HPLC Separation of Chlorophyll and Carotenoid Pigments of Four Kiwi Fruit Cultivars

M. Pilar Cano

Freezing of Vegetable Products Department, Instituto del Frío, Spanish Council for Scientific Research, Ciudad Universitaria, 28040 Madrid, Spain

Major pigment constituents of the extracts of four kiwi fruit (*Actinidia chinensis*, Planch) cultivars (Hayward, Abbot, Bruno, and Monty) have been separated by high-performance liquid chromatography (HPLC) on a C-18 reversed-phase column. At the ripe stage, three classes of compounds were shown to be present: xanthophylls, chlorophylls and their derivatives, and only one hydrocarbon carotenoid (β-carotene). The xanthophylls were identified as violaxanthin, neoxanthin, neochrome, auroxanthin, lutein epoxide, and lutein. Neo A and neo B, mono-cis isomers of lutein, were observed in all kiwi fruit extracts. The chlorophylls were identified as chlorophyll *b* and chlorophyll *a* and the decomposition product pheophytin *a*. A chlorophyll *a* derivative with unknown structure was detected in the four cultivars, and only Abbot kiwi fruit extracts showed the presence of the epimeric forms, chlorophyll *b′* and chlorophyll *a′*. The only hydrocarbon carotenoid present in this fruit was β-carotene with very low content in all cultivars, especially in Monty kiwi fruits.

INTRODUCTION

The production of kiwi fruit (*Actinidia chinensis*, Planch) in Spain was increased. The composition of kiwi fruit cultured in Spain has been studied by Cano et al. (1989). The green color, which is a major attraction to consumers, is mainly due to chlorophylls, but carotenoids (xanthophylls, oxygenated carotenoids, and hydrocarbon carotenoids) contributed to the appearance of kiwi fruit pericarp (Gross, 1987).

Pigment changes during ripening in the pericarp of the kiwi fruit cv. Bruno were investigated by Gross (1982). The total carotenoid content of the ripe fruit was 6.3 $\mu g\ g^{-1}$. The major carotenoid pattern described in this work contained neochrome as an additional fifth major pigment (6%). Besides the five main carotenoid pigments (violaxanthin, neoxanthin, neochrome, lutein, and β-carotene), and additional 30 pigments in either small or trace amounts produced a carotenoid pattern of unusual complexity. This complexity may be attributed both to the special type of chloroplasts found in the fruit (Possingham et al., 1980), which may have a special biosynthetic capacity, and to the high acidity of the fruit.

The retention of the bright green color of vegetables during heat processing has long been the goal of research in the canning and freezing industry. The degradation of chlorophyll pigments involves a number of reactions. The olive green color of canned and several nonblanched frozen vegetables has been attributed to the formation of pheophytin (Campbell, 1937). Recently it has been shown that not only the formation of pheophytins is involved but also the formation of pyropheophytins (Elbe et al., 1986), the isomerization of chlorophylls (Schwartz et al., 1981), or the considerable formation of chlorophyllides and some pheophorbides during low-temperature (60–70 °C) blanching treatment (Buckle and Edwards, 1970).

Pulp or puree is one of the processed kiwi fruit products finding use in international markets. Although heat can be used to destroy microorganisms in the production of kiwi fruit pulp, undesirable changes take place in the color and flavor of the product. Consequently, it is recommended that the pulp be frozen immediately after manufacture and stored at −18 °C (Lodge et al., 1985). The changes in product quality after prolonged storage of commercial frozen kiwi fruit pulp have been suggested as being responsible for some of its marketing difficulties. Robertson (1985) studied changes in chlorophyll content of frozen kiwi fruit pulp stored at −18 °C. However, very little other information exists on the nature of changes, their significance in establishing product quality, and the conditions under which they occur. So the kiwi fruit was chosen for investigation of their pigment composition as the first step to study the chemical and biochemical pathways of color deterioration in the frozen product during storage.

Several methods of isolation and detection of chlorophylls, carotenes, and xanthophylls in fruits and other organs by high-performance liquid chromatography (HPLC) have been described in the literature. A method for extraction and quantitation of chloroplast pigments in fruits and other organs by HPLC has been reported by Eskins et al. (1977), but they determined quantitatively only the chlorophylls *b* and *a*. Perhaps the closest example to the work presented in this text has been reported by Khachik et al. (1986b), who developed a method for the separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of five green vegetables (broccoli, cabbage, spinach, Brussels sprouts, and kale) by HPLC using a C-18 reversed-phase column. The major carotenoids separated by this method were identified as β-carotene, lutein, zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin.

In this paper I have investigated the four kiwi fruit cultivars grown in Spain for the presence of the major carotenoid and chlorophyll pigments by HPLC. I have developed HPLC conditions that separated 18 components in extracts from kiwi fruits that were assigned to three classes of compounds: xanthophylls, chlorophylls and their derivatives, and hydrocarbon carotenoids. I also have studied the compositional differences in the pigment pattern between cultivars of kiwi fruit to further analyze their suitability for freezing preservation without important changes in fruit color.

EXPERIMENTAL PROCEDURES

Apparatus. A Hewlett-Packard Model 1040 quaternary solvent delivery system equipped with a Hewlett-Packard 1040A rapid-scanning UV–visible photodiode array detector was em-

0021-8561/91/1439-1786$02.50/0 © 1991 American Chemical Society

Table I. Characteristics of Ripe Kiwi Fruits

	cultivar			
characteristic	Hayward	Abbot	Bruno	Monty
weight, g	120.32	102.04	85.42	80.19
pressure test (0.794-cm plunger), N	87.49	70.21	78.48	75.26
pH	3.35	3.22	3.24	3.36
acidity, % citric acid	1.54	1.61	1.62	1.56
total solids, %	17.88	17.53	15.53	17.81
soluble solids, Brix at 20 °C	16.00	16.10	14.70	17.10

ployed. The data were stored and processed by means of a Hewlett-Packard Model 9000/300 computing system and Color Pro plotter. The absorption spectra of the carotenoids were recorded between 300 and 600 nm at the rate of 12 spectra/min. The HP 9000 computer with a built-in integration program was used to evaluate the peak area and peak height. Absorption spectra of isolated components in various solvents were recorded on a Perkin-Elmer Lambda 15 UV-visible spectrophotometer.

Column. Separations were performed on a stainless steel (10 cm × 4.6 mm i.d.) Hypersil ODS (5-μm spherical particles) column (Hewlett-Packard), which was protected with a Hibar guard cartridge (3-cm length × 4.6-mm i.d.) packed with Spherisorp C18 (5-μm particle size).

Reagents and Materials. The reference samples of chlorophyll *a* and chlorophyll *b* (Sigma-Aldrich, S.A.) were used without further purification. Lutein, zeaxanthin, and *all-trans*-β-carotene were provided by Hoffman-La Roche, Basel, Switzerland. HPLC grade solvents, methanol and ethyl acetate (Promochem), were used without further purification. Kiwi fruits, cvs. Hayward, Bruno, Abbot, and Monty, were obtained from the Villaviciosa experimental Farm in Asturias (Spain) and transported to the pilot plant facility at Instituto del Frio. Forty kilograms of each variety of kiwi fruit was stored at 1-2 °C under 90% relative humidity for near 2 months until the best maturity level for processing was reached (Table I). Kiwi fruits were hand-peeled, sliced (6–8 mm), and frozen at −40 °C and 5.5 m/s air rate in an air blast freezer until the temperature of the product was −20 °C. Frozen slices were packed in polyethylene bags, vacuum sealed, and stored at −18 °C.

Chromatographic Procedure. The analytical separations were carried out according to a modified procedure of Schwartz et al. (1981). A combination of isocratic and gradient chromatography separated the oxygenated carotenoids (xanthophylls) from chlorophylls and the hydrocarbon carotenoids. A gradient mixture of methanol/water (75:25), eluent A, and ethyl acetate, eluent B, was used, beginning at time 0 until time 10 (minutes) with a semifinal composition of eluent B (70%). The gradient eluent composition was followed at time 10 until time 14 (minutes) with a final composition of eluent B (100%). The flow rate employed was 1.7 mL/min, and the chromatographic runs were monitored at 430 nm. At the end of the gradient the column was reequilibrated under the initial conditions by a new gradient condition beginning at time 14 until time 20 (minutes) with a final composition of eluent B (0%) at the same flow rate (1.7 mL/min).

Preparation of Samples for Extraction. Fruits were prepared for analysis in the same way they are prepared for consumption, i.e., inedible parts removed. The frozen samples were thawed in a refrigerator until the pigment extraction.

Extraction. The extraction procedure was similar to that employed by Fuke et al. (1985). Sodium carbonate (1–2 g) was added to 50 g of kiwi fruit paste (made for homogenization of small pieces of fruit with seeds and cores removed) to adjust the pH to 8–9 to prevent conversion of chlorophyll to pheophytin. Chilled acetone was then added to the sample to produce an approximately 80% solution. This was mixed in a homogenizer and centrifuged at 4000*g* for 10 min (0–5 °C); this operation was repeated until the green color was completely removed from the residue. The supernatants were collected and transferred to a separatory funnel and diethyl ether and cold deionized water added. After vigorous shaking and standing, the aqueous layer was discarded. The washing procedure was repeated 5–10 times to remove acetone. The diethyl ether layer was dehydrated with fluorometric analytical grade sodium sulfate anhydride. This

Table II. TLC Bands Separated in the Order of Chromatographic Elution of Kiwi Fruit Extracts

band	color	R_f	chem class
1	yellow	0.96	carotenes
2	gray-green	0.79	chlorophylls
3	green	0.68	chlorophylls
4	light green	0.65	chlorophylls
5	yellow-green	0.60	chlorophylls
6	orange-yellow	0.55	xanthophylls
7	yellow	0.34	xanthophylls
8	light yellow	0.32	xanthophylls
9	yellow	0.05	xanthophylls

solution was transferred to a beaker and evaporated under a stream of nitrogen. The residue was dissolved and diluted to 5 mL with chromatographic grade acetone. Duplicate 20-μL samples were injected for each extract for the HPLC analysis.

Separation of Pigments by Semipreparative TLC. A concentrated solution of each sample extract in acetone was chromatographed on semipreparative thin-layer plates (20 × 20 cm, layer thickness 200 μm; Kieselgel 60F 254, Merck, Darmstadt, W. Germany) under a continuous stream of nitrogen using a solution containing petroleum ether (bp 30–60 °C) (50%) and acetone (50%) and allowed to condition for 30 min. The plate was then lowered into the solvent and the chromatogram developed for 1.5 h in darkness at room temperature. The resulting bands were scraped from the plate in the appropriate solvent for HPLC and spectrophotometric analyses. The bands exposed in Table II were separated in this procedure. This preparative TLC was employed to separate the individual kiwi fruit pigments in a relatively pure form so that standards were available for subsequent optimization of HPLC conditions.

Identification of the Pigments. Identification was based on chromatographic behavior on HPLC and TLC, visible absorption spectra, and specific chemical reactions.

Preparation of Calibration Curves. The analytically pure samples obtained from TLC and HPLC were employed for the preparation of the calibration curves. The calibration curves for xanthophylls and carotenes were obtained by area measurement of pure reference compounds at various concentrations. In general, the procedure employed for the pigment quantification was similar to that previously reported by Khachik et al. (1986b). The calibration curves prepared gave good linearity over a wide range of concentration and had relative standard deviations less than 5%.

RESULTS AND DISCUSSION

The major pigment constituents of kiwi fruit consist of three classes of compounds. In order of chromatographic elution on a C-18 reversed-phase column these are (a) xanthophylls (oxygenated carotenoids), (b) chlorophylls and their derivatives, and (c) hydrocarbon carotenoids (β-carotene). The chromatograms of the four kiwi fruit cultivars (Figures 1–4) show the presence of the typical components of these various classes of compounds. The chromatographic conditions employed separated the components within 11 min. The xanthophylls (peaks 1–10) are eluted under the first step of the proposed gradient, a composition of eluent B (70%) and ethyl acetate, at time 10 min, which is followed by a second gradient step that elutes the chlorophylls and their derivatives (peaks 11–17) as well as the single hydrocarbon carotenoid, β-carotene, found (peak 18). Under these conditions the above components are well separated, with a greater abundance of chlorophylls with respect to xanthophylls and β-carotene (Table III).

Peak Identification. The xanthophylls, chlorophylls, and the hydrocarbon carotenoid found in the four cultivars of kiwi fruit and their corresponding HPLC peaks as identified by spectroscopy and chromatography as well as chemical methods are shown in Table III. The details of the identification and the chemistry of these compounds will be described later.

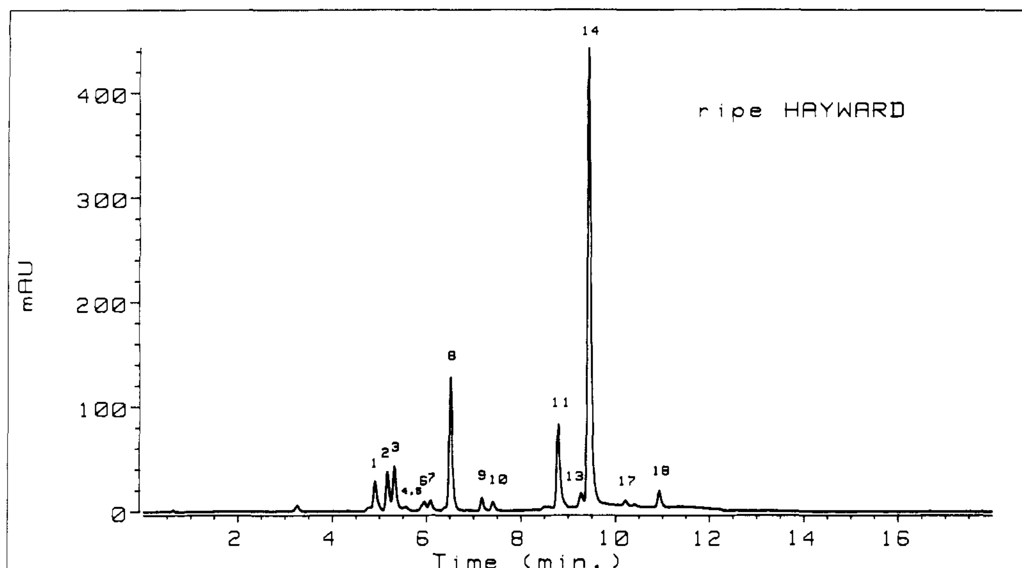

Figure 1. HPLC chromatogram of ripe Hayward kiwi fruit extract. Peak identifications are given in Table III.

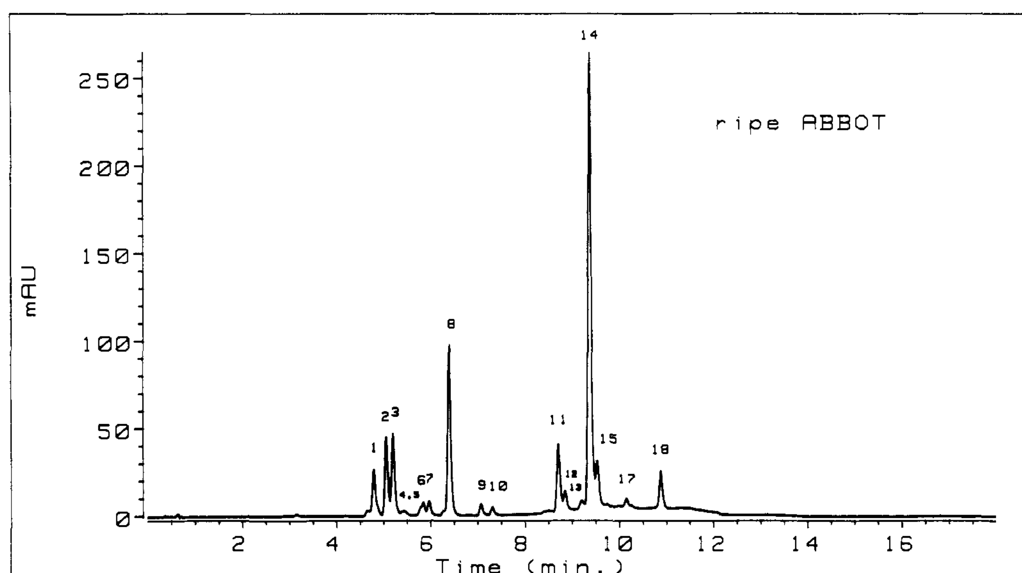

Figure 2. HPLC chromatogram of ripe Abbot kiwi fruit extract. Peak identifications are given in Table III.

Qualitative Distribution of Carotenoids and Chlorophylls. The general chromatographic profiles of the four kiwi fruit cultivars are very similar. The major differences among cultivars appear to be the level of concentration at which the various components are present. For example, the chromatograms from extracts of ripe kiwi fruits show the presence of the same components; however, the relative concentration of β-carotene (peak 18) with respect to other compounds present in Abbot and Bruno cultivars appears to be higher than the β-carotene content in Monty and Hayward kiwi fruits. The carotenoids of kiwi fruit, cv. Bruno, have been characterized as β-carotene, lutein, violaxanthin, neoxanthin, and neochrome (Gross, 1982). The ratio of the five main pigments differed from that of green leaves in lower β-carotene, very low violaxanthin level, and the presence of neochrome at levels twice as high as violaxanthin (Gross, 1987).

This author describes an additional 30 pigments in either small or trace amounts (characterized only by their electronic spectra) which produced a carotenoid pattern of unusual complexity. However, in this study HPLC chromatograms of kiwi fruit extracts showed the presence of 10 xanthophylls identified by their spectral characteristics and chemical behavior as well as comparison of the HPLC retention times of unknowns with those of reference compounds. TLC separation of pigment extract produced nine bands (Table II) using petroleum ether (50%) and acetone (50%) as eluent. The components of each band were rechromatographed to identify their retention time and spectral absorption. Isolation of each compound and in some cases establishment of their structures by chemical reactions produced the structural elucidation comparing with the literature data about these compounds.

(*a*) *Xanthophylls.* The major xanthophylls found in kiwi fruits are listed in order of chromatographic elution in Table III. The identification of these compounds by mass spectroscopy was often accompanied by confusing results (Khachik et al., 1986b). The UV–visible light absorption properties of individual xanthophylls provided valuable information on the nature of the end group and the chromophores involved.

Table III shows the absorption maxima in ethanol of these compounds. 9′-*cis*-Neoxanthin (natural neoxanthin) (λ_{max} 438 nm) was identified from its UV–vis light absorption and the hypsochromic shift of 4 nm in comparison to its all-trans compound. The addition of a few drops of ethanolic hydrogen chloride produced the

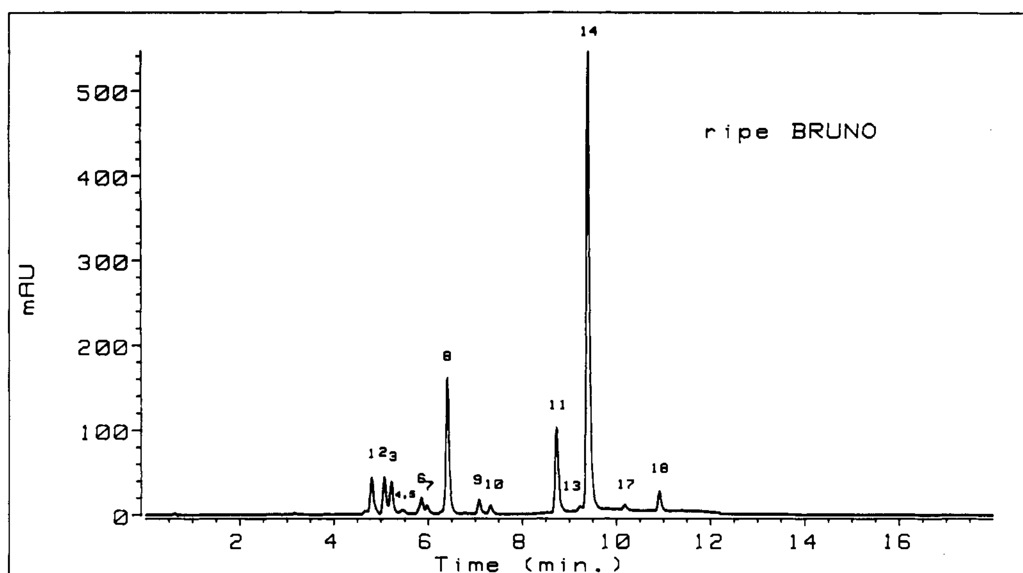

Figure 3. HPLC chromatogram of ripe Bruno kiwi fruit extract. Peak identifications are given in Table III.

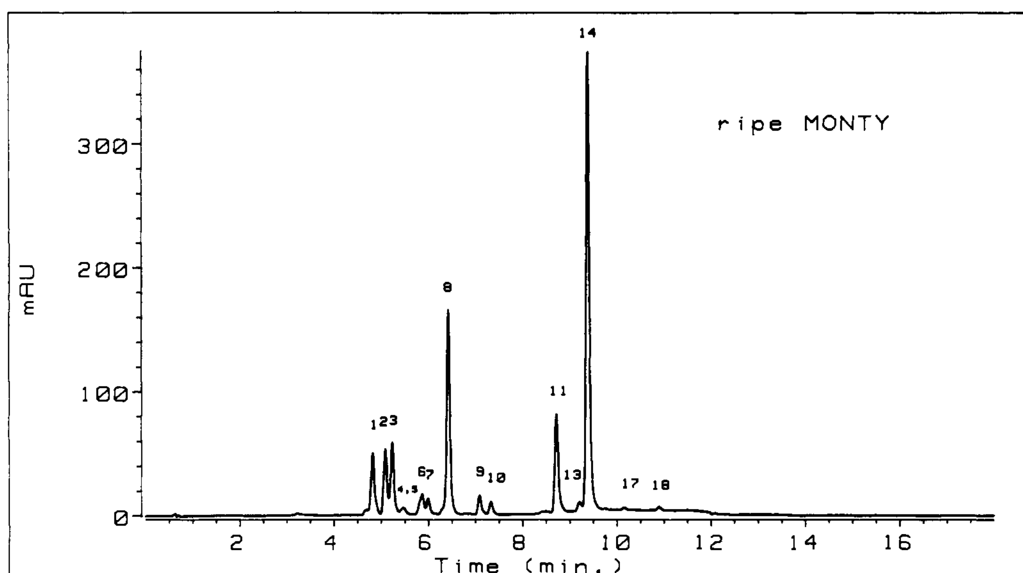

Figure 4. HPLC chromatogram of ripe Monty kiwi fruit extract. Peak identifications are given in Table III.

Table III. Peak Identification of the Various Components of the Kiwi Fruit Extracts Separated by HPLC

chem class	peak	component	retention time, min	λ_{max}, nm (ethanol)
xanthophylls	1	9′-*cis*-violaxanthin	4.92	418, 442, 470
	2	9′-*cis*-neoxanthin	5.18	412, 436, 464
	3	violaxanthin	5.32	418, 442, 470
	4	neochrome (*cis*)	5.52	398, 422, 449
	5	neochrome (*trans*)	5.60	398, 422, 449
	6	auroxanthin	5.96	380, 402, 426
	7	lutein epoxido	6.07	418, 440, 468
	8	*all-trans*-lutein	6.49	442, 446, 474
	9	neolutein B	7.16	418, 442, 470
	10	neolutein A	7.40	420, 438, 466
chlorophylls	11	chlorophyll *b*	8.80	(438), 462
	12	chlorophyll *b*′	8.84	(438), 462
	13	chlorophyll *a* derivative	9.29	334, 382, 412, 430
	14	chlorophyll *a*	9.46	334, 382, 412, 430
	15	chlorophyll *a*′	9.61	334, 382, 412, 430
	16	pheophytin *b*	10.08	414, 434
	17	pheophytin *a*	10.25	324, 372, 408
hydrocarbon carotenoids	18	*all-trans*-β-carotene	10.98	422, 446, 474

expected hypsochromic shift of 16–20 nm in its absorption maxima characteristic of an epoxide–furanoxide rearrangement to afford neochrome (λ_{max} 422 nm). The presence of *all-trans*-neoxanthin and/or neochrome in green vegetables may be an artifact due to extraction and/or chromatography; however, the occurrence of neoxanthin (9′-*cis*) and neochrome in the pericarp of kiwi fruit has been established by Gross (1982). The HPLC retention

time and the absorption spectrum of neochrome (*cis* and *trans*) were identical with those of an authentic sample of this compound obtained from neoxanthin.

Violaxanthin was identified from its visible light absorption and chemical transformations. Isolation of this compound by TLC was shown by HPLC to have resulted in the formation of luteoxanthin (λ_{max} 422 nm) and auroxanthin (λ_{max} 402 nm); this behavior was also confirmed by acid treatment. In this work, a good separation of *trans*-violaxanthin and *cis*-violaxanthin showing the same absorption maxima at 442 nm with retention times of 4.90 and 5.32 min, respectively, was obtained (Table III).

Auroxanthin (λ_{max} 402 nm) and *all-trans*-lutein epoxide (λ_{max} 442 nm) isolated by semipreparative TLC were shown in HPLC chromatograms. On treatment with trace amounts of acid the absorption maximum of lutein epoxide showed a 20-nm hypsochromic shift. I have not observed the presence of antheraxanthin, a structurally related isomer of lutein epoxide, in the four kiwi fruit cultivars that were investigated in the present work. In analogy to the biosynthesis of antheraxanthin from zeaxanthin, the biosynthetic pathway to lutein epoxide would be expected to involve lutein. Therefore, the occurrence of lutein epoxide is not surprising, since its precursor, lutein, is the most abundant xanthophyll in kiwi fruit.

Lutein, in these extracts, was shown to be accompanied by two minor cis isomers, which were identified from their absorption. Neolutein B (λ_{max} 442 nm) and neolutein A (λ_{max} 438 nm) peaks showed a good separation at 7.130 and 7.367 min, respectively. The visible absorption of neolutein A contained an intense cis peak in the near-UV region. The visible light absorption spectra and retention time of *all-trans*-lutein were identical with those of an authentic sample of this compound and with the literature values (Ritter and Purcell, 1981).

(*b*) *Chlorophylls.* The major chlorophylls found in kiwi fruit extracts were identified as chlorophylls *a* and *b* and five derivatives from their HPLC retention times and absorption spectra. Only in Abbot kiwi fruits, chlorophylls *b* and *a* were both accompanied by minor quantities of their C-10 epimeric isomers, which are known as chlorophylls *b′* and *a′*, respectively; the chromatogram are shown in Figure 2. These epimeric isomers have also been reported by Khachik et al. (1986) in the HPLC separation of carotenoids and chlorophyll constituents in green vegetables such as broccoli, cabbage, spinach, Brussels sprouts, and kale. Pheophytin *a*, the most common derivative of chlorophyll *a*, was also present in all kiwi fruit extracts. Pheophytin *b* was not present in ripe fruits, but it could be detected in preclimacteric fruits (cv. Abbot) and in frozen kiwi fruit slices. The conversion of chlorophylls to pheophytins, which is readily effected as result of heat or acid treatment, could be propiciated in kiwi fruit tissues due to its high acidity. The peak with a retention time of 9.29 min observed in all kiwi fruit cultivars was an unknown chlorophyll *a* derivative with spectral characteristics like chlorophyll *a*. This compound has been previously reported in the literature (Kost, 1988).

Quantitative Distribution of Xanthophylls, Chlorophylls, and Carotenes. The quantitative distribution of xanthophylls, chlorophylls, and carotenes in kiwi fruit cultivars is shown in Table IV. The quantitative data shown in Table IV for each kiwi fruit cultivar were obtained from three consecutive extractions from one homogeneous batch of sample (see Table I for ripeness characteristics). The kiwi fruit cultivars studied in this work contain the same constituents, but the concentration of each class (xanthophylls, chlorophylls, and β-carotene) varies over a fairly wide range depending upon variation attributable to the fruit ripeness and cultivar because the season and growing location were the same for the four kiwi fruit cultivars.

Table IV. Quantitative Distribution of Xanthophylls, Carotenes, and Chlorophylls in Kiwi Fruit Cultivars

component	kiwi fruit cultivars, mg/100 g of edible food			
	Hayward	Abbot	Bruno	Monty
	Xanthophylls			
9′-*cis*-violaxanthin	0.079	0.079	0.085	0.104
9′-*cis*-neoxanthin	0.078	0.115	0.075	0.097
violaxanthin	0.093	0.129	0.072	0.117
neochrome (*cis* + *trans*)	0.012	0.015	0.016	0.017
auroxanthin	0.024	0.028	0.039	0.043
lutein epoxido	0.021	0.024	0.019	0.029
all-trans-lutein	0.290	0.259	0.281	0.316
neolutein B	0.029	0.017	0.029	0.029
neolutein A	0.021	0.013	0.019	0.022
total	0.647	0.679	0.635	0.770
	Chlorophylls			
chlorophyll *b*	0.437	0.294	0.450	0.458
chlorophyll *b′*		0.095		
chlorophyll *a* derivative	0.076	0.049	0.024	0.042
chlorophyll *a*	1.723	1.127	1.592	1.420
chlorophyll *a′*		0.225		
pheophytin *b*				
pheophytin *a*	0.031	0.027	0.030	0.010
total	2.267	1.817	2.096	1.930
	Carotenes			
all-trans-β-carotene	0.036	0.065	0.041	0.005

There are some similarities in the distribution percentage of various components of xanthophylls between different cultivars. It appears that the lutein percentages of total xanthophylls are more or less the same in the four cultivars of ripe kiwi fruit, although the conversion of lutein to lutein epoxide may be more efficient in cv. Abbot, which showed a higher level of this xanthophyll related to lower lutein content.

There was not found α-carotene in the kiwi fruit extracts. Some authors (Khachik and Beecher, 1986a) explain this circumstance due to the hydroxylation of α- and β-carotene known to be responsible for the formation of 3-hydroxy cyclic carotenoids and epoxy carotenoids; the absence of α-carotene in kiwi fruit may therefore be related to the complete conversion of this compound to lutein.

The occurrence of neoxanthin, which is the most common allene in green fruits, has also been associated with violaxanthin and lutein. The differences between percentages of neoxanthin, violaxanthin, and lutein from one cultivar to another may be attributed to their biosynthetic transformations. The photosynthetic mechanism by which these xanthophylls are formed would be expected to be greatly affected by the sources of sample variance (Goodwin, 1980).

The presence of cis isomers of xanthophylls (*cis*-violaxanthin, see chromatograms) in the fruit extracts was initially expected to be due to an artifact of extraction and/or chromatography. Such artifacts usually result in the formation of an equilibrium mixture in which the final ratio of cis to trans isomers at equilibrium is constant. However, the quantitative evaluation of the ratios of the cis and trans isomers in different kiwi fruit cultivars, obtained under identical extraction and chromatographic procedures, indicates that the cis isomers may not be an artifact. These changes in the ratio of cis to trans isomers are particularly noticeable in the case of 9′-*cis*- and *all-trans*-violaxanthin, which varies from 1.63 (cv. Abbot) and 1.17 to 1.12 (cv. Hayward and cv. Monty, respectively) to 0.84 (cv. Bruno). The presence of neochrome (*cis* + *trans*)

is probably an artifact, because very small quantities of these compounds were observed in all kiwi fruit extracts. Gross (1982) reported the presence of neochrome as a fifth main xanthophyll pigment in Bruno kiwi fruits; this may be due to the chromatographic procedure employed (TLC), as well as to the usual variability between samples of Bruno cultivar studied in both works.

The total xanthophyll content in Hayward, Abbot, and Bruno kiwi fruit cultivars is about 10–15 times greater than the β-carotene content, the only hydrocarbon carotenoid present; Monty cultivar shown only traces of β-carotene (Table IV). There are similarities between the total carotenoid content of the Bruno kiwi fruits presented in this paper and those of the same cultivar reported by Gross (1982), but there is no information about the xanthophylls and β-carotene content in the other three kiwi fruit cultivars.

The quantitative data on chlorophylls and derivatives are shown in Table IV. The loss of magnesium in chlorophyll *a*, which converts this compound to pheophytin *a*, is taking place much more efficiently than in chlorophyll *b* for all kiwi fruit cultivars studied. The Hayward kiwi fruits show the higher values in total chlorophylls at the ripe stage (2.26 mg/100 g of edible food). The other cultivars (Bruno, Monty, and Abbot) show values between 1.81 and 2.09 mg/100 g of edible food. The presence of a chlorophyll *a* derivative, peak 13, with a chemical structure similar to that of chlorophyll *a* but unknown, in all kiwi fruit cultivars is coincident with the results of Brauman and Grimme (1981), who studied extracts of other produce.

The epimeric chlorophyll derivatives, chlorophyll *a′* and chlorophyll *b′*, only appeared in Abbot kiwi fruit extracts (Figure 2). The presence of these compounds in the extract was initially expected to be an artifact of extraction. However, the extraction and chromatographic procedure for the analysis of all kiwi fruit cultivars was rigorously the same, and these epimeric isomers have also been reported by Schwartz et al. (1981) in the HPLC separation of chlorophylls and their derivatives in fresh and processed spinach.

Nomenclature. For convenience, the trivial names of several naturally occurring carotenoids have been used throughout this text. The trivial and the systematic names as well as the chemical structures of the xanthophylls, chlorophylls, and hydrocarbon carotenoids have been given by Kost (1988).

ACKNOWLEDGMENT

I thank Dr. W. Schuep and Dr. H. E. Keller and the F. Hoffman-La Roche Co. (Basel, Switzerland) for their support in providing a number of carotenoid reference samples. This research was supported by the Comisión Interministerial de Ciencia y Tecnologia (Spain) through Project ALI88-0180.

LITERATURE CITED

Brauman, T.; Grimme, L. H. Reversed-phase high performance liquid chromatography of chlorophylls and carotenoids. *Biochim. Biophys. Acta* **1981**, *637*, 8–11.

Buckle, K. A.; Edwards, R. A. Chlorophyll degradation and lipid oxidation in frozen unblanched peas. *J. Sci. Food Agric.* **1979**, *21*, 307–312.

Campbell, H. Undesirable color changes in frozen peas stored at insufficiently low temperatures. *Food Res.* **1937**, *9*, 140–145.

Cano, M. P.; Fuster, C.; Prestamo, G. Suitability to freezing of four spanish kiwifruit cultivars. In *Tecnical Innovations in Freezing and Refrigeration of Fruits and Vegetables*; International Institute of Refrigeration, University of Davis: Davis, CA, 1989; pp 262–267.

Elbe, J. H.; Juang, A. S.; Attoe, E. L.; Kenneth Nank, W. Pigment composition and color of conventional and very green canned beans. *J. Agric. Food Chem.* **1986**, *34*, 52–54.

Eskins, K.; Schofield, C. R.; Dutton, H. J. High-performance liquid chromatography of plant pigments. *J. Chromatogr.* **1977**, *135*, 217–220.

Fuke, Y.; Sasago, K.; Matsuoka, H. Determination of chlorophylls in kiwifruit and their changes during ripening. *J. Food Sci.* **1985**, *50*, 1220–1223.

Goodwin, T. W. *The Biochemistry of the Carotenoids*; Chapman and Hall: New York, 1980; Vol. 1, Chapter 4, p 96.

Gross, J. Pigment changes in the pericarp of the Chinese gooseberry of kiwi fruit (*Actinidia chinensis*) cv. Bruno during ripening. *Gartenbauwissenschaft* **1982**, *47*, 162–167.

Gross, J. *Pigments in Fruits*; Schweigert, B. S. S., Ed.; Academic Press: London, 1987.

Khachik, F.; Beecher, G. R. Separation and identification of carotenoids and carotenoid precursors found in green vegetables by liquid chromatography. Presented at the Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy, Atlantic City, NJ, March 10–14, 1986a.

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.* **1986b**, *34*, 603–616.

Kost, H. P. *Plant Pigments*; Zweig, G., Sherma, J., Eds.; CRC Press: Boca Raton, FL, 1988; Vol. 1.

Lodge, N.; Hogg, M. G.; Fletcher, G. C. Gamma-irradiation of frozen kiwifruit pulp. *J. Food Sci.* **1985**, *50*, 1224–1226.

Possingham, J. V.; Coote, M.; Hawker, J. S. The plastids and pigments of fresh and dried Chinese gooseberries (*Actinidia chinensis*). *Ann. Bot.* **1980**, *45*, 524–533.

Ritter, E. D.; Purcell, A. E. *Carotenoids as colorants and vitamin A precursors*; Bauernfeind, J. C., Ed.; Academic: New York, 1981; Chapter 10, p 883.

Robertson, G. L. Changes in the chlorophyll and pheophytin concentrations of kiwifruit during processing and storage. *Food Chem.* **1985**, *17*, 25–32.

Schwartz, S. J.; Woo, S. L.; 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.

Received for review April 11, 1991. Accepted July 24, 1991.