertheless, to reinforce the color of refined seed oil to look like an olive oil, it is not a new practice (*3*).

European regulations (*4*) do not permit the addition of colorants to oils and fats of animal or vegetable origin. However, for other food products two natural colorants structurally related with the chlorophylls are authorized—those denominated E 140 and E 141. Because they are natural (pigments obtained from an animal or vegetable raw material)—and although being subject to rigorous examination—they are not as a rule subject to regulatory specific criteria of purity. E 140 comprises direct chlorophyll derivatives; it is marketed according to its solubility: E 140i is liposoluble, chlorophylls derivatives, and E 140 ii is hydrosoluble, composed of chlorophyllins. E 140i is obtained from natural sources—alfalfa, nettles, and other edible plant materials—via solvent extraction. During this process, part of the chlorophylls can be transformed into their magnesium-free derivatives: the pheophytins. This means (see later) a drastic change in the color, which goes from green to brown. The end product can contain other pigments (such as carotenoids), oils, and waxes, so that the final aspect of the colorant is waxy. The colorant E 140ii (also known as sodium or potassium chlorophyllins) is obtained by saponification of the solvent-extracted products from edible plant material. Saponification breaks the ester-phytol bond and can even rupture the so-called isocyclic ring. Following saponification, the acids are neutralized, forming the potassium and/or sodium salts. Unlike E 140i, this colorant is marketed as powder or aqueous solution (*5*).

The colorant E 141 is composed of copper complexes of chlorophyll derivatives—that is, the corresponding copper derivative of E 140. The product is marketed as E 141i, which is liposoluble and known as "copper chlorophylls", and E 141ii, which is hydrosoluble and known as "copper chlorophyllins"; each results from the addition of copper to the respective pigment solutions.

There is also an artificial green colorant (E 142), which is authorized in Europe, but not in the United States. Being artificial, it has to pass strict toxicological controls. The FDA, with regard to green colorants in foods, allows the use of E 141 only in citrus-based dry beverage mixes, and never exceeding 2%.

Copper chlorophyllins (E 141ii) are the colorings most used in food technologies, due to their hydrophyllic character, easily soluble in ice creams, jellies, soft drinks, vegetables in vinegar, etc. Therefore, there is more bibliography for the detection of this colorant (*6*−*8*).

But, the colorant used to boost the color in olive oils is E 141i—that is, the liposoluble form of the copper derivative of chlorophylls, making it readily soluble in the oil. The cupro-derivatives are used preferentially because they are much more stable than the original chlorophyll, as the insertion of the Cu^{2+} ion in the

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macrocycle of the chlorophyll generates a highly stable complex that remains green despite the processing and storage of the food (*9*). In contrast, the naturally chelated magnesium in the chlorophyll macrocycle is readily substituted by hydrogen during the storage or processing to form other, nongreen chlorophyll derivatives (pheophytins).

Because of the limited use of this colorant, the bibliography for this respect is scarce. Inoue et al. (*10*) proposed a chromatographic method to identify Cu−pheophytin *a* and *b* standards. Scotter et al. (*8*) described a method able to separate all the pigments present in two colorant samples of E141i, but without identification. The aim of the present work is to propose a specific analytical method for the detection of the colorant E 141i in olive oil, identifying all the chlorophyll pigments present in the colorant E 141i.

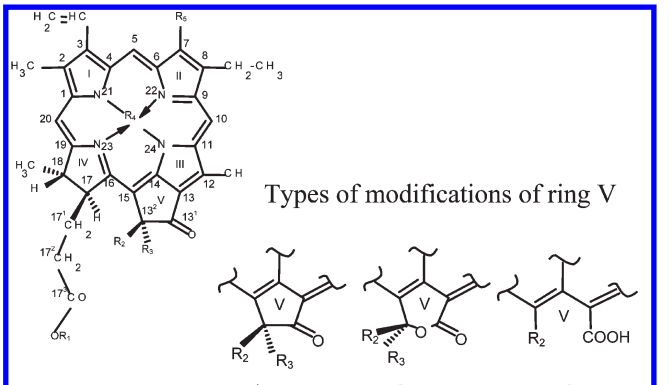
MATERIALS AND METHODS

Chemicals and Standards. Tetrabutylammonium acetate and ammonium acetate were supplied by Fluka (Zwijndrecht, The Netherlands), HPLC reagent grade solvents were purchased from Teknokroma (Barcelona, Spain), and analytical grade solvents were supplied by Panreac (Barcelona, Spain). For the preparation, isolation, and purification of chlorophyll pigments, analytical grade reagents were used (Panreac, Barcelona, Spain). The deionized water used was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA).

Chlorophyll *a* and *b* were purchased from Sigma-Aldrich Co. (Madrid, Spain). Chlorophyllide was formed by enzymatic de-esterification of chlorophylls. The reaction mixture contained 100 mM Tris-HCl (pH 8.5) containing 0.24% (w/v) Triton X-100, chlorophyll *a* dissolved in acetone and crude enzymatic extract from *Ailanthus altissima* (Mill.) leaves in a 5:1:5 ratio (*11*). C-13 epimer of chlorophyll *a* and *b* were prepared by treatment with chloroform (*12*). The 13^2-OH-chlorophyll *a* and *b* was obtained by selenium dioxide (37 mg, 0.34 mmol) oxidation of chlorophyll *a* at reflux-heating for 4 h in pyridine (5 mL) solution under argon (*13*). 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% (w/v) NaOH, and exposed to atmospheric oxygen at room temperature for 10 min. The resulting oxidation products were transferred to diethyl ether by the addition of water saturated with NaCl, and 15^1-OH-lactone chlorophylls were isolated by NP-TLC and semipreparative HPLC (*14*). Pyropheophytin *a* and *b* were obtained from the respective pheophytins by reflux-heating at 100 °C in collidine (*15*). All Mg-free derivatives were obtained from the corresponding chl parent dissolved in diethyl ether by acidification with 2−3 drops of 5 M HCl (*16*). 15-Glyoxilic acid pheophytin *b* was obtained by alkaline treatment in aqueous media (0.5% w/v sodium hydroxide) (*17*). The copper complexes were prepared following a procedure similar to that reported by Jones et al. (*18*). The pigments were dissolved in acetone for the chelation reaction with an excess of copper(II) ions as chloride and with ascorbic acid to minimize oxidative changes.

Plant Material. Different samples of commercial olive oils (mixture of virgin olive oil and refined olive oil) were purchased in a supermarket. The oils have a 75% of monounsaturated fatty acids, 15% of saturated fatty acids, and 10% of polyunsaturated fatty acids. The oils were selected in a wide margin, between high pigment content olive oils (25 mg/kg of total chlorophylls) and low pigment content olive oils (1 mg/kg of total chlorophylls), from the beginning of the picking season (November) to the end (December). The olive oils samples corresponded with different olive varieties: Hojiblanca, Picual, Arbequina, and mixtures between them. The samples of colorants were supplied by different firms engaged in the production and marketing of food colorants. The adulterated sample was obtained by mixing the olive oil with one of these colorants (E 141i) at 0.1%.

Pigment Extraction. Samples of 15 g of olive oil were dissolved with *N*,*N*-dimethylformamide (DMF) saturated with $MgCO_3$ (*19*). The extracts combined in a funnel were repeatedly treated with hexane (5 × 50 mL). Chlorophylls, chlorophyll derivatives, and xanthophylls were retained in DMF phase. The hexane phase contained lipids and carotenes. The DMF phase was treated with 10% (w/v) NaCl solution at 0 °C, and



Pigment	Peak nº	I.ring[a]	R_1	R_2	R_3	R_4	R_5
Pheophytin *b*	1-1′	1	Phytol	H	$COOCH_3$	2H	CHO
Pheophytin *a*	2-2′	1	Phytol	H	$COOCH_3$	2H	CH_3
Pyropheophytin *a*	3	1	Phytol	H	H	2H	CH_3
Cu-pheophorbide *a*	4	1	H	H	$COOCH_3$	Cu	CH_3
15-glyoxylic acid pheo *b*	5	3	Phytol	COCOOH		2H	CHO
Cu-pheophytin *b*	6-6′	1	Phytol	H	$COOCH_3$	Cu	CHO
13^2-OH-pheophytin *b*	7-7′	1	Phytol	OH	$COOCH_3$	2H	CHO
Cu-13^2-OH-pheo *b*	8	1	Phytol	OH	$COOCH_3$	Cu	CHO
Cu-13^2-OH-pheo *a*	9-9′	1	Phytol	OH	$COOCH_3$	Cu	CH_3
Cu-15^1-lactone pheo *a*	10	2	Phytol	OH	$COOCH_3$	Cu	CH_3
Pyro-pheophytin *b*	11	1	Phytol	H	H	2H	CHO
Cu-pheophytin *a*	12-12′	1	Phytol	H	$COOCH_3$	Cu	CH_3
Cu-pyro-pheophytin *b*	13	1	Phytol	H	H	Cu	CHO
Cu-pyro-pheophytin *a*	14	1	Phytol	H	H	Cu	CH_3

[a] I. ring: isocyclic ring (ring V)

Figure 1. Structures of the chlorophyll pigments described in the text. Peaks numbers as in **Tables 1** and **2**; pheo, pheophytin.

the chls 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 filtrated 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. For colorants (E 141i), different aliquots were dissolved in acetone. Analysis was immediate or followed storage at −20 °C not more 18 h. Data are means of triplicate analysis.

Analysis of Chlorophylls Compounds 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 HP1100 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 precolumn (1 × 0.4 cm i.d.) packed with the same material. Separation was performed using an elution gradient (flow rate 1.250 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 and 1 M ammonium acetate in water. The column was stored in methanol/water (1/1, v/v). The gradient scheme is a modification of that of Mínguez-Mosquera et al. (*19*), and briefly is initially 75% A and 25% B, then changes to 25% A in 8 min, isocratic 2 min, change to 10% A in 8 min, then to 100% B in 5 min, isocratic 15 min, and return to initial conditions in 5 min. Sequential detection was performed with a photodiode array detector at 430 nm. Data were collected and processed with a LC HP ChemStation (revision 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.

Limit of Detection (LOD) Calculations. The chlorophyll composition varies between colorants, depending on the initial raw material, and the extraction process, because the regulation does not specify a standard

Table 1. Chromatographic and Spectroscopic Properties of Chlorophyllic Pigments Presents in Olive Oils

				I		II		III		IV		V		VI	
pigment	peak no. (**Figures 2,3**)	K_c[a]	Soret	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*
pheophytin *b*	1 (**Figure 2a**)	11.50	435			412	2.27	524	13.62	(558)	20.42	598	16.33	654	3.83
pheophytin *b*′	1′ (**Figure 2a**)	11.96	435			412	2.27	524	13.62	(558)	21.80	595	17.63	654	4.87
pheophytin *a*	2 (**Figure 2b**)	12.46	409	(400)	1.07	(376)	1.31	505	8.78	537	9.62	609	10.63	666	1.85
pheophytin *a*′	2′ (**Figure 2b**)	12.98	409	(400)	1.07	(376)	1.31	505	9.18	537	10.10	609	11.22	666	2.35
pyro−pheophytin *a*	3 (**Figure 2b**)	14.41	409	(400)	1.07	(376)	1.31	505	8.78	537	9.62	609	10.63	666	1.85

[a] Retention factor, $k_c = (t_R - t_M)/t_M$ where t_R is the retention time of the pigment peak and t_M is the retention time of an unretained component. *M*, maximum aborbance (nm); *R*, quotient of absorbance at Soret band divided by absorbance at wavelenght indicated. The values in parentheses indicate inflection points in the absorption spectrum.

protocol. For this reason, Cu−pyropheophytin *a* standard, as the major chlorophyll derivative present in all the colorants have been used to calculate the LOD. The calibration equations were obtained by least-squares linear regression analysis over a concentration range according to the levels of these pigments in VOO. Injections in duplicate were made for seven different volumes at each standard solution ($r^2 = 0.9955$).

Statistical Analysis. All the analyses were carried out in triplicate. Data were expressed as percentage ± SE. All statistical analyses were performed using Statistica for Windows (StatSoft, Inc., 2001).

RESULTS AND DISCUSSION

Olive Oil. The chlorophyll fraction of olive oil is determined mainly by pheophytins (*a* and *b*). During the olive oil extraction process, the native chlorophylls (present in the olive fruit) are transformed into pheophytins when the central Mg^{2+} ion of the porphyrin ring is substituted by H+. This reaction proceeds by acids released during milling and beating and is visually very striking, because it directly affects the chromophore group of the chlorophyll, and the color changes from bright green to olive brown. Some oils may retain traces of the original chlorophylls. The pheophytin ($a + b$) content makes up more than 90% of the chlorophyll fraction of an olive oil. Even in a virgin olive oil, after 3−4 months of storage all the chlorophylls have been transformed into pheophytins (*20*).

Figure 1 shows the structures of the chlorophylls derivatives described in the work. In some olive oils, traces have been detected of easily formed chlorophyll derivatives oxidized on carbon-13 (OH-pheophytins and lactone-pheophytins), whose presence may be due both to the possible activity of the oxidatives enzymes in the fruit (*11*) and to the conditions inherent to the extraction process. In oils from fruits with a high chlorophyllase activity, as is the case of the Arbequina variety, chlorophyllides and pheophorbides also have been found. These compounds are products of the enzymatic deesterification of the alcohol phytol in the molecules of chlorophyll and pheophytin, respectively (*2*). If the storage is lengthy, pyropheophytin *a* can also be formed. This compound comes from the demethoxycarbonylation of C-13[2]. Its appearance in foods has always been related with strong heat treatments, such as sterilization. However, Gallardo-Guerrero et al. (*20*) have demonstrated the formation of this compound during the storage of virgin olive oil at 15 °C for one year—although in amounts never more than 3%. Consequently, olive oil should contain only pigments from the fruit plus derivatives associated with the extraction process and storage: specifically, pheophytins (*a* and *b*), traces of oxidized chlorophylls, and pyropheophytin *a*, and, in certain cases, de-esterified chlorophyll derivatives.

Table 1 and **Figure 2a**,**b**, solid line, show the main spectroscopic characteristics of the chlorophyll compounds present in olive oil, and **Figure 1** describes its structures. **Figure 3a** displays a typical chromatogram at 430 nm of olive oil, although it is an analysis of several oil samples of different varieties (Hojiblanca, Picual, Arbequina, and mixtures between them), from the beginning to the end of the picking season. At this wavelength, the major peak

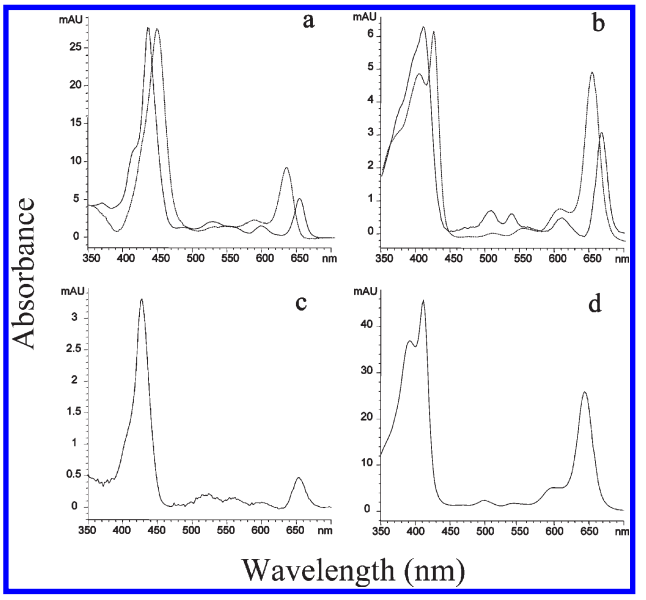


Figure 2. Spectra of the chlorophyll derivatives presents in the colorants E 141i: (**a**) pheophytin *b* (solid line) and Cu−pheophytin *b* (dotted line); (**b**) pheophytin *a* (solid line) and Cu−pheophytin *a* (dotted line); (**c**) 15-glyoxylic acid pheophytin *b*; (**d**) Cu−15[1]-OH lactone pheophytin *a*. Structures are as in **Figure 1**.

is that of lutein, the major carotenoid in olive oil, (*21*), which hinders visualization of the chromatographic region of interest (retention times above 22 min). An amplification of this region (**Figure 2b**) reveals more clearly the major chlorophyll derivatives in an olive oil. The results shown in this figure are supported by years of research in the field of olive oil pigments, and in which chlorophyll pigments other than those described have never been detected (except in cases of adulteration or poor processing) (*19*−*24*). The presence of chlorophyll derivatives other than those described indicates that the olive oil has been adulterated with the addition of a colorant.

E 141i colorants. Figure 4 shows the profile of four actual examples of E 141i colorants marketed under different brands as authorized food colorants (for another type of food). The chlorophyll and carotenoid composition varies between samples, depending on the initial raw material and the extraction process, as the regulation does not specify a standard protocol. In the carotenoid fraction, lutein and β-carotene predominate, accompanied by other minor xanthophylls. **Table 2** and **Figure 2** show the chromatographic and spectroscopic properties of all the chlorophyll derivatives present in the four samples of E 141i colorants analyzed, and **Figure 1** describe its structures. The insertion of the copper ion in the chlorophyll macrocycle induces a bathochromic displacement of the Soret band (between 3 and 15 nm, depending on the pigment) and a hypsochromic displacement of band VI (between 12 and 30 nm, depending on the pigment), as can be seen in **Table 2**. In contrast, alterations

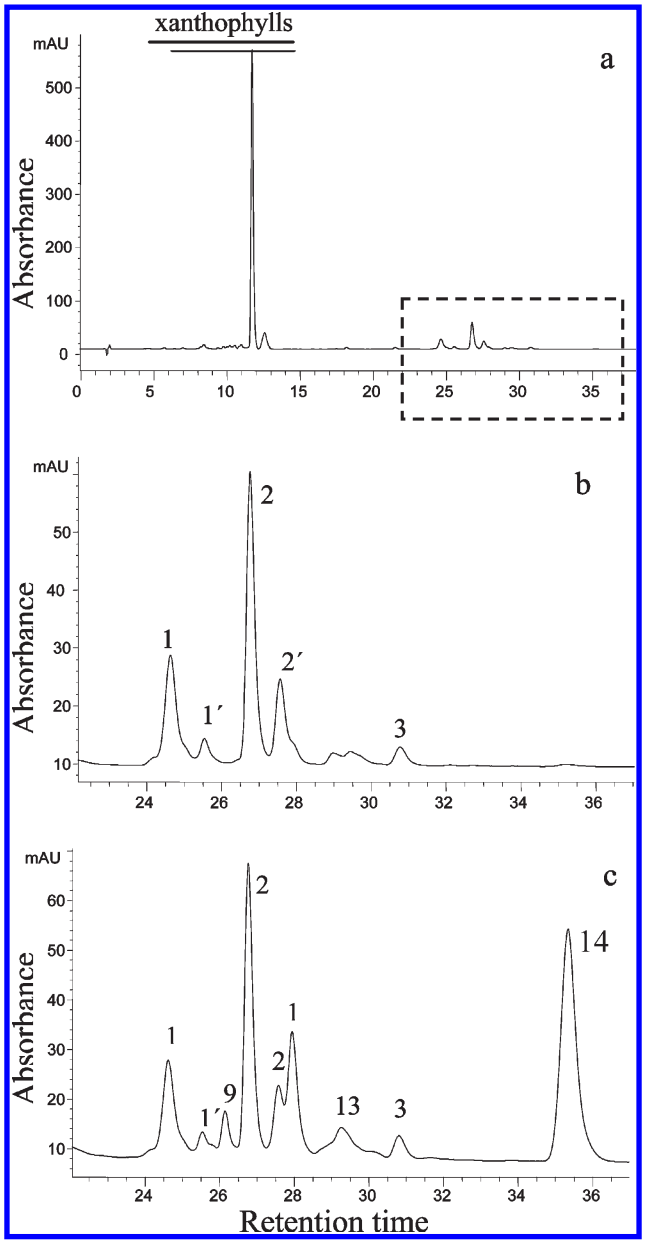


Figure 3. Chromatograms (at 430 nm) of olive oil (**a**, **b**) and mixture of olive oil and colorant E 141i (**c**). Enlargement of chromatograms of the frame of **Figure 3a** for olive oil (**b**) and mixture of olive oil and colorant 141i (**c**). Peaks as in **Tables 1** and **2**.

of the chlorophyll molecule that do not affect the conjugated double-bond system of the macrocycle do not alter the absorption spectrum. Thus, the hydroxylation of C13 (13^2-OH), the loss of the phytol chain, the epimers, and the demethoxycarbonylation that leads to the formation of the pyroderivatives, do not alter the absorption spectrum of the corresponding precursor pigment, although they do alter the polarity, and—in consequence—the retention times (**Table 2** and **Figure 4**).

In the chlorophyll fraction, the most striking feature is that $99.59 \pm 0.52\%$ (in area basis) of the chlorophyll pigments present in the different samples of E 141i colorant are not those of an olive oil. Thus, the simple detection of one of these compounds in an unknown olive oil indicates adulteration. The copper treatment and the conditions during the extraction process of the colorant E 141i (see Introduction) forms copper complexes and chlorophyll compounds that are not present in the olive oil. Such juice is obtained simply by physical means and with smooth

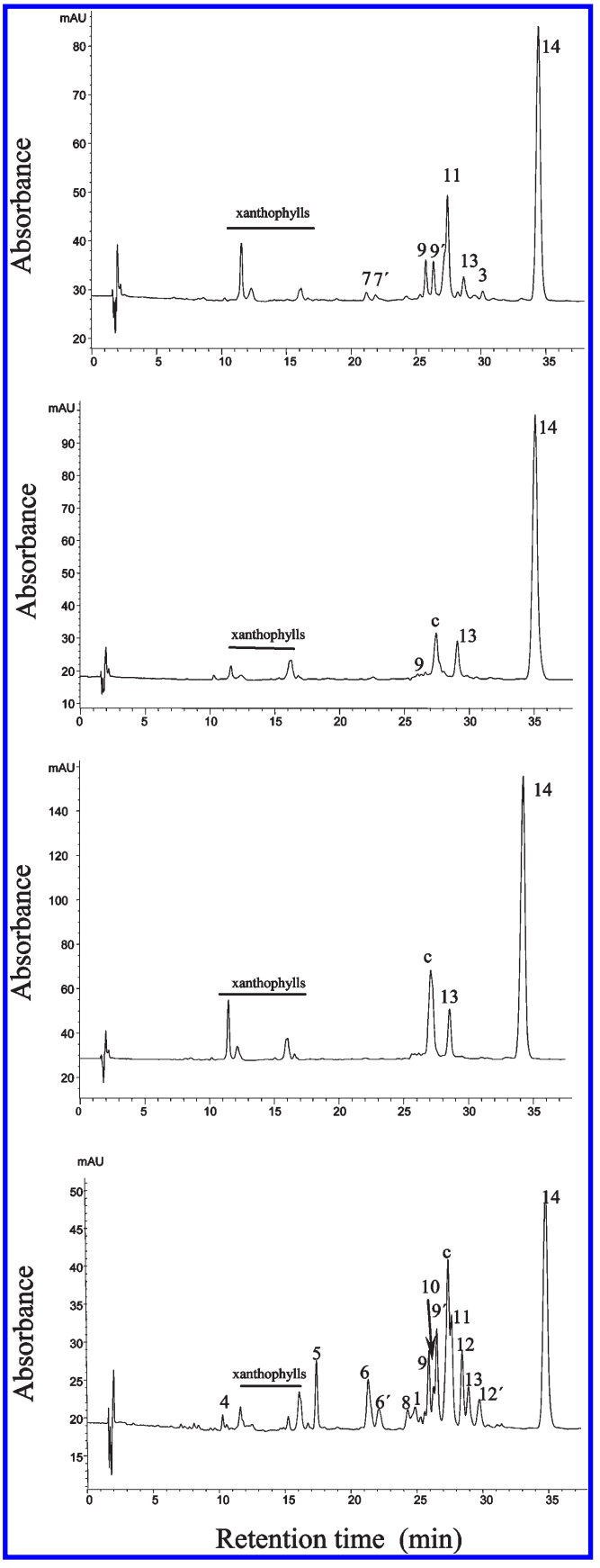


Figure 4. Chromatograms at 430 nm of four different samples of natural colorants E 141i, obtained as described in Materials and Methods. Peaks as in **Table 1** and **2**; c, β-carotene.

temperatures, which minimally modified the original chlorophylls present in the olive fruit (see before). The first three samples of E

Table 2. Chromatographic and Spectroscopic Properties of Chlorophyllic Pigments Present in Colourants 141i Samples

				I		II		III		IV		V		VI	
pigment	peak no. (**Figure 2**−**4**)	K_c[a]	Soret	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*
Cu−pheophorbide *a*	4 (**Figure 2b**)	4.17	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00
glyoxy−pheo *b*[b].	5 (**Figure 2c**)	7.77	426	350	7.00	(408)	2.85	(530)	15.31	(570)	14.41	(596)	17.50	651	6.45
Cu−pheophytin *b*	6 (**Figure 2a**)	9.75	443					540	15.53			590	6.47	633	2.38
Cu−pheophytin *b*′	6′(**Figure 2a**)	10.15	443					540	17.36			590	7.36	633	2.89
13^2-OH−pheophytin *b*	7 (**Figure 2a**)	9.82	435			412	2.27	524	13.62	(558)	20.42	598	16.33	654	3.83
13^2-OH−pheophytin *b*′	7′ (**Figure 2a**)	10.18	435			412	2.27	524	13.62	(558)	21.80	595	17.63	654	4.87
Cu−13^2-OH pheo *b*	8 (**Figure 2a**)	11.27	443					540	15.53			590	6.47	633	2.38
pheophytin *b*	1 (**Figure 2a**)	11.50	435			412	2.27	524	13.62	(558)	20.42	598	16.33	654	3.83
Cu−13^2-OH pheo *a*	9 (**Figure 2b**)	12.07	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00
Cu−13^2-OH pheo *a*′	9′ (**Figure 2b**)	12.38	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00
Cu−15^1-OH-lac- pheo *a*	10 (**Figure 2d**)	12.26	411	392	1.20			500	16.50	(540)	17.71	599	7.75	645	1.75
Pyro−pheophytin *b*	11 (**Figure 2a**)	12.94	435			412	2.27	524	13.62	(558)	20.42	598	16.33	654	3.83
Cu−pheo *a*	12 (**Figure 2b**)	13.35	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00
Cu−pheo *a*′	12′ (**Figure 2b**)	14.02	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00
Cu−yro-pheo *b*	13 (**Figure 2a**)	13.60	443					540	15.53			590	6.47	633	2.38
pyro-pheophytin *a*	3 (**Figure 2b**)	14.41	409	(400)	1.07	(376)	1.31	505	8.78	537	9.62	609	10.63	666	1.85
Cu−pyro-pheo *a*	14 (**Figure 2b**)	16.54	424	(384)	1.65	400	1.02	506	20.11	548	12.07	606	3.29	654	1.00

[a] As in **Table 1**. [b] Glyoxy, 15-glyoxilic acid; pheo, pheophorbide.

141i colorants are similar one to another; the fourth (**Figure 4d**) presents a more complex chromatogram. In any case, however, the major chlorophyll derivative in all of them is Cu−pyropheophytin *a* (peak 14), which makes up, depending on the sample, between 39% and 91% of the chlorophyll fraction. The limit of detection (LOD) defined at a signal-to-noise ratio of about 3 was 6.58 ng/g for Cu−pyropheophytin *a*. Meanwhile, 76−100% of the chlorophyll derivatives present in the colorant samples are cupro-derivatives. As has been seen (**Figure 3a**,**b**), the cupro−chlorophyll derivatives are absent from olive oils, so that detection of the adulteration is relatively easy, among other things because the insertion of the copper ion in a chlorophyll pigment alters its polarity, enabling a chromatographic separation of the original pigment in 1−2 min. The identification is also helped by the fact that the absorption spectra of the cupro−chlorophyll derivatives are substantially different from those of the precursor chlorophyll derivatives, as mentioned above.

Olive Oil Adulterated with E 141i. Figure 3c shows the chromatographic profile of the pigment extract obtained from a sample of olive oil adulterated with E 141i colorant. The assay was conducted with different olive oils, obtaining the same results. The chromatogram has been amplified in the most apolar region, where the cupro−chlorophyll derivatives are concentrated. It is clearly observed how the proposed chromatographic method enables separation of the natural pigments of olive oil (peaks 1−3, **Figure 3b**) from the chlorophyll derivatives coming from the colorant (peaks 9, 11, 13, and 15). As conclusion and practical recommendation, the presence of cupro−chlorophyll derivatives in an olive oil sample implies that the oil has been adulterated. The chromatographic method and information (spectroscopic characteristics, **Table 2** and **Figure 3**) proposed in the present paper, allow the separation and identification of the pigments present in the colorant E 141i. The method also distinguishes between chlorophyll compounds of E141i and chlorophylls inherent to olive oil. Besides the general detection at 430 nm, simultaneous detection at the specific wavelengths of the major copper complexes is recommended: 654 nm for Cu−pyropheophytin *a* and 633 nm for Cu−pyropheophytin *b*.

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