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Carotenoids in White- and Red-Fleshed Loquat Fruits

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Fruits of 23 loquat (Eriobotrya japonica Lindl.) cultivars, of which 11 were white-fleshed and 12 redfleshed, were analyzed for color, carotenoid content, and vitamin A values. Color differences between two loguat groups were observed in the peel as well as in the flesh. β -Carotene and lutein were the major carotenoids in the peel, which accounted for about 60% of the total colored carotenoids in both red- and white-fleshed cultivars. β -Cryptoxanthin and, in some red-fleshed cultivars, β -carotene were the most abundant carotenoids in the flesh, and in total, they accounted for over half of the colored carotenoids. Neoxanthin, violaxanthin, luteoxanthin, 9-cis-violaxanthin, phytoene, phytofluene, and ζ -carotene were also identified, while zeaxanthin, α -carotene, and lycopene were undetectable. Xanthophylls were highly esterified. On average, 1.3- and 10.8-fold higher levels of colored carotenoids were observed in the peel and flesh tissue of red-fleshed cultivars, respectively. The percentage of β -carotene among colored carotenoids was higher in both the peel and the flesh of red-fleshed cultivars. Correlations between the levels of total colored carotenoids and the color indices were analyzed. The a^* and the ratio of a^*/b^* were positively correlated with the total content of colored carotenoids, while L^* , b^* , and H° correlated negatively. Vitamin A values, as retinol equivalents (RE), of loquat flesh were 0.49 and 8.77 μ g/g DW (8.46 and 136.41 μ g/100 g FW) on average for whiteand red-fleshed cultivars, respectively. The RE values for the red-fleshed fruits were higher than fruits such as mango, red watermelon, papaya, and orange as reported in the literature, suggesting that loquat is an excellent source of provitamin A.

KEYWORDS: Carotenoids; provitamin A; color; high-performance liquid chromatography; *Eriobotrya japonica*

INTRODUCTION

Carotenoids are important components of fruits. They are the principal pigments responsible for color, one of the most important aspects of fruit external quality, of many yellow to red fruits such as citrus and tomato. They also play multiple roles in maintaining human health such as disease prevention, free radical removal, immune enhancement, and senescence retardation (1–3). It is especially noteworthy that vitamin A, which is critically required for vision, resistance to infectious disease, epithelial cell integrity, bone remodeling, and reproduction (4), is formed solely from dietary provitamin A carotenoids in animal and human bodies. The provitamin A carotenoids are those that contain at least one unsubstituted β -ionone ring and

a polyene side chain. β -Carotene, α -carotene, and β -cryptoxanthin are the most common provitamin A carotenoids found in some yellow- or red-colored plant tissues such as carrot root, tomato fruit, mango fruit, and pepper fruit (5).

Loquat (*Eriobotrya japonica* Lindl.) is a Rosaceae plant with carotenoids as principal pigments in mature fruit. The plant originated in southeastern China and has been commercially cultivated world wide in countries such as China, Japan, India, Spain, Brazil, the United States, Australia, and South Africa (6, 7). Fruits can be consumed fresh or processed into jam, juice, wine, syrup, or candied fruits; seeds are rich in starch and have been used to make wine; and leaves, flowers, and fruits are traditional Chinese medicines for their high medicinal value (6).

The color of fruits varies with cultivars from yellow to orange red in the peel and white to orange in the flesh. According to the flesh color, loquat is usually sorted into two groups, whitefleshed, or Baisha, and red-fleshed, or Hongsha. The flesh color of the former is pale yellow or ivory white, while that of the latter is saffron yellow. Carotenoids were found to be mainly

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Table 1. Main Fruit Quality Indices of Loguat Cultivars^a

			vertical diameter	horizontal diameter	fruit shape	TSS	total		water co	ontent(%)
group	cultivar	weight (g)	(cm)	(cm)	index	(°Brix)	acid (%)	pН	peel	flesh
white-fleshed	90-1	44.96 ± 6.90	4.05 ± 0.29	$\textbf{4.13} \pm \textbf{0.20}$	0.981	11.83 ± 1.74	0.61 ± 0.01	3.74 ± 0.04	$\textbf{79.69} \pm \textbf{2.48}$	85.43 ± 0.09
	Baiyu	25.19 ± 4.00	$\textbf{3.06} \pm \textbf{0.18}$	3.69 ± 0.21	0.829	17.54 ± 2.44	0.41 ± 0.01	4.13 ± 0.03	68.80 ± 0.86	80.17 ± 0.29
	Bingtangzhong	21.80 ± 3.10	3.21 ± 0.21	3.31 ± 0.34	0.970	21.38 ± 2.63	0.16 ± 0.00	5.19 ± 0.02	70.42 ± 3.87	76.51 ± 0.38
	Dazhong	$\textbf{31.04} \pm \textbf{3.29}$	3.78 ± 0.20	$\textbf{3.83} \pm \textbf{0.16}$	0.987	16.40 ± 1.94	$\textbf{0.38} \pm \textbf{0.01}$	4.23 ± 0.01	$\textbf{76.19} \pm \textbf{0.38}$	82.64 ± 0.10
	Guanyu	43.58 ± 4.02	3.92 ± 0.19	4.35 ± 0.14	0.901	12.93 ± 1.30	0.57 ± 0.01	3.81 ± 0.02	78.03 ± 0.40	85.27 ± 0.10
	Jidanbai	32.01 ± 7.02	3.58 ± 0.44	3.80 ± 0.25	0.942	16.33 ± 1.73	0.48 ± 0.01	3.95 ± 0.02	73.22 ± 0.80	81.36 ± 0.14
	Luqiaobaisha	$\textbf{38.92} \pm \textbf{3.78}$	4.34 ± 0.25	4.04 ± 0.12	1.074	11.55 ± 1.11	0.51 ± 0.02	3.86 ± 0.01	$\textbf{79.32} \pm \textbf{0.12}$	87.37 ± 0.08
	Qingzhong	26.21 ± 5.57	$\textbf{3.26} \pm \textbf{0.23}$	3.67 ± 0.26	0.888	14.72 ± 1.41	$\textbf{0.42}\pm\textbf{0.01}$	3.97 ± 0.01	77.80 ± 1.35	82.67 ± 0.07
	Ruantiaobaisha	$\textbf{30.58} \pm \textbf{6.11}$	3.55 ± 0.24	3.73 ± 0.26	0.952	15.52 ± 2.74	0.77 ± 0.01	3.67 ± 0.02	74.75 ± 1.95	82.40 ± 0.25
	Tianzhong	21.18 ± 3.08	$\textbf{3.20} \pm \textbf{0.24}$	3.37 ± 0.25	0.950	19.02 ± 1.90	$\textbf{0.16} \pm \textbf{0.01}$	5.03 ± 0.02	$\textbf{73.84} \pm \textbf{1.28}$	78.40 ± 0.07
	Tongpi	$\textbf{23.99} \pm \textbf{4.80}$	$\textbf{3.39} \pm \textbf{0.23}$	3.54 ± 0.25	0.958	13.46 ± 1.96	0.59 ± 0.01	$\textbf{3.84} \pm \textbf{0.01}$	76.85 ± 3.70	84.94 ± 0.15
	average	$30.86 \pm 8.39^{\#}$	$3.58 \pm 0.41^{\#}$	$3.77\pm0.31^{\#}$	$0.948 \pm 0.062^{\text{\#}}$	$15.52 \pm 3.05^{\#}$	0.46 ± 0.19	4.13 ± 0.51	$75.36 \pm 3.54^{\#}$	$82.47 \pm 3.25^{\#}$
red-fleshed	90-2	46.35 ± 4.73	4.22 ± 0.27	4.36 ± 0.18	0.966	12.80 ± 1.06	$\textbf{0.29} \pm \textbf{0.01}$	4.22 ± 0.02	77.38 ± 1.85	86.01 ± 0.09
	Algeie	35.64 ± 5.25	3.93 ± 0.31	4.07 ± 0.17	0.966	13.62 ± 1.20	1.23 ± 0.01	$\textbf{3.43} \pm \textbf{0.01}$	74.62 ± 4.51	84.35 ± 0.16
	Baozhu	22.28 ± 5.79	3.56 ± 0.29	3.44 ± 0.20	1.035	12.54 ± 0.70	$\textbf{0.48} \pm \textbf{0.01}$	3.98 ± 0.02	76.13 ± 1.34	84.72 ± 0.24
	Bahong	43.35 ± 5.98	4.03 ± 0.22	4.32 ± 0.22	0.933	12.15 ± 1.03	$\textbf{0.29} \pm \textbf{0.01}$	4.22 ± 0.02	80.51 ± 1.21	86.53 ± 0.11
	Dahongpao	41.15 ± 5.52	4.21 ± 0.16	4.23 ± 0.24	0.995	10.92 ± 0.62	$\textbf{0.35} \pm \textbf{0.01}$	4.47 ± 0.04	76.26 ± 1.52	82.32 ± 0.07
	Dayeyangdun	$\textbf{33.08} \pm \textbf{2.83}$	3.76 ± 0.18	$\textbf{3.83} \pm \textbf{0.12}$	0.982	10.25 ± 0.99	0.61 ± 0.01	3.64 ± 0.04	$\textbf{78.85} \pm \textbf{1.75}$	86.88 ± 0.17
	Jiajiao	40.37 ± 6.17	4.55 ± 0.37	3.98 ± 0.19	1.143	11.03 ± 0.78	0.76 ± 0.03	3.52 ± 0.02	80.23 ± 1.69	87.79 ± 0.11
	Jidanhong	$\textbf{36.21} \pm \textbf{4.01}$	4.43 ± 0.32	$\textbf{3.93} \pm \textbf{0.21}$	1.127	11.72 ± 1.37	$\textbf{0.43} \pm \textbf{0.01}$	$\textbf{3.92} \pm \textbf{0.02}$	81.56 ± 0.27	86.94 ± 0.03
	Luoyangqing	46.79 ± 5.11	4.60 ± 0.22	4.29 ± 0.20	1.072	10.87 ± 0.67	$\textbf{0.26} \pm \textbf{0.01}$	4.44 ± 0.01	80.43 ± 0.97	86.41 ± 0.07
	Marc	$\textbf{37.63} \pm \textbf{6.22}$	$\textbf{4.23} \pm \textbf{0.34}$	3.99 ± 0.12	1.060	16.71 ± 1.59	0.46 ± 0.01	4.00 ± 0.01	77.64 ± 0.29	81.79 ± 0.12
	Pelusheis	$\textbf{36.12} \pm \textbf{9.09}$	4.20 ± 0.41	4.00 ± 0.36	1.050	15.74 ± 2.83	0.78 ± 0.01	3.69 ± 0.01	$\textbf{79.00} \pm \textbf{0.91}$	79.50 ± 0.10
	Zaozhong	48.52 ± 3.63	5.53 ± 0.31	4.25 ± 0.16	1.301	11.15 ± 0.13			77.67 ± 3.54	86.09 ± 0.15
	average	$\textbf{38.98} \pm \textbf{7.23}^{\text{\#}}$	$4.27 \pm 0.50^{\#}$	$4.06\pm0.26^{\#}$	$1.053 \pm 0.102^{\text{\#}}$	$12.46\pm2.01^{\#}$	0.51 ± 0.30	4.03 ± 0.42	$78.36\pm2.10^{\text{\#}}$	$84.94\pm2.02^{\#}$

^a Data are expressed as means \pm SD (n = 10). For each fruit quality index, data marked with # and ## refer to significant differences between two groups at P < 0.05 and P < 0.01 levels, respectively, with Student's t test.

responsible for the color of loquat fruits, and the most abundant ones were identified as β -carotene, β -cryptoxanthin, lutein, violaxanthin, α -carotene, and γ -carotene (8–14). Most previous reports on the carotenoid composition of loquat were based on either an open column or a combination of column and thinlayer chromatography (8-12). High-performance liquid chromatography (HPLC), coupled with a UV/vis detector and a C_{18} reversed phase HPLC column, was applied later, but only one cultivar was covered and only the content of β -carotene and β -cryptoxanthin was analyzed (13, 14). More detailed information on loquat carotenoid composition can be obtained by HPLC when coupled with a C₃₀ reversed phase HPLC column and a photodiode array (PDA) detector. Therefore, by applying HPLC coupled with a C₃₀ column and a PDA detector, the present study was carried out to analyze the carotenoid composition of white- and red-fleshed loquat fruits and its relation to peel color and flesh provitamin A content.

MATERIALS AND METHODS

Plant Material. Eleven white-fleshed cultivars and 12 red-fleshed ones (**Table 1**) of loquat (*E. japonica* Lindl.) were used in this study. Fruits were harvested at commercial maturity from Yuhang Loquat Science Institute (Hangzhou, China) and Taihu Technological Popularization Center of Evergreen Fruit (Suzhou, China). Ten unblemished fruit from each cultivar were selected for analysis. Some main fruit quality indices, including size, shape, color, contents of sugar, and titrable acids, were based on unpublished data from previous studies and are shown in **Table 1**.

Color Measurement. The peel color was measured with a HunterLab MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) at opposite sides of each fruit. The CIE 1976 $L^*a^*b^*$ color scale was adopted. The hue angle $[H^\circ, = \arctan(b^*/a^*)]$ and chroma $[C^*, = (a^{*2} + b^{*2})^{1/2}]$ were calculated according to methods previously reported (15, 16).

Sample Preparation. After color measurement, the peel and the flesh of fruit were separated, cut into small pieces, frozen in liquid nitrogen, and ground into a fine powder. Carotenoids were extracted and saponified as previously described (*17*). Either 200 mg of peel or

500 mg of flesh powder was extracted with 1.4 mL of chloroform/ methanol/50 mM Tris buffer, pH 7.5, containing 1 M NaCl (2:1:1, v/v/ v) in a 2 mL tube. After centrifugation, the chloroform phase was recovered and the aqueous phase was re-extracted with 700 μ L of chloroform. The pooled chloroform extracts were dried under nitrogen and dissolved in 350 μ L of 6% (w/v) KOH in methanol. The mixture was incubated at 60 °C for 30 min in darkness, and 350 μ L of water and 700 μ L of chloroform were then added. After centrifugation, the chloroform phase was recovered and partitioned with water until the aqueous phase became neutral. The chloroform extracts were dried under nitrogen and dissolved in ethyl acetate. For each sample, three replicates were performed.

HPLC Analysis of Carotenoids. HPLC analysis of carotenoids in peel and flesh of loquats was carried out on Waters Alliance 2695 system (Waters Corp., Milford, MA) consisting of a 2695 separation module and a 2996 PDA detector, equipped with a 250 mm × 4.6 mm i.d., 5µm, YMC reverse-phase C_{30} column and a 20 mm × 4.6 mm i.d., YMC C_{30} guard (Waters). Chromatography was carried out at 25 °C with the elution program as previously described (17). Carotenoids were identified on the basis of the same retention times and the same spectroscopic characteristics as standards. Carotenoid standards lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO). Neoxanthin, violaxanthin, luteoxanthin, 9-*cis*-violaxanthin, phytoene, phytofluene, ζ-carotene, and lycopene were prepared as previously reported (17–19). Methods for carotenoid quantification have previously been reported (17).

Determination of Water Content. Carotenoid contents of fresh fruit were converted to a dry weight basis by determining the water content of peel and flesh of each cultivar by drying in an oven at 70 °C until the weight was unchangeable.

RESULTS AND DISCUSSION

Fruit Quality Indices and Peel Color Evaluation. Loquat fruits varied with cultivars in size, shape, sugar, acid, and water contents (**Table 1**). A significantly higher total soluble solids (**TSS**) content was observed for white-fleshed cultivars, and as a result, the water content was significantly lower for these cultivars. The size and shape index were significantly higher

Table 2.	Peel	Color	of	White-	and	Red-Fleshed	Loquat	Fruits ^a
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group	cultivar	L*	a*	<i>b</i> *	a*/b*	H°	C^{\star}
white-fleshed	90-1	64.16 ± 1.59	11.40 ± 2.12	45.65 ± 2.07	$\textbf{0.25} \pm \textbf{0.05}$	75.97 ± 2.64	47.09 ± 2.01
	Baiyu	66.21 ± 2.58	9.17 ± 3.14	46.65 ± 2.43	0.20 ± 0.07	78.86 ± 3.76	47.64 ± 2.61
	Bingtangzhong	63.44 ± 3.55	9.64 ± 2.26	45.96 ± 2.90	0.21 ± 0.05	78.08 ± 3.02	47.02 ± 2.73
	Dazhong	67.13 ± 2.31	10.15 ± 2.26	49.93 ± 3.11	$\textbf{0.20}\pm\textbf{0.04}$	78.54 ± 2.36	50.99 ± 3.22
	Guanyu	67.15 ± 1.83	11.30 ± 2.15	47.08 ± 3.09	0.24 ± 0.04	76.56 ± 2.18	48.45 ± 3.29
	Jidanbai	63.88 ± 2.65	9.35 ± 3.04	45.24 ± 2.18	0.21 ± 0.07	78.31 ± 3.88	46.29 ± 2.04
	Luqiaobaisha	66.99 ± 1.54	10.84 ± 2.82	46.38 ± 0.99	0.23 ± 0.06	76.89 ± 3.28	47.71 ± 1.23
	Qingzhong	61.74 ± 2.77	8.19 ± 3.41	46.95 ± 2.40	$\textbf{0.18} \pm \textbf{0.07}$	80.11 ± 4.04	47.78 ± 2.42
	Ruantiaobaisha	66.03 ± 2.59	11.64 ± 2.85	43.66 ± 3.12	0.27 ± 0.08	74.95 ± 4.23	45.31 ± 2.65
	Tianzhong	64.90 ± 3.52	6.92 ± 3.23	46.83 ± 2.78	0.16 ± 0.05	81.51 ± 4.21	47.45 ± 2.52
	Tongpi	61.99 ± 3.45	13.70 ± 3.33	46.44 ± 4.15	0.30 ± 0.08	$\textbf{73.49} \pm \textbf{4.12}$	48.54 ± 4.01
	average	$64.87 \pm 1.99^{\#}$	$10.21 \pm 1.85^{\#}$	$46.43 \pm 1.52^{\#}$	$0.22 \pm 0.04^{\#}$	$77.57 \pm 2.30^{\#}$	47.66 ± 1.44
red-fleshed	90-2	58.83 ± 2.05	23.85 ± 2.16	42.71 ± 2.34	0.55 ± 0.05	60.83 ± 2.36	48.96 ± 2.48
	Algeie	59.69 ± 1.50	16.64 ± 1.54	43.48 ± 3.23	0.38 ± 0.04	69.03 ± 1.91	46.58 ± 3.23
	Baozhu	61.68 ± 1.46	18.12 ± 2.62	42.24 ± 2.41	0.42 ± 0.06	66.85 ± 2.96	46.02 ± 2.76
	Bahong	60.91 ± 1.82	19.21 ± 2.54	41.56 ± 2.58	0.47 ± 0.08	65.14 ± 3.71	45.88 ± 2.09
	Dahongpao	57.62 ± 1.34	24.09 ± 1.82	43.05 ± 2.05	0.56 ± 0.05	60.76 ± 2.22	49.37 ± 1.97
	Dayeyangdun	61.64 ± 1.43	19.45 ± 2.19	43.09 ± 2.77	0.45 ± 0.05	65.71 ± 2.41	47.32 ± 2.92
	Jiajiao	59.15 ± 1.91	19.28 ± 2.09	43.23 ± 1.72	0.45 ± 0.06	65.97 ± 2.65	47.36 ± 1.59
	Jidanhong	60.56 ± 2.65	23.19 ± 2.00	43.50 ± 1.84	0.53 ± 0.05	61.94 ± 2.42	49.34 ± 1.75
	Luoyangqing	61.83 ± 0.94	26.17 ± 2.66	44.71 ± 2.61	0.59 ± 0.06	59.68 ± 2.75	51.86 ± 2.37
	Marc	58.34 ± 1.69	15.92 ± 1.95	28.84 ± 0.99	0.55 ± 0.06	61.19 ± 2.54	32.97 ± 1.62
	Pelusheis	60.25 ± 1.63	13.51 ± 1.74	30.98 ± 0.54	0.43 ± 0.05	66.51 ± 2.45	33.83 ± 1.08
	Zaozhong	63.50 ± 1.33	24.84 ± 2.53	38.22 ± 6.83	0.62 ± 0.09	57.54 ± 2.36	46.49 ± 3.62
	average	$60.33 \pm 1.69^{\text{\#}}$	$20.36 \pm 4.01^{\text{\#}}$	$40.47 \pm 5.20^{\text{\#}}$	$0.50 \pm 0.08^{\text{\#}}$	$63.43 \pm 3.53^{\text{\#}}$	45.50 ± 5.91

^a Data are expressed as means \pm SD (n = 10). For each color index, data marked with ## refer to significant differences between two groups at the P < 0.01 level with Student's *t* test.

for red-fleshed cultivars, while the content of total acids as well as the pH of flesh tissues were similar among the two groups.

Loquat cultivars are usually sorted into white- and red-fleshed groups according to the flesh color. In this study, the differences in peel color of these two groups were also observed (Table 2). The lightness values (L^*) and the chroma values (C^*) of red-fleshed cultivars were lower than those of white-fleshed ones, reflecting the more intense color, which must be from the higher accumulation of carotenoids. The a^* values were much greater, while the b^* values were slightly smaller, and as a result, the ratios of a^* to b^* were also higher in red-fleshed cultivars. The a^*/b^* ratio was negative for green fruits, zero for yellow fruits, and positive for orange fruits (20). The higher positive a^*/b^* value indicates a redder color; therefore, the peel color of the red-fleshed cultivars is also redder. Alternatively, the color was well-described by hue angle (H°) as follows: 0° for red-purple and 90° for yellow (15). Therefore, with hue angles of 78° and 64°, the peel color of white- and red-fleshed cultivars tends to be yellow and orange, respectively.

HPLC Carotenoid Profiles of Loquat Fruits. Carotenoids can be classified into carotenes (pure hydrocarbons) and xanthophylls (oxygenated derivatives of carotenes), and most xanthophylls contain at least one hydroxyl group in the molecule. The xanthophylls may be present in fruits as esterified or nonesterified or sometimes partially esterified. Although saponification was included in the extractions of carotenoids from loquat fruits in previous studies (8–14), the degree of xanthophyll esterification was not reported. In this study, it was obvious that the xanthophylls in both peel and flesh of loquat fruits were highly esterified. As shown in **Figure 1**, very little nonesterified lutein (peak 9) or β -cryptoxanthin (peak 14) was observed.

A total of 23 carotenoids were detected, 10 of which were identified. Zeaxanthin, α -carotene, and lycopene were undetectable (**Table 3**). β -Carotene is the most abundant carotenoid in peel tissues of both Ruantiaobaisha, a white-fleshed cultivar, and Dahongpao, a red-fleshed one. β -Carotene is the third most abundant in flesh tissues of Dahongpao but seventh in Ruantiaobaisha. Lutein is second and most abundant in the peel and

flesh of Ruantiaobaisha but quite low in both tissues of Dahongpao. β -Cryptoxanthin, the third and the most abundant carotenoid in the flesh of Ruantiaobaisha and Dahongpao, respectively, was quite low in the peel of both cultivars (Table 3). The preferential accumulation of some individual carotenoids in tissues of loquat fruits is similar to that in citrus (17), although the underlying mechanisms have not been elucidated in this study. The colorless carotenoids phytoene and phytofluene exist in loquat fruit tissues, but their contribution to total carotenoids was near or less than 5%. Unidentified carotenoids with peak numbers 15 and 19 have similar spectra to α -carotene, but their retention times differ. They are also not α -carotene-derived xanthophylls since the chromatograms for saponified and unsaponified samples are the same for these peaks (Figure 1). It is suggested that these two carotenoids are *cis* isomers of α or β -carotenes because a *cis* peak with maximum absorbance around 340 nm was observed, and the occurrence of geometric isomer of β -carotene has been reported earlier (8). The carotenoid with peak number 5, being second most abundant in the flesh of Dahongpao (Table 3), was not identified in this study but deserves further analysis. The presence of major carotenoids was similar for the peel or flesh of both cultivars (Figure 1), except for the high abundance of lutein in the flesh of Ruantiaobaisha. The occurrence of lutein in the flesh tissues was rare but has also been reported in pummelo (17) and kiwi fruit (21). Its occurrence in other nongreen tissues has also been reported for potato tuber, marigold flower, and various seeds, including sunflower, canola, and maize (22).

Carotenoid Contents in Loquat Peel and Their Relationships to Color. Different carotenoids have varied color and color intensities; lycopene is red, β -carotene and β -cryptoxanthin are orange, lutein is yellow, auroxanthin is light green-yellowish, and phytoene is colorless. To consider the different contribution to color of individual carotenoids, the total amounts of colored carotenoids were estimated by calculating the total peak areas recorded in the chromatograms at 450 nm as β -carotene equivalents. β -Carotene and lutein are major carotenoids in peel, and they accounted for about 60% of total colored carotenoids

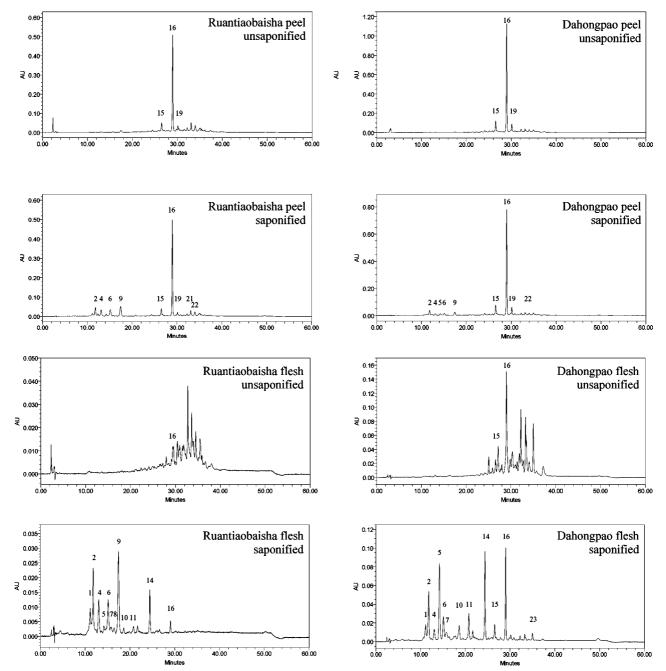


Figure 1. HPLC of saponified or unsaponified carotenoids in peel and flesh of Ruantiaobaisha and Dahongpao loquat fruits, monitored at 450 nm. The peaks are numbered according to the elution sequence, as detailed in **Table 3**.

of both red- and white-fleshed cultivars (**Table 4**). The total percentage of these two carotenoids was similar in the peel of both loquat groups; however, β -carotene was 10% higher in red-fleshed cultivars, while lutein was 13% lower. In consideration of the color difference of these two carotenoids, the result might well explain why the peel of red-fleshed cultivars was also redder. β -Carotene was most abundant in all cultivars analyzed, which was quite extraordinary since very few other fruits, except for apricot (23), another Rosaceae fruit, have been reported to accumulate β -carotene as the principal carotenoid in peel and flesh.

Consistent with the color intensity difference between redand white-fleshed loquats (**Table 2**), a lower content of total colored carotenoids was observed in white-fleshed cultivars, which is only 44% of that of red-fleshed ones (**Table 4**). All red-fleshed cultivars have a higher content (214.50–475.22 μ g/g DW) than that of all white-fleshed ones (91.52–202.28 μ g/g DW). A 4.2-fold difference was observed between the highest and the lowest contents, with an average of 1.3-fold. When compared to other fruits, the total amounts of carotenoids in white- and red-fleshed loquat peel were similar to, and higher than, orange peel (17), respectively.

Correlations between the levels of total colored carotenoids and the color indices were analyzed. The a^* values and the ratios of a^* to b^* correlated positively, but b^* negatively, to the contents (**Figures 2B–D**), suggesting that the higher accumulation of colored carotenoids tended to make fruit redder. L^* correlated negatively to the content, and no significant correlation was observed for C^* (**Figures 2A,F**). The hue angle has been reported to be closely correlated to the presence of color and accumulation of pigments such as carotenoids, chlorophylls, and anthocyanins (23, 24). This conclusion was confirmed in this study, since fruits with higher colored carotenoids had lower hue angles (**Figure 2E**).

					abundand	ce ^d (%)	
				Ruantia	aobaisha	Daho	ngpao
peak no.	carotenoids	Rt ^b (min)	$\lambda_{\max}{}^c$ (nm)	peel	flesh	peel	flesh
1	neoxanthin	11.1	416, 440, 469	1.59	4.67	0.34	2.71
2	violaxanthin	11.8	(415), 439,468	5.79	17.62	3.77	10.91
3	unidentified ^a	12.4	416, 437, 465	1.20	е	0.54	0.18
4	luteoxanthin	13.0	401, 420, 449	4.86	9.46	1.30	2.64
5	unidentified	14.2	417, 440, 470	1.27	1.50	1.28	18.02
6	9- <i>cis</i> -violaxanthin	15.1	413, 436, 465	6.37	12.37	2.03	6.32
7	unidentified	15.8	416, 441, 469		1.16		1.40
8	unidentified	16.4	417, 441, 476	0.22	1.54		0.39
9	lutein	17.4	(420),445, 473	9.85	30.05	3.18	0.21
10	unidentified	18.6	419, 446, 476			0.10	3.92
11	unidentified	20.8	419, 446, 475	0.61		0.33	5.34
12	phytoene	22.2	276, 286, 297	0.51		2.95	4.28
13	phytofluene	23.7	331, 348, 365	0.12		0.80	0.87
14	β -cryptoxanthin	24.4	(426),451, 479	0.52	12.84	0.27	18.82
15	unidentified	26.6	(429),446, 471	3.51		6.09	2.52
16	β -carotene	29.0	(427),452, 479	49.54	2.60	62.45	14.59
17	ζ-carotene	29.4	380, 400, 425			0.10	0.18
18	unidentified	29.8	(419),450, 475	0.30		0.33	0.27
19	unidentified	30.1	(429),446, 471	1.26		3.48	0.58
20	unidentified	30.8	(419),448, 475	0.65		1.04	0.45
21	unidentified	33.1	(429),446, 476	2.21		1.13	
22	unidentified	34.0	(429),446, 472	2.50		1.42	
23	unidentified	35.0	(428),453, 482	1.45		1.38	1.19

^a Unidentified compounds with carotenoid spectra. ^b *R*_t, retention time. ^c Obtained with photodiode array detection in mobile solvents. Values in parentheses indicate shoulders instead of peaks. ^d Expressed as a percentage of total carotenoids. ^e Under the detection limit, that is, 0.08, 0.05, and 0.03 µg/g DW, respectively, for phytoene, phytofluene, and other carotenoids in peel tissue. The limits were half less for flesh tissue since more starting material was applied. When expressed as percentages, the limits were 0.02% for phytoene and 0.01% for other carotenoids in flesh tissue of Ruantiaobaisha, and the limits were 0.001% for all carotenoids in the other three materials.

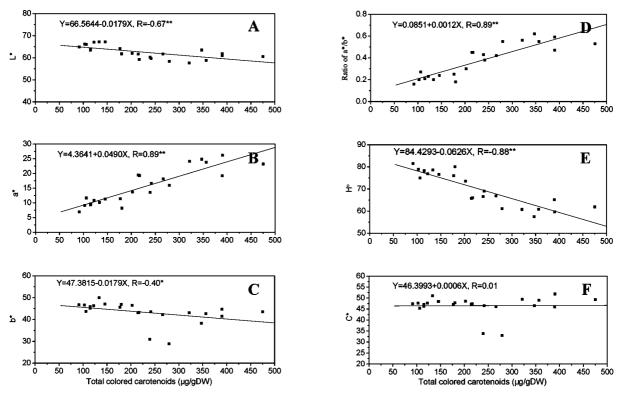


Figure 2. Correlations of content of total colored carotenoids with color indices, including L^* (A), a^* (B), b^* (C), ratio of a^*/b^* (D), H° (E), and C^* (F) (n = 23).

Carotenoid and Provitamin A Values in Loquat Flesh. β -Cryptoxanthin and, in some red-fleshed cultivars, β -carotene were the most abundant carotenoids in flesh of both loquat groups, and in total, they accounted for over half of the colored carotenoids (**Table 5**). The lutein content was similar for both groups, but the percentage of lutein among total colored carotenoids was quite different, resulting from the big difference in the content of total colored carotenoids. The levels in all white-fleshed cultivars (2.85–27.39 μ g/g DW) were much lower than those in all red-fleshed cultivars (50.88–152.66 μ g/g DW). The difference between the highest and the lowest contents was 52.6- and 10.8-fold for the average contents, which is much

group	cultivar	lutein	eta-cryptoxanthin	eta-carotene	carotenoids	<i>p</i> -caroterie equivalent	RE1	RE2
white-fleshed	90-1	44.50 ± 3.89	4.69 ± 0.31	83.38 ± 6.12	176.91 ± 10.24	85.73	14.29	290.23
	Baiyu	27.46 ± 1.67	3.95 ± 0.24	41.85 ± 2.77	102.83 ± 6.90	43.83	7.30	227.76
	Bingtangzhong	26.09 ± 2.17	7.84 ± 0.62	44.39 ± 4.19	115.13 ± 11.93	48.31	8.05	238.12
	Dazhong	38.79 ± 3.74	3.39 ± 0.36	56.04 ± 3.07	133.41 ± 9.92	57.74	9.62	229.05
	Guanyu	31.03 ± 1.62	6.32 ± 0.23	70.56 ± 2.30	145.64 ± 3.39	73.72	12.29	270.01
	Jidanbai	28.06 ± 1.37	5.82 ± 0.42	49.49 ± 3.09	114.94 ± 5.35	52.40	8.73	233.79
	Luqiaobaisha	31.34 ± 1.42	2.76 ± 1.32	52.53 ± 5.23	122.21 ± 5.35	53.91	8.99	185.91
	Qingzhong	35.56 ± 6.15	11.90 ± 2.39	75.08 ± 14.41	180.05 ± 24.50	81.03	13.51	299.92
	Ruantiaobaisha	25.12 ± 1.33	3.63 ± 0.34	49.58 ± 2.93	106.06 ± 5.70	51.40	8.57	216.39
	Tianzhong	22.63 ± 0.82	5.82 ± 0.26	34.27 ± 2.51	91.52 ± 4.70	37.18	6.20	162.19
	Tongpi	37.08 ± 3.39	8.49 ± 1.18	91.71 ± 11.47	202.28 ± 20.55	95.96	15.99	370.17
	average	31.61 ± 6.68 (23.32%)	$5.88 \pm 2.71^{##}$ (4.34%)	$58.99 \pm 18.45^{ m ##}$ (43.52%)	$135.54\pm 36.26^{#\!\!+}$	$61.92 \pm 19.07^{##}$	$10.32 \pm 3.18^{\#\#}$	$247.60 \pm 57.60^{##}$
red-fleshed	90-2	29.23 ± 2.59	17.45 ± 1.17	193.34 ± 9.40	356.5 ± 18.10	202.07	33.68	761.84
	Algeie	37.28 ± 3.28	18.47 ± 1.31	115.59 ± 9.64	241.71 ± 19.78	124.83	20.80	527.90
	Baozhu	30.65 ± 0.28	15.09 ± 0.01	158.92 ± 2.32	266.51 ± 1.06	166.47	27.74	662.15
	Bahong	52.37 ± 2.39	26.75 ± 0.62	207.27 ± 6.15	390.11 ± 14.99	220.65	36.77	716.65
	Dahongpao	28.95 ± 1.69	18.50 ± 1.48	186.19 ± 7.85	321.58 ± 17.78	195.44	32.57	773.21
	Dayeyangdun	29.12 ± 3.11	9.79 ± 1.04	115.55 ± 17.47	214.50 ± 18.24	120.45	20.07	424.48
	Jiajiao	32.41 ± 4.70	6.74 ± 0.61	119.28 ± 13.29	217.00 ± 26.38	122.65	20.44	404.10
	Jidanhong	37.99 ± 1.04	50.78 ± 2.70	246.94 ± 14.11	475.22 ± 23.68	272.33	45.39	836.99
	Luoyangqing	40.13 ± 2.02	24.57 ± 2.20	215.79 ± 13.06	390.25 ± 24.19	228.08	38.01	743.86
	Marc	17.08 ± 2.66	32.30 ± 2.81	135.40 ± 12.68	279.59 ± 28.19	151.55	25.26	564.81
	Pelusheis	25.81 ± 2.53	22.35 ± 1.65	130.17 ± 8.81	239.39 ± 18.77	141.35	23.56	494.76
	Zaozhong	33.37 ± 1.50	27.62 ± 1.22	196.35 ± 2.02	347.24 ± 3.31	210.16	35.03	782.22
	average	$32.87\pm 8.66~(10.55\%)$	$22.53 \pm 11.55^{\#}$ (7.23%)	$168.40 \pm 45.01^{\#\#} (54.04\%)$	$311.63 \pm 82.04^{##}$	$179.67 \pm 49.07^{##}$	$29.94 \pm 8.18^{\#\#}$	$641.08 \pm 150.81^{\#}$

Table 4. Carotenoid Content in Peel of Loquat Fruits^a

group cultivar white-fleshed 90-1							
	lutein	eta-cryptoxanthin	eta-carotene	carotenoids	equivalent	RE1	RE2
	0.97 ± 0.07	3.56 ± 0.26	1.56 ± 0.09	11.19 ± 1.54	3.34	0.56	8.16
Baiyu	0.34 ± 0.05	0.59 ± 0.09	0.28 ± 0.03	2.85 ± 0.47	0.58	0.10	1.98
Bingtangzhong		3.43 ± 0.23	0.57 ± 0.06	8.35 ± 0.29	2.29	0.38	8.93
Dazhong		3.21 ± 0.05	1.05 ± 0.04	8.96 ± 0.15	2.66	0.44	7.64
Guanyu	0.42 ± 0.13	3.32 ± 0.50	1.01 ± 0.02	8.47 ± 0.25	2.67	0.45	6.63
Jidanbai	0.43 ± 0.04	2.06 ± 0.20	0.27 ± 0.03	4.59 ± 0.52	1.30	0.22	4.10
Luqiaobaisha	sha 0.07 ± 0.01	0.83 ± 0.05	0.67 ± 0.15	3.71 ± 0.49	1.09	0.18	2.27
Qingzhong	1.46	15.94 ± 0.15	2.34 ± 0.08	27.39 ± 0.81	10.31	1.72	29.81
Ruantiaobaisha		0.79 ± 0.07	0.71 ± 0.17	3.71 ± 0.21	1.11	0.18	3.17
Tianzhong		4.26 ± 0.19	0.99 ± 0.05	11.46 ± 0.72	3.12	0.52	11.23
Tongpi		5.21 ± 0.52	1.04 ± 0.00	10.33 ± 0.26	3.65	0.61	9.19
average	$0.65\pm 0.22~(7.08\%)$	$3.93 \pm 4.26^{##}$ (42.81%)	$0.95\pm0.59^{**}$ (10.35%)	$9.18\pm 6.81^{\#\#}$	$2.92 \pm 2.66^{##}$	$0.49 \pm 0.44^{##}$	$8.46 \pm 7.72^{##}$
red-fleshed 90-2	0.68 ± 0.24	38.28 ± 3.25	28.48 ± 2.12	109.05 ± 9.94	47.62	7.94	111.08
Algeie	0.43 ± 0.11	28.61 ± 0.31	12.70 ± 0.80	50.88 ± 0.36	27.01	4.50	70.43
Baozhu	0.40 ± 0.04	37.94 ± 1.00	28.33 ± 0.52	98.94 ± 6.25	47.30	7.88	120.41
Bahong	0.42 ± 0.02	33.58 ± 2.94	31.40 ± 2.75	104.52 ± 9.59	48.19	8.03	108.16
Dahongpao	to 0.60 ± 0.05	54.91 ± 3.84	32.96 ± 2.08	152.66 ± 10.00	60.42	10.07	178.04
Dayeyangdun	dun 1.32 ± 0.03	33.02 ± 1.18	68.35 ± 3.58	147.76 ± 3.84	84.86	14.14	185.52
Jiajiao		14.36 ± 0.42	40.45 ± 2.84	75.51 ± 4.09	47.63	7.94	96.95
Jidanhong		61.92 ± 3.12	20.92 ± 1.02	115.30 ± 5.95	51.88	8.65	112.97
Luoyangqing	ing 0.70 ± 0.02	42.90 ± 1.53	39.09 ± 0.61	143.85 ± 5.01	60.54	10.09	137.12
Marc	2.19 ± 0.16	52.21 ± 4.74	29.07 ± 2.41	110.00 ± 9.32	55.18	9.20	167.53
Pelusheis	0.22 ± 0.01	40.86 ± 1.35	29.57 ± 1.34	86.11 ± 4.64	50.00	8.33	170.77
Zaozhong	0.58	30.44 ± 6.16	31.50 ± 0.65	102.81 ± 21.33	46.72	7.79	108.36
average	0.71 ± 0.54 (0.66%)	$39.09 \pm 12.89^{ m # m t}$ (36.15%)	$32.74 \pm 13.39^{ m # m t}$ (30.28%)	$108.12 \pm 29.91^{#\!$	$52.28 \pm 13.36^{##}$	$8.71 \pm 2.23^{##}$	$130.61\pm 36.72^{##}$

Table 5. Carotenoid Content of Flesh of Loquat $\mathsf{Fruits}^{\mathsf{a}}$

Carotenoids in Loquat Fruits

The total colored carotenoid content was lower in flesh than in peel for all cultivars analyzed, and such a difference was even more obvious for the white-fleshed ones. Peel tissues accumulated 5.6–35.1 and 0.5–3.8 times, respectively, for whiteand red-fleshed cultivars, a higher content of colored carotenoids than flesh tissues. The results were quite consistent with the color differences in peel and flesh. The differential accumulation of carotenoids in peel and flesh of loquat has been reported (9, 10) and observed in other fruits such as citrus (17, 25), indicating that biosynthesis of carotenoids in these two tissue types might be independent to some extent. Therefore, elucidation of the underlying mechanisms for differential accumulation of carotenoids in two loquat groups and two tissue types, as well as breeding of a high carotenoid loquat, especially from an originally white-fleshed one, would be of great interest.

Loquat is a fruit source of provitamin A because the two most abundant carotenoids, β -cryptoxanthin and β -carotene, can be converted into vitamin A in animal and human bodies. Vitamin A values in fruits can be expressed as μg retinol equivalents (μ g RE), with 1 μ g RE equal to 6 μ g of β -carotene or 12 μ g of other provitamin A carotenoids, such as β -cryptoxanthin (26). Because of the great differences in the content of provitamin A carotenoids, the RE values in flesh tissue of loquats ranged from 0.10–1.72 μ g/g DW (1.98–29.81 μ g/100 g FW) to 4.50–14.14 μ g/g DW (70.43–185.52 μ g/100 g FW), with averages of 0.49 and 8.77 μ g/g DW (8.46 and 136.41 μ g/100 g FW) for white- and red-fleshed cultivars, respectively. On a fresh weight basis, 100 g of the edible portion of red-fleshed loquat fruit contains on average 131 μ g RE; thus, a daily consumption of 200 g of loquat flesh can meet about 30% of the recommended dietary requirement of vitamin A, 800-1000 RE/day (27), for adults.

It is obvious that red-fleshed cultivars are superior to whitefleshed ones as far as the provitamin A value is considered. However, white-fleshed cultivars had better fruit quality aspects such as a high content of TSS (**Table 1** of this study, (28)). On the other hand, the peel, an inedible part, has a much higher RE value than the flesh (**Tables 4** and **5**). Apart from carotenoids, the peel is also rich in triterpenoids, polyphenols, and other bioactive components (29). Therefore, the peel can be potentially processed for functional foods or value-added ingredients.

Because of the importance of vitamin A in human health, many fruits and vegetables have been evaluated for their provitamin A values. The average value, on both dry and fresh weight bases, for flesh of red-fleshed loquat was higher than common fruits such as mango, red watermelon, papaya, and orange (30). Even the average provitamin A value for the flesh of the white-fleshed loquat was similar to that of oranges (30). Therefore, loquat flesh, especially that of red-fleshed cultivars, is an excellent source of provitamin A.

In conclusion, an overview of the carotenoid composition of loquat fruits, in both peel and flesh tissues, white- and redfleshed cultivars, as well as the relationships between the total colored carotenoid contents and the color of peel and the provitamin A value of the flesh, has been presented in this study. The results suggest that the carotenoids contribute greatly to orange color of the peel and that the flesh, especially that of red-fleshed cultivars, is an excellent source of provitamin A.

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