JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 117−121 **117**

Color in Fruit of the Genus *Actinidia*: Carotenoid and Chlorophyll Compositions

TONY K. MCGHIE* AND GARY D. AINGE

The Horticulture and Food Research Institute of New Zealand Ltd., Private Bag 11030, Palmerston North, New Zealand

The carotenoid and chlorophyll contents in the fruit of four species of *Actinidia* were measured to determine the chemical basis of color in kiwifruit and related *Actinidia* species. The species studied were the two commercial fruits *Actinidia deliciosa* cv. Hayward and a yellow-fleshed genotype *Actinidia chinensis* cv. Hort16A (known commercially as ZESPRI Gold kiwifruit), the yellow fruit of *Actinida polygama*, and the orange fruit of *Actinida macrosperma*. As reported previously, ripe fruit of *A. deliciosa* contain chlorophylls *a* and *b* and the carotenoids normally associated with photosynthesis, β-carotene, lutein, violaxanthin, and 9′-*cis*-neoxanthin. The carotenoids in *A. chinensis* were similar to those in *A. deliciosa* but also contained esterified xanthophylls. Only trace amounts of chlorophyll were present in *A. chinensis*. The major carotenoid in both *A. macrosperma* and *A. polygama* was β-carotene, with no chlorophyll detected. The yellow color of *A. chinensis* was mostly due to the reduction of chlorophyll rather than an increase in carotenoid concentration. In contrast to the three yellow/orange species, the green fruit of *A. deliciosa* retains chlorophyll during maturation and ripening, and esterified xanthophylls are not produced. This suggests that in fruit of *A. deliciosa* chloroplasts are not converted to chromoplasts as is typical for ripening fruit.

KEYWORDS: Kiwifruit; *Actinidia*; carotenoids; chlorophyll; color

INTRODUCTION

The health benefits of consuming fruit-derived antioxidants such as vitamin C, carotenoids, and flavonoids are increasingly being recognized (*1*). Carotenoids are a diverse group of lipophilic antioxidant compounds found in abundance in fruits and vegetables. The consumer does not always appreciate that green vegetables contain carotenoids; yellow, orange, and red fruits are more widely recognized to contain higher concentrations of carotenoids, which accumulate during fruit ripening (*2*, *3*). Thus, carotenoids contribute to both the appearance and attractiveness of fruit as well as provide additional nutritional value in the form of dietary antioxidants.

Until recently the only species of the genus *Actinidia* grown commercially was *A. deliciosa* cv. Hayward, the well-known kiwifruit or "kiwi". In this genotype, the flesh of the ripe fruit is green and contains chlorophyll and various carotenoids including lutein and β-carotene (*4*). Recently, a yellow-fleshed kiwifruit (cv. Hort16A), of the species *Actinidia chinensis*, has come into commercial production and is being marketed under the name ZESPRI Gold Kiwifruit. Production of this new kiwifruit is expected to rise rapidly over the next 5−10 years.

Within the genus *Actinidia* a range of fruit colors occurs, including green, red, purple, yellow, and orange. To date, the compositions of carotenoid and chlorophyll pigments have been reported only for the green fruit of one species, *A. deliciosa* (*4*−*6*). To determine the chemical basis of highly colored ripe fruit in the genus *Actinidia*, we investigated the major chlorophyll and carotenoid components and concentrations in four genotypes representing four different *Actinidia* species.

MATERIALS AND METHODS

Samples of fruit of four genotypes, each from a different *Actinidia* species, were studied. Fruits of the commercial cultivars, *A. chinensis* Planch. cv. Hort16A and *A. deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson cv. Hayward were bought at a local supermarket in June 1999. Fruits of *Actinidia macrosperma* C.F. Liang (MA03_01) and *Actinidia polygama* (Sieb. et Zucc.) Maxim. (PC01_02) were collected at the Kerikeri Research Orchard in February 1999. The HortResearch germplasm genotype number, shown in parentheses above, consists of a two-letter prefix, which denotes the taxon, the next two numbers denoting the accession of that particular taxon in chronological order, and the two numbers following the subscript dash denote the particular genotype or selection studied. Voucher specimens have been deposited at the Auckland Institute and Museum (AK) and Landcare Research (CHR): *A. chinensis* cv. Hort16A ARF 336, *A. deliciosa* cv. Hayward ARF 37, 121, and *A. macrosperma* MA03_01, ARF 248, 313. Specimens of *A. polygama* PC01_02 are being collected.

Extraction. Fruits of *A. chinensis*, *A. deliciosa*, and *A. macrosperma* were ripe when received and extracted immediately; fruits of *A. polygama* were not ripe when received and were left to ripen at room temperature. Approximately 50 g of whole, ripe (soft and edible) fruit of *A. polygama* and *A. macrosperma* and portions of flesh from five separate fruits each of *A. chinensis* and *A. deliciosa* were extracted with 200 mL of acetone/tetrahydrofuran (1:1) in the presence of 15 g of anhydrous Na_2SO_4 and 3 g of $NaCO_3$ in a Polytron blender. After filtering, the residue was further extracted with another 200 mL of

10.1021/jf010677l CCC: $22.00
Published on Web 11/27/2001

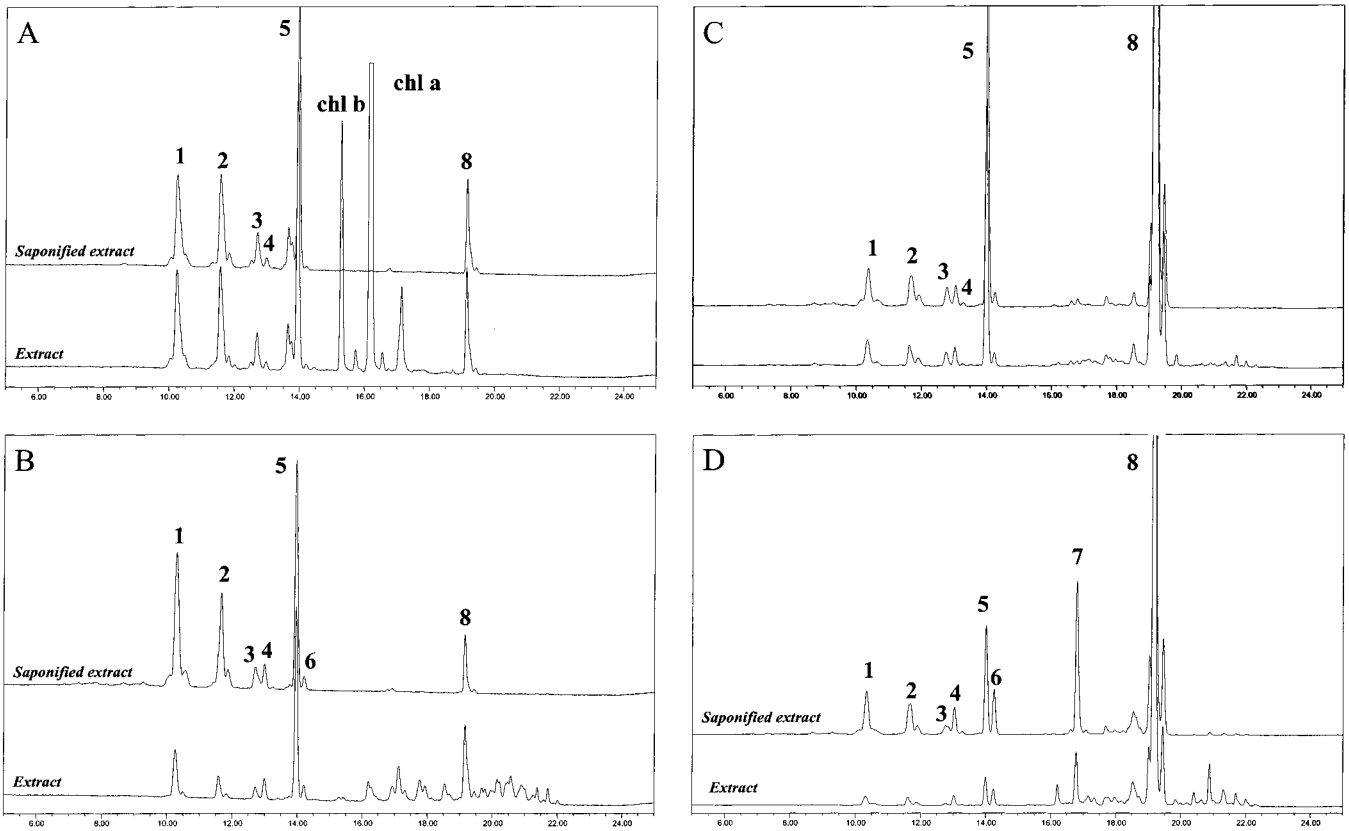

Figure 1. HPLC chromatogram traces of carotenoids and chlorophylls from fruit of *A. deliciosa* (A), *A. chinensis* (B), *A. macrosperma* (C), and *A. polygama* (D). Detection is at 430 nm. Peak identifications are provided in Table 1.

acetone/tetrahydrofuran (1:1), and the two extracts were combined. The solvent in each extract was removed under vacuum on a rotary evaporator at 40 °C and transferred to a separating funnel; the remaining residue was transferred with 2 × 50 mL rinses of diethyl ether. Fifty milliliters of 10% NaCl was added, the ether layer was collected, and the remaining aqueous layer was extracted twice more with 100 mL of diethyl ether. The combined ether extracts were evaporated to dryness under vacuum, and the residue was dissolved in a minimal amount of acetone. During storage at −20 °C a precipitate formed in the extracts, which dissolved when a small quantity of diethyl ether was added.

Saponifcation. For the saponification experiments, 2 mL of acetone/ether extract was taken to dryness, flushed with N_2, and treated with 1.5 mL of diethyl ether/methanol/30% NaOH (0.2:1:0.3) overnight at room temperature in the dark. After 18 h, 10% NaCl and ether was added, and the carotenoids were recovered. After evaporation, the residue was dissolved in 2 mL of acetone and analyzed by HPLC.

HPLC Separation. All extracts were analyzed by reversed-phase HPLC with a system composed of JASCO HPLC components (LG-980-02 ternary gradient controller, DG-980-50 degassing unit, PU-980 HPLC pump, AS-950 autosampler, and UV-975 UV−vis detector). Separation of carotenoids and chlorophylls was achieved using a Waters Spherisorb ODS2 column, and the ternary gradient system was as described by Wright et al. (*7*). Carotenoid and chlorophyll profiles were monitored at 430 nm and the chromatographic data processed on a Millennium Chromatography Data System.

Carotenoid Identification and Quantification. Chromatographic peaks were identified by comparison with authentic standards of β-carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, chlorophyll *a*, and chlorophyll *b*. Further confirmation of peak identity was obtained by manually collecting fractions corresponding to HPLC peaks at the exit of the detector and comparison of the visible and mass spectra to published information (Table 2). Visible spectral data were collected using a Beckman model DU 640. A VG 70S mass spectrometer was used to collect positive electron impact (70 eV) mass spectral data. Carotenoids were quantified using standard solutions prepared from authentic standards. When standards were not available or the peak was not conclusively identified, as for 9′-*cis*-neoxanthin, peak 3, and peak 7, concentrations were calculated using the lutein calibration curve. The concentration of esterified carotenoids was determined by summing the area of all the peaks eluting between 16.5 and 23 min and for which the peak area was reduced substantially on saponification. The concentration was calculated using the calibration curve generated for lutein.

RESULTS AND DISCUSSION

Representative chromatogram traces showing the carotenoid and chlorophyll distribution found in the fruit of each of the *Actinidia* genotypes are presented in Figure 1. Carotenoid and chlorophyll concentrations are listed in Table 1.

***A. deliciosa* Cv. Hayward.** Green flesh is a distinguishing feature of ripe kiwifruit (*A. deliciosa*) and is due to the retention of chlorophyll during ripening. Chlorophylls *a* and *b* were both present in *A. deliciosa*, with chlorophyll *a* present at the greater concentration. Two additional small chlorophyll-related peaks were detected, but we assumed these to be the epimers of chlorophylls *a* and *b*, and these, as they are probably artifacts of the analysis procedure, were not studied further. As expected, saponification removed the chlorophyll peaks from the chromatogram. Neither pheophytin *a* nor pheophytin *b*, which result from the substitution of Mg^{2+} in the tetrapyrrole ring system with H^+ from chlorophylls *a* and *b*, respectively, in acidic conditions, was detected.

The carotenoids detected in this sample of *A. deliciosa* were those generally associated with chlorophyll-containing tissues (*8*) and included β-carotene, lutein, violaxanthin, and 9′-*cis*-neoxanthin, with the β-carotene concentration greatest. In contrast to the results of Cano (*4*, *5*) and Gross (*6*), we did not detect neochrome, 9′-*cis*-violaxanthin, or aurothanxin in *A. deliciosa* or, indeed, any of the other *Actinidia* genotypes

Table 1. Concentrations of Chlorophylls and Carotenoid Detected in the Fruit of Four *Actinidia* Genotypes

		concentration (mg/100 g of fresh wt							
		A. deliciosa		*A. chinensis*		*A. macrosperma*		*A. polygama*	
peak	compound		after saponification		after saponification		after saponification		after saponification
1	9′-*cis*-neoxanthin	0.12	0.11	0.04	0.15	0.10	0.14	0.09	0.38
2	violaxanthin	0.10	0.09	0.02	0.09	0.07	0.13	0.09	0.27
3	peak 3	0.03	0.03	0.01	0.02	0.04	0.05	0.02	0.08
4	antheraxanthin	0.01	0.01	0.02	0.02	0.06	0.08	0.10	0.19
5	lutein	0.16	0.15	0.11	0.13	0.57	0.74	0.19	0.49
6	zeaxanthin	nd[a]	nd	0.01	0.01	0.02	0.02	0.08	0.14
7	peak 7	0.01	nd	0.01	nd	0.01	0.02	0.40	0.70
8	β-carotene	0.17	0.15	0.17	0.09	25.2	24.1	21.6	18.78
	acyl esters[b]	nd		0.24		1.16		0.20	
	total	0.59	0.53	0.62	0.50	26.3	25.2	23.7	21.0
	chlorophyll *a*	0.55	nd	0.02	nd	nd	nd	0.13	nd
	chlorophyll *b*	0.44	nd	nd	nd	nd	nd	0.03	nd
	total	0.99	0.02					0.16	

[a] nd, not detected. [b] As defined under Materials and Methods.

studied. Although the reasons for the differences are not known, it is possible that the presence of neochrome and aurothanxin in the previous studies by Cano (*4*, *5*) are a result of acid-induced furanoid rearrangement occurring during extraction and purification (*2*).

Neither the composition nor the concentrations of the carotenoids changed markedly following saponification, indicating that the carotenoids were present in an unesterified form as is usual for carotenoids associated with chlorophyll-containing tissues. Thus, the chlorophyll and carotenoid composition of flesh from the fruit of *A. deliciosa* appears to be similar to those found in the chloroplasts of normal photosynthetically active tissue (*8*).

***A. chinensis* Cv. Hort16A.** Fruit from different selections of *A. chinensis* have flesh colors varying from yellow to green with different levels of brightness. The recently commercialized selection ZESPRI Gold kiwifruit cv. Hort16A has bright yellow flesh and was therefore expected to contain a greater concentration and possibly a different composition of carotenoids compared with the green-fleshed species (*A. deliciosa*). As shown in Table 1, the Hort16A genotype of *A. chinensis* contained the same carotenoids as the Hayward genotype of *A. deliciosa*, namely, β-carotene, lutein, violaxanthin, and 9′-*cis*-neoxanthin, and the concentrations were similar. In contrast to *A. deliciosa*, only trace concentrations of chlorophyll *a* were detected and no chlorophyll *b* was detected.

The chromatogram obtained for cv. Hort16A (Figure 1B) shows a series of peaks eluting immediately before and after β-carotene in a region expected for acyl esterified carotenoids (*9*, *10*). Saponification removed these peaks providing further evidence that these peaks represent esterified carotenoids. Furthermore, following saponification, the xanthophyll components identified as 9′-*cis*-neoxanthin, violaxanthin, and lutein all increased, indicating these carotenoids are accumulating in the yellow fruit of *A. chinensis* as esterified compounds. The accumulation of xanthophyll carotenoids esterified with acyl fatty acids is often found as chlorophyll-containing chloroplasts are converted to carotenoid-containing chromoplasts during fruit ripening (*11*). These results show that the total carotenoid concentration in this particular selection of *A. chinensis*, either before or after saponification, was similar to that found in *A. deliciosa* (Table 1), suggesting that the yellow color of *A. chinensis* is mainly due to the absence of chlorophylls in the fruit rather than a significant increase in the carotenoid concentration.

***A. macrosperma*.** Ripe fruit of *A. macrosperma* are bright orange, suggesting the presence of high concentrations of carotenoids. Both lutein and β-carotene were present in greater concentrations than in any of the other *Actinidia* genotypes examined, whereas the concentrations of the other carotenoids (9′-*cis*-neoxanthin, violaxanthin, and antheraxanthin) were similar to those found in *A. deliciosa* and *A. chinensis*. Concentrations of β-carotene were particularly high at ~20 mg/100 g of fresh weight, providing a total carotenoid concentration higher than that of most other yellow fruit (*2*, *3*) and suggesting that *A. macrosperma* may represent a good source of dietary provitamin A. The carotenoid composition and concentrations (~40 times greater than that of *A. deliciosa* and *A. chinensis*) suggests that the bright orange color of *A. macrosperma* is due to the high concentrations of β-carotene and that the other carotenoids make minimal contribution to the fruit color. Moreover, *A. macrosperma* fruits have a relatively thick highly colored skin, suggesting that the skin may contain greater concentrations of carotenoid than the flesh. Chlorophyll was absent in *A. macrosperma*, although fruits are initially green before turning bright orange on ripening. Like *A. chinensis*, *A. macrosperma* contains esterified carotenoids, as indicated by the disappearance of peaks that elute in the region of β-carotene and an increase in the xanthophyll carotenoids peaks following saponification. However, the concentration of the esterified carotenoids is low compared with β-carotene, suggesting that during ripening the developing chromoplast accumulates high concentrations of β-carotene rather than esterified xanthophylls.

***A. polygama*.** Both the flesh and skin of ripe *A. polygama* fruit are yellow to light orange, often with a slight striping. As the skin is smooth, thin, and hairless, it is considered to be edible, so the whole fruit was included in the analysis. Even though the chlorophyll-related carotenoids (β-carotene, lutein, violaxanthin, and 9′-*cis*-neoxanthin) were present, the concentrations of xanthophyll carotenoids in *A. polygama* were greater than *A. deliciosa*, *A. chinensis*, or *A. macrosperma*. Fruits of *A. polygama* contained elevated concentrations of β-carotene, an additional carotenoid peak (peak 7), and comparatively high concentrations of zeaxanthin. Although fruits of *A. polygama* and *A. macrosperma* both contained similar concentrations of β-carotene, *A. polygama* fruits have a yellow appearance,

Table 2. Spectral Data of Carotenoids Detected in *Actinidia* Fruit

peak	compound	retention time	mol wt (*m*/*z*)	spectral data[a] (peak max, nm)
1	9′-*cis*-neoxanthin	10.32	600	416, 438, 466 (a)
2	violaxanthin	11.66		
3	unknown peak 3	12.76		422, 444, 469 (e)
4	antheraxanthin	13.02		423, 447, 469 (e)
5	lutein	13.99	568	424, 447, 475 (a)
6	zeaxanthin	14.24	568	427, 450, 477 (a)
7	unknown peak 7	16.78	552	425, 450, 476 (e)
8	β-carotene	19.16	536	452, 477 (a)

[a] Solvent: (a) acetone; (e) ethanol.

whereas *A. macrosperma* fruits are bright orange. This difference in color may in part be due to the relatively greater concentrations of carotenoids such as violaxanthin and 9′-*cis*-neoxanthin in *A. polygama* fruit. These carotenoids have a lower number of double bonds in the light-absorbing chromopore and tend to be more yellow compared with β-carotene, which is yellow-orange (*2*). Initially peak 7 was believed to be β-cryptoxanthin, but comparison of the retention time with the β-cryptoxanthin peak in an extract of corn discounted this possibility. In an effort to identify this compound, fractions corresponding to this peak were collected from multiple HPLC injections and visible and MS spectroscopic properties were determined (Table 2). The molecular weight of *m*/*z* 552 (β-carotene + oxygen) and the fact that this compound elutes from the HPLC between chlorophyll *a* and β-carotene suggests that this compound may be a monohydroxy cyclic carotene; however, a definitive identification remains to be completed. Like the fruit of *A. chinensis*, the fruit of *A. polygama* contained a number of peaks that disappeared following saponification, suggesting that these compounds are likely to be esterified carotenoids. Following saponification the peaks corresponding to 9′-*cis*-neoxanthin, violaxanthin, lutein, zeaxanthin, and peak 7 all increased, indicating that these carotenoids accumulate as esters in fruit of *A. polygama* during ripening.

Recently it has been suggested that the presence of lutein and zeaxanthin in the diet may be beneficial for reducing the incidence of age-related macular degeneration, a disease which significantly impairs vision and affects ~70% of the aged population. Unfortunately, high concentrations of zeaxanthin are relatively rare in foods. The fruits of *A. polygama* contain both lutein and appreciable amounts of zeaxanthin and may therefore represent a good source of dietary zeaxanthin.

Carotenoid Esterification. Esterified carotenoids were detected in ripe fruit of three (*A. chinensis*, *A. macrosperma*, and *A. polygama*) of the four *Actinidia* genotypes. No carotenoid esterification was apparent in the ripe green fruit of *A. deliciosa*. Although the biosynthetic route to esterified carotenoids is not known in detail (*12*), the accumulation of esterified carotenoids is associated with the transition of chloroplasts to chromoplasts in senescent tissues such as leaves and ripening fruit (*11*, *13*). The observation that esterified carotenoids did not accumulate during the ripening of *A. deliciosa* fruit suggests that these fruit can be classified as "stay-green", as the transformation of chloroplasts to chromoplasts does not appear to occur. In contrast, during ripening of *A. chinensis* fruit the transition from chloroplast to chromoplast appears to occur as indicated by the disappearance of chlorophyll and the presence of small amounts of esterified carotenoid. On the other hand, the ripe fruit of both *A. macrosperma* and *A. polygama*, in addition to not containing chlorophyll, contained high concentrations of β-carotene and evidence of accumulating esterified carotenoids. Thus, the chlorophyll and carotenoid composition of these four *Actinidia* species appears to represent a continuum of chloroplast to chromoplast development with *A. deliciosa* having no development of chromoplasts, *A. macrosperma* and *A. polygama* a high-level development of chromoplasts, and *A. chinensis* partial development of the chromoplast. Presumably these differences in the propensity to develop chromoplasts and accumulate highly colored acylated carotenoid pigments during fruit ripening can be attributed to the genetic makeup of each of these species.

Fruit Color. A primary goal of this research was to develop an understanding of the pigment composition of kiwifruit and the effect on color. It appears that the yellow color of *A. chinensis* is due mainly to the absence of chlorophyll from the fruit. In contrast, the fruit of *A. deliciosa* contains similar concentrations of carotenoid but also ~1 mg of chlorophyll/100 g of fresh weight. This concentration of chlorophyll appears to be sufficient to mask the yellow color of the carotenoids and provide a green color to the fruit.

Higher concentrations of carotenoids, particularly β-carotene, are associated with the brighter colors of *A. macrosperma* and *A. polygama* fruit; therefore, new kiwifruit cultivars with brighter colors than ZESPRI Gold kiwifruit will require greater accumulation of carotenoids. Furthermore, although the β-carotene compositions of *A. macrosperma* and *A. polygama* fruits were similar, the difference in the flesh color and xanthophyll composition suggests that it may be possible to develop new kiwifruit cultivars with specific hues from bright yellow through dark orange.

ACKNOWLEDGMENT

We thank Dr. Ross Ferguson for collecting the *Actinidia* fruit samples and for providing background information on the selections and John Allen, HortResearch, for performing the mass spectral analysis. We gratefully acknowledge the provision of carotenoid standards by Dr. Peter Molnar, University Medical School of Pecs.

LITERATURE CITED

(1) Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

(2) Rodriguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Food*; ILSI Press: Washington, DC, 1999.

(3) USDA-NCC Carotenoid Database for U.S. Foods−1998; http://www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.html

(4) Cano, M. P. HPLC separation of chlorophyll and carotenoid pigments of four kiwifruit cultivars. *J. Agric. Food Chem.* **1991**, *39*, 1786−1791.

(5) Cano, M. P.; Marin, M. A. Pigment composition and color of frozen and canned kiwifruit slices. *J. Agric. Food Chem.* **1992**, *40*, 2141−2146.

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

(7) Wright, S. W.; Jeffrey, S. W.; Mantoura, R. F. C.; Llewellyn, C. A.; Bjornland, T.; Repeta, D.; Welschmeyer, N. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* **1991**, *77*, 183−196.

(8) Khachik, F.; Beecher, G. R. Separation of carotenol fatty esters by high performance liquid chromatography. *J. Chromatogr.* **1988**, *449*, 119−133.

(9) Lichtenthaler, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350−382.

(10) Khachik, F.; Beecher, G. R.; Lushby, W. R. Separation, identification and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe and pink grapefruit by liquid chromatography. *J. Agric. Food Chem.* **1989**, *37*, 1465−1473.

(11) Britton, G. Overview of carotenoid biosynthesis. In *Carotenoids Vol. 3: Biosynthesis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser: Basel, Switzerland, 1998; pp 13−147.

(12) Bonora, A.; Pancaldi, S.; Gualandri, R.; Fasulo, M. P. Carotenoid and ultrastructure variations in plastids of *Arum italicum* Miller fruit during maturation and ripening. *J. Exp. Bot.* **2000**, *51*, 873−884.

(13) Hornero-Mendez, D.; Minguez-Mosquera, M. I. Xanthophyll esterification accompanying carotenoid overaccumulation in chromoplast of *Capsicum annuum* ripening fruit is a constitutive process and useful for ripeness index. *J. Agric. Food Chem.* **2000**, *48*, 1617−1622.

Received for review May 22, 2001. Revised manuscript received October 10, 2001. Accepted October 11, 2001.

JF010677L