Rapid Method of Quantification of Chlorophylls and Carotenoids in Virgin Olive Oil by High-Performance Liquid Chromatography

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The fatty material of olive oil was separated, yielding a fat-free concentrate of pigments, by solid-phase extraction on octadecyl (C_{18}) columns. The study of recovery carried out with lutein and pheophytin *a* gave an excess error lower than 4%. A total of 17 pigments was separated from this extract and quantified by HPLC using a reversed-phase C_{18} column. The method consisted of an elution gradient of 2 mL/min of water–ionic reagent–methanol (1:1:8) and methanol–acetone (1:1). Detection was performed by absorption at 410 and 430 nm and quantification from the straight lines of calibration of each analytically pure standard. The application of this technique to five monovariety virgin olive oils showed that pigment content among them is very different, depending on the variety of olive.

INTRODUCTION

The technique of HPLC has been widely applied to the study of photosynthetic pigments since Evans et al. (1975), who used normal-phase columns (Iriyama, 1978; Abaychi and Riley, 1979). HPLC using reversed-phase columns has been used in the analysis of marine phytoplankton (Brauman and Crime, 1979; Wright, 1984), natural waters (Mantoura and Llewellyn, 1983), and vegetables and fruits (Schwartz et al., 1981; Fisher and Rouseff, 1986; Khachik et al., 1986; Fisher and Kocis, 1987; Heinonen et al., 1989).

In edible oils, Fraser and Frankl (1985) isolated the chlorophyllic derivatives in soybean oil by chromatography on a cellulose column and separated them by reversed-phase HPLC. Recovery on the column was 60–70% of the pigments present. Aitzetmüller (1989) proposed the freezing-out technique for the analysis of chlorophyll degradation products in fats and oilseeds. This technique permits the obtaining of a pigment solution in acetone that is largely free of triglycerides and can be injected directly into the liquid chromatograph for analysis by reversed-phase HPLC. Rahmani and Saari (1985) proposed a quantitative method for the determination of chlorophylls, pheophytins, and β-carotene in vegetable oils by normal-phase HPLC, directly injecting the oil sample, diluted 20 times (w/v) in hexane–2-propanol (98.5:1.5). However, this method did not detect lutein, which is the major carotenoid in virgin olive oil (Mínguez-Mosquera et al., 1990a).

The HPLC study of carotenoids in oil presents fewer problems than that of chlorophyllic derivatives, as saponification is an easy method for purification of chlorophylls and lipids. Stancher et al. (1987) developed a nonaqueous reversed-phase HPLC method to determine carotenoids in olive oil. They found lutein and β-carotene as majority pigments, in concentrations varying with the type of oil and the process of manufacture.

Although chlorophylls and derivatives are easily separated from xanthophylls and carotenes by saponification, some alkali-sensitive carotenoids are known (Davies, 1976; Liaaen-Jensen, 1971). Khachik et al. (1986) investigated the effect of saponification on the qualitative and quantitative distribution of carotenoids in certain vegetables. They showed that saponification was accompanied by significant losses of xanthophylls—particularly the epoxycarotenoids—while the carotenes were not significantly affected.

The present work proposes the use of octadecyl (C_{18}) solid-phase extraction columns to obtain a fat-free pigment extract from the oil sample. This purified extract may be determined quantitatively by reversed-phase HPLC—separating chlorophylls, xanthophylls, and derivatives individually.

MATERIALS AND METHODS

Apparatus. A Büchi rotavapor, Model R 110; a Desaga UV–vis lamp, provided with white light and ultraviolet $UV_{254,366}$; a Hewlett-Packard UV–vis spectrophotometer, Model 8450, provided with a Hewlett-Packard recorder, Model 7225 A; a Waters 600 E multisolvent delivery system; a Waters 994 programmable photodiode array detector; and a Waters 5200 printer-plotter were used.

Reagents. HPLC grade solvents (methanol and acetone) were used without further purification. The water used was deionized and filtered through a nylon membrane of 0.45 μm.

Procedure. *Pigment Extraction.* Two methods were used to obtain the pigment extract: liquid-phase distribution (LPD), perfected by Mínguez-Mosquera and Garrido-Fernández (1989), and solid-phase extraction (SPE) according to the procedure used by Gutiérrez et al. (1989).

The method of liquid-phase distribution (LPD) is based on a selective separation of pigments between *N,N*-dimethylformamide and hexane. The sample of virgin oil (10–15 g) was dissolved directly in 150 mL of *N,N*-dimethylformamide and treated with five 50-mL successive portions of hexane in a decanting funnel. Chlorophylls, chlorophyllic derivatives, and xanthophylls were retained in the *N,N*-dimethylformamide phase. The hexane phases contained lipids and carotenes.

The *N,N*-dimethylformamide phase was treated with 2% Na_2SO_4 solution at 0 °C and transferred to 100 mL of a mixture of hexane/ethyl ether (1:1 v/v). The aqueous phase was discarded, eliminating polyphenols and other water-soluble compounds. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness in a rotavapor at 30 °C. The dry residue was dissolved in an appropriate volume of acetone, which was used for the pigment analysis by HPLC.

The five hexane phases were combined in a rotavapor flask, concentrated, filtrated, and eluted in a known volume of hexane to measure directly the β-carotene concentration, using the coefficient of extinction $E^{1\%}_{454} = 2592$ (Foppen, 1971) as this phase only contains this pigment (Mínguez-Mosquera et al., 1990a).

For the solid-phase extraction (SPE), octadecyl (C_{18}) disposable extraction columns were used. The column was conditioned first with methanol and then with hexane. The sample, 1 g of oil dissolved in 4 mL of hexane, was injected and washed with a further 3 mL of hexane. Lastly, after checking that there were no lipid remains, the pigments were eluted with 5 mL of acetone.

0021-8561/92/1440-0060$03.00/0

Table I. Gradient Scheme Used for the Separation of Pigments

time, min	mobile phase % A	mobile phase % B	curve[a]
initial	75	25	
8	25	75	linear (6)
10	25	75	isocratic
18	10	90	convex (4)
23	0	100	concave (10)
30	75	25	concave (10)

[a] The numbers in parentheses correspond to the curve type included in the program from water 600 E liquid chromatograph.

The hexane phase retained the carotene fraction and part of the pheophytin *a*, which were measured directly in this solution as detailed above for β-carotene and for pheophytin *a* using the coefficient of extinction in hexane $E^{1\%}_{660} = 613$ (Mínguez-Mosquera et al., 1990b). The acetone phase was taken to dryness and collected in 0.2 mL of acetone for HPLC.

Liquid Chromatography. The chromatographic method was perfected for the table olive in a previous work (Mínguez-Mosquera et al., 1991) and is based (with some modifications) on that of Mantoura and Llewellyn (1983). The latter authors used reversed-phase ion-pair chromatography to achieve good resolution with chlorophyllides and pheophorbides from acetone extracts of algal culture and natural waters. They obtained the best results when the ion-pair reagent was present in both the sample and the mobile phase. Our work has shown, however, that addition of the reagent to the sample is not necessary for good component separation.

The sample dissolved in acetone and filtered through a nylon membrane of 0.45 μm was injected into a liquid chromatograph. Separation was performed on a column packed with Spherisorb ODS-2 (25 cm × 4 mm i.d.; 5-μm particle size) protected with a guard cartridge (3 cm × 4 mm i.d.) packed with the same material as the column. The eluents used were (A) water–ion-pair reagent–methanol (1:1:8 v/v) and (B) acetone–methanol (1:1 v/v). The ion-pair reagent was a solution of tetrabutylammonium (0.05 M) and ammonium acetate (1 M) in water. The pigments were eluted at a rate of 2 mL/min following the gradient scheme shown in Table I.

Pigment detection was at 430 and 410 nm using a programmable array detector; the chromatogram was obtained on a printer-plotter.

Standards. Chlorophylls *a* and *b* were supplied by Sigma (St. Louis, Mo.). Pheophytins *a* and *b* were obtained by acidification with 13% hydrochloric acid from the respective solutions of chlorophylls (Sievers and Hynninen, 1977). Chlorophyll *a* and *b* C-10 epimers were obtained according to the method of Watanabe et al. (1984). β-Carotene, lutein, violaxanthin, and neoxanthin were obtained from a pigment extract of green olives saponified with methanolic KOH (Mínguez-Mosquera and Garrido-Fernández, 1989) and separated by TLC (silica gel GF_{254} plates 20 × 20 cm, thickness 0.7 mm) using petroleum ether (65–95 °C)–acetone–diethylamine (10:4:1). β-Carotene was purified with petroleum ether (40–65 °C) and lutein with dichloromethane–ethyl acetate (4:1). Violaxanthin and neoxanthin were purified with benzene–acetone (4:1) (Foppen, 1971; Mínguez-Mosquera et al., 1991). Auroxanthin and neochrome were obtained from violaxanthin and neoxanthin by acidification with ethanolic hydrochloric acid (0.1 M) (Khachik et al., 1986).

Quantification. The analytically pure samples were used to obtain the calibration slopes representing the area of the peak obtained with different injected volumes of pure solutions of known concentration. The approximate detection limits were calculated from calibration slopes obtained as a function of peak height, taking as lower limit a peak height equal to twice the noise signal. Antheraxanthin and mutatoxanthin were obtained in insufficient quantities to estimate the calibration line.

RESULTS AND DISCUSSION

Obtaining a fat-free extract of pigments enabled the use of reversed-phase HPLC, offering great advantage over normal-phase HPLC. It also allowed the sample to be concentrated, thus considerably decreasing the detection limits compared with those offered by other methods (Rahmani and Saari-Csallany, 1985).

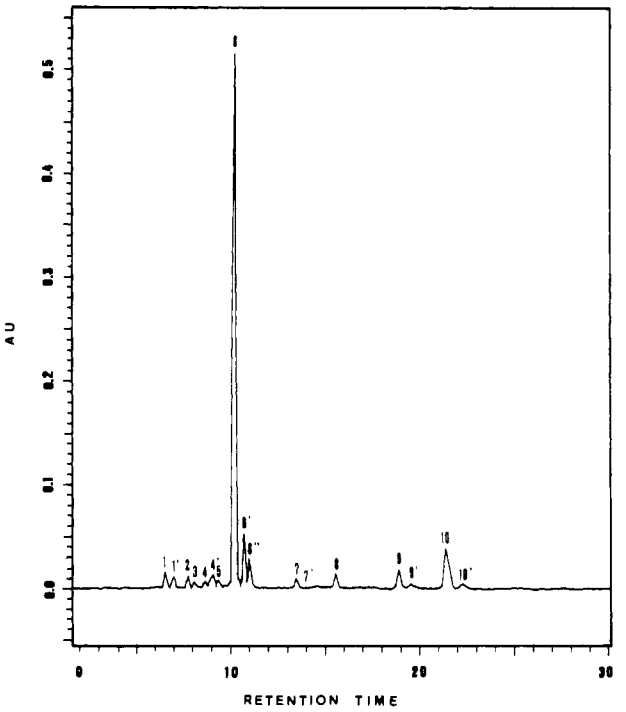


Figure 1. Chromatogram of pigment extract from virgin olive oil. Peak identities: 1, neoxanthin; 1′, neoxanthin isomer; 2, violaxanthin; 3, luteoxanthin; 4, antheraxanthin; 4′, antheraxanthin isomer; 5, mutatoxanthin; 6, lutein; 6′, lutein isomer; 6″, lutein isomer; 7, chlorophyll *b*; 7′, chlorophyll *b*′; 8, chlorophyll *a*; 9, pheophytin *b*; 9′, pheophytin *b*′; 10, pheophytin *a*; 10′, pheophytin *a*′.

Identification was made by comparing retention times and absorption spectra with those of authentic standards. For detection by absorbance, 430 nm was chosen, being a wavelength intermediate between the absorption maximum of pheophytin *a* (410 nm) and that of chlorophyll *b* (466 nm). Since the other components have characteristic absorption maxima within these two extremes, this wavelength is appropriate for the simultaneous detection of chlorophylls and carotenoids. However, another chromatogram was performed in parallel at 410 nm, allowing the quantification of pheophytin *a* with a lower detection limit.

Figure 1 shows the typical chromatogram of pigment extract from virgin olive oil. Table II shows the chromatographic and spectrophotometric characteristics that allowed identification of the pigments. In order of elution, these were neoxanthin, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, and lutein in the xanthophyll group and chlorophyll *b*, chlorophyll *a*, pheophytin *b*, and pheophytin *a* in the chlorophyllic pigments.

HPLC showed that neoxanthin consists of two components (peaks 1 and 1′) which were not separated by TLC. The absorption spectrum of peak 1′, obtained with the photodiode array detector, presented absorption maxima and peak ratios (percent III/II) identical to those of standard neoxanthin. Therefore, the peak corresponded to an isomer of neoxanthin.

Violaxanthin, peak 2, did not show isomers. The electronic absorption maxima of peak 3 showed a hypsochromic displacement of 16–20 nm with respect to the maximum of violaxanthin. Its identification as luteoxanthin was confirmed by its having the same retention time and identical absorption spectrum as standard lu-

Table II. Chromatographic and Spectroscopic Characteristics of Pigments from Olive Oil Separated by HPLC

peak	k^a	$r_{i,st}{}^b$	pigment	spectral data in the eluent, maxima, nm I	II	III	IV	V	VI	peak ratio	detection limits, ng
1	3.74	0.59	neoxanthin	414	438	466				90	3
1′	4.04	0.64	neoxanthin isomer	414	438	466				90	3
2	4.60	0.73	violaxanthin	416	440	470				94	20
3	4.86	0.77	luteoxanthin	400	424	450				107	
4	5.24	0.83	antheraxanthin	(420)	444	474				22	
4′	5.54	0.88	antheraxanthin isomer	(420)	444	474				22	
5	5.77	0.92	mutatoxanthin	(404)	426	452				39	
6	6.30	1.00	lutein	424	446	474				60	2.5
6′	6.72	1.07	lutein isomer	418	440	468				42	2.5
6″	6.91	1.10	lutein isomer	416	438	466				27	2.5
7	8.71	1.38	chlorophyll *b*	466	600	650				3.3 (I/III)	17
7′	9.06	1.44	chlorophyll *b*′	466	600	650				3.3 (I/III)	17
8	10.19	1.62	chlorophyll *a*	(384)	(412)	432	(580)	616	664	1.3 (III/VI)	19
9	12.62	2.00	pheophytin *b*	(412)	436	524	598	654		5.1 (II/V)	8
9′	13.06	2.07	pheophytin *b*′	(412)	436	524	598	654		5.1 (II/V)	8
10	14.41	2.29	pheophytin *a*	410	(470)	506	534	608	666	1.8 (I/VI)	33.5[d]
10′	15.06	2.39	pheophytin *a*′	410	(470)	506	534	608	666	1.8 (I/VI)	33.5[d]

[a] Capacity factor (k) = $(tr - t_m)/tr$. [b] Relative retention ($r_{i,st}$) = k_i/k_{st}; st = lutein. [c] For carotenoids peak ratio is % III/II. [d] Detection limits at 410 nm.

Table III. Study of Recovery of Lutein and Pheophytin *a* by Liquid-Phase Distribution (LPD) and Solid-Phase Extraction (SPE) in Virgin Olive Oil

sample	lutein, mg/kg LPD	lutein, mg/kg SPE	pheophytin *a*, mg/kg LPD	pheophytin *a*, mg/kg SPE
virgin olive oil	1.86	2.40	4.62	6.45
standard solution added	2.22		10.60	
enriched oil[a]	4.33	4.80	14.60	17.24
% recovery	106	104	95.9	101.1

[a] Average of three samples.

teoxanthin obtained from violaxanthin by treatment with 0.1 M ethanolic HCl.

Peak 4 was identified tentatively as antheraxanthin by its similarity in absorption maxima and peak ratios. Khachik et al. (1986) found lutein epoxide in vegetables, which differs from antheraxanthin only in the position of the double bond of the terminal group. Although its absorption maxima were similar to those we have found in this work, neither the form of the spectrum nor the peak ratios were. In our case, the ratio percent III/II was markedly lower, and the lower band was reduced to an inflection point, as is characteristics of carotenoids with two β-ionone residues (Davies, 1975). This may incline its identification toward antheraxanthin. Peak 4′ is the isomer of antheraxanthin; it presents identical absorption maxima and peak ratios.

The electronic absorption maxima of peak 5 showed a hypsochromic displacement of 18 nm with respect to the maxima of antheraxanthin. This points to its identification as mutatoxanthin.

Lutein, peak 6, was accompanied by two other minority peaks (6′ and 6″) whose absorption spectra were similar to those of lutein, but with hypsochromic displacements of 4–6 and 6–8 nm, respectively. This small hypsochromic change points to their identification as mono-cis isomers of lutein (Khachik et al., 1986) and rules out the possibility of isolutein, as the epoxide test was not positive.

Chlorophyll *b* was also shown to consist of two components (peaks 7 and 7′). Comparison with the retention time of the standard prepared according to the method of Watanabe et al. (1984) identified them as epimers of chlorophyll *b*. The retention time of peak 8 agreed with that of chlorophyll *a*. Pheophytin *b* and pheophytin *a* proved to be two isomers, peaks 9 and 9′ and peaks 10 and 10′, respectively.

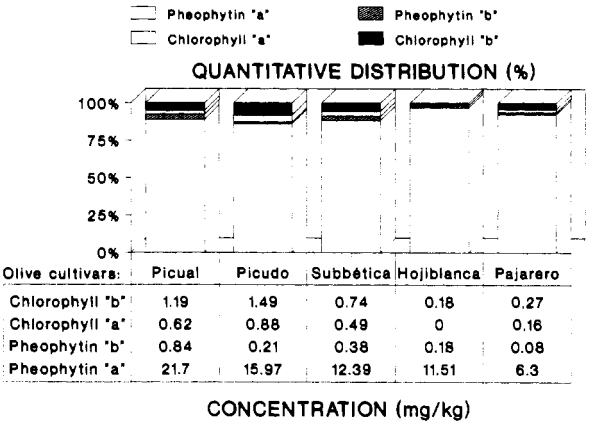
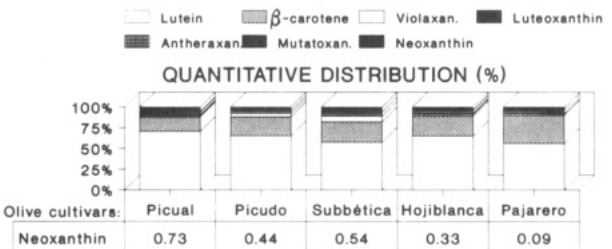


Figure 2. Individual concentration and quantitative distribution of chlorophylls and pheophytins of pigment extracts from five monovariety olive oils.

The pigments identified in virgin olive oil included those found naturally in the fresh olive, together with those formed during the extraction process. Pheophytins are derivatives of the chlorophylls by substitution of the Mg^{2+} ion by H^+, and xanthophylls with furanoid groups such as luteoxanthin and mutatoxanthin are degradation products of their respective 5,6-epoxide precursors, violaxanthin and antheraxanthin. This type of acid-catalyzed reaction is produced by the free acidity due to the fatty acids liberated during the virgin olive oil obtention.

Quantification. Table III shows the results of the study of recovery of lutein and pheophytin *a* by the two extraction procedures used. The standard technique of enrichment was used to study the efficiency of the proposed methods of pigment purification. A virgin olive oil was enriched with lutein (2.22 mg/kg) and pheophytin *a* (10.60 mg/kg), obtained and purified as described in the previous section. The concentration of lutein and pheophytin *a* was determined by HPLC in both the standard solution of pigments and the oil. The pigment concentration in the initial virgin olive oil was determined using LPD and SPE. The percentage recovery was calculated from the expression % recovery = $E/(NE + S) \times 100$, where E is the concentration of lutein or pheophytin *a* found in the enriched oil sample, NE is the concentration of lutein or pheophytin found in the nonenriched virgin oil sample,



Olive cultivars:	Picual	Picudo	Subbética	Hojiblanca	Pajarero
Neoxanthin	0.73	0.44	0.54	0.33	0.09
Mutatoxan.	0.11	0	0	0.05	0
Antheraxan.	0.4	0.36	0.45	0.31	0.16
Luteoxanthin	0.14	0	0	0.11	0
Violaxan.	0.18	0.42	0.64	0.19	0.06
β-carotene	2.33	2.4	2.23	2.19	1.08
Lutein	9.34	6.87	5.26	5.99	1.78

CONCENTRATION (mg/kg)

Figure 3. Individual concentration and quantitative distribution of carotenoids of pigment extracts from five monovariety olive oils.

and S is the concentration of lutein or pheophytin in the standard solution added to the oil.

It was found that the SPE method achieved a recovery of 104% for lutein and 101% for pheophytin *a*. LPD gave recoveries of 106% for lutein and 95.9% for pheophytin *a*.

From these results, it is considered that SPE combined with HPLC offers a clear advantage. It enables the individual quantitative analysis of chlorophyllic and carotenoid pigments in a maximum of 1 h with an excess error of some 4%. Consequently, the method proposed allows routine control of pigments in different vegetable oils, offering broad possibilities for better quality control.

Figures 2 and 3 present individual concentration (milligrams per kilogram) and quantitative distribution (percent) of pheophytins–chlorophylls and carotenoids, respectively, using this methods in five monovariety virgin olive oils. Epimers and isomers of the same compounds were evaluated jointly. Although in all samples pheophytin *a* and lutein are the main pigments, total content of these shows clear differences between varieties that are transferred to the color of the corresponding oils obtained (Mínguez-Mosquera and Garrido-Fernández, 1989; Mínguez-Mosquera et al., 1990a).

ACKNOWLEDGMENT

We express our sincere gratitude to CICYT for supporting this research project and to M. Bodineau-Bada for technical assistance.

LITERATURE CITED

Abaychi, J. K.; Riley, J. P. The Determination of Phytoplankton Pigment. *Anal. Chim. Acta* **1979**, *107*, 1–11.

Aitzetmüller, K. Analysis of Chlorophyll Degradation Products in Fats and Oilseeds. *Abstracts of Papers*, Congrés International "Chevreul" pour l'étude des Corps Gras, Angers, France, 1989; pp 177–184.

Brauman, T.; Crimme, L. H. Single-Step Separation and Identification of Photosynthetic Pigments by High-Performance Liquid Chromatography. *J. Chromatogr.* **1979**, *170*, 264–268.

Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic: London, 1976.

Evans, N.; Games, D. E.; Jackson, A. H.; Matlin, S. A. Applications of High-Pressure Liquid Chromatography and Field Desorption Mass Spectrometry in Studies of Natural Porphyrins and Chlorophyll Derivatives. *J. Chromatogr.* **1975**, *115*, 325–333.

Fisher, C.; Kocis, J. A. Separation of Paprika Pigments by HPLC. *J. Agric. Food Chem.* **1987**, *35*, 55–57.

Fisher, J. F.; Rouseff, R. L. Solid-Phase Extraction and HPLC Determination of β-criptoxanthin and α- and β-carotene in Orange Juice. *J. Agric. Food Chem.* **1986**, *34*, 985–989.

Foppen, F. H. Tables for Identification of Carotenoid Pigments. *Chromatogr. Rev.* **1971**, *14*, 133–298.

Fraser, M. S.; Frankel, G. Detection of Chlorophyll Derivatives in Soybean Oil by HPLC. *J. Am. Oil Chem. Soc.* **1985**, *62*, 113–121.

Gutiérrez, F.; Albi, M. A.; Palma, R.; Rios, J. L.; Olías, J. M. Bitter Taste of Virgin Olive Oil: Correlation of Sensory Evaluation and Instrumental HPLC Analysis. *J. Food Sci.* **1989**, *54*, 68–73.

Heinonen, M. I.; Ollilainen, V.; Linkola, E. K.; Varo, P. T.; Koivistoinen, P. E. Carotenoids in Finnish Foods: Vegetables, Fruits and Berries. *J. Agric. Food Chem.* **1989**, *37*, 655–659.

Iriyama, K.; Yoshiura, M.; Shiraki, M. Micromethod for the Qualitative and Quantitative Analysis of Photosynthetic Pigments Using High-Performance Liquid Chromatography. *J. Chromatogr.* **1978**, *154*, 302–305.

Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, Identification and Quantification of the Major Carotenoid and Chlorophyll Constituents in Extracts of Several Green Vegetables by Liquid Chromatography. *J. Agric. Food Chem.* **1986**, *34*, 603–616.

Liaaen-Jensen, S. Isolation, Reactions. In *Carotenoids*; Isler, O., Ed.; Birkhäuser Verlag: Basel, 1971.

Mantoura, R. F. C.; Llewellyn, C. A. The Rapid Determination of Algal Chlorophyll and Carotenoid Pigments and their Breakdown Products in Natural Waters by Reverse-Phase High-Performance Liquid Chromatography. *Anal. Chim. Acta* **1983**, *151*, 297–314.

Mínguez-Mosquera, M. I.; Garrido-Fernández, J. Chlorophyll and Carotenoid Presence in Olive Fruit (Olea europaea). *J. Agric. Food Chem.* **1989**, *37*, 1–7.

Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Garrido-Fernández, J.; Gallardo-Guerrero, L. Pigments Present in Virgin Olive Oil. *J. Am. Oil Chem. Soc.* **1990a**, *67*, 192–196.

Mínguez-Mosquera, M. I.; Garrido-Fernández, J.; Gandul-Rojas, B. Quantification of Pigments in Fermented Manzanilla and Hojiblanca Olives. *J. Agric. Food Chem.* **1990b**, *38*, 1662–1666.

Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Montaño-Asquerino, A.; Garrido-Fernández, J. Determination of chlorophylls and carotenoids by HPLC During Olive Lactic Fermentation. *J. Chromatogr.* **1991**, *585*, 259-266.

Rahmani, M.; Saari-Csallany, A. *Rev. Fr. Corps Gras* **1985**, *6–7*, 257–259.

Schwartz, S. J.; Woo, S. L.; von Elbe, J. H. High-Performance Liquid Chromatography of Chlorophylls and their Derivatives in Fresh and Processed Spinach. *J. Agric. Food Chem.* **1981**, *29*, 533–535.

Sievers, G.; Hynninen, P. H. Thin-layer Chromatography of Chlorophylls and their Derivatives on Cellulose Layers. *J. Chromatogr.* **1977**, *134*, 359–364.

Stancher, B.; Zonta, F.; Bogoni, P. Determination of Olive Oil Carotenoids by HPLC. *J. Micronutr. Anal.* **1987**, *3*, 97–106.

Watanabe, T.; Hongu, A.; Hond, K.; Nakazato, M.; Konno, M.; Saitoh, S. Preparation of Chlorophylls and Pheophytins by Isocratic Liquid Chromatography. *Anal. Chem.* **1984**, *56*, 251–256.

Wright, S. W.; Shearer, J. D. Rapid Extraction High-Performance Liquid Chromatography of Chlorophylls from Marine Phytoplankton. *J. Chromatogr.* **1984**, *294*, 281–295.

Received for review July 1, 1991. Accepted September 24, 1991.

Registry No. Neoxanthin, 14660-91-4; violaxanthin, 126-29-4; luteoxanthin, 1912-50-1; antheraxanthin, 68831-78-7; mutatoxanthin, 31661-06-0; lutein, 127-40-2; chlorophyll *b*, 519-62-0; chlorophyll *b'*, 22309-14-4; chlorophyll *a*, 479-61-8; pheophytin *b*, 3147-18-0; pheophytin *b'*, 75498-61-2; pheophytin *a*, 603-17-8; pheophytin *a'*, 75598-38-8.