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ANALYSIS ON PIGMENTS IN THE EXOCARP OF ORANGE FRUIT

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ABSTRACT: We examined the chlorophyll (Chl) and carotenoid contents, and the three intermediates (protoporphyrin IX (PPIX), magnesium protoporphyrin IX (MGPP) and protochlorophyllide (Pchlide)) of Chl biosynthetic pathway in the exocarp of orange fruit during its late ripening stage. While Chl content declines by 12 folds, carotenoid content always remains similar level. Meanwhile, the ratios of Chl a/b, carotenoid/Chl and Pchlide/Chl alter from 1.51 to 4.42, from 0.22 to 2.74 and from 1.00 to 0.07, respectively. It is, therefore, concluded: (a) the deficiency of carotenoid is not directly accompanied by the deficiency of Chl; (b) the degradation rate of Chl *b* is faster than that of Chl *a*; (c) the degradation rate of PPIX is slower than that of MGPP and Pchlide; and (d) as ripening takes place, the residual Pchlide is more easily forwarded to Chl.

KEYWORDS: Chlorophyll, Carotenoid, Magnesium protoporphyrin, Pigment, Protochloro-phyllide, Protoporphyrin IX.

INTRODUCTION

The fruit of *Citrus* is derived from the carpel or gynoecium. Its pericarp is divided into three parts: exocarp, mesocarp, and endocarp. Several layers of cell underneath epidermis of exocarp contain chloroplasts during the developing stage and contain chromoplasts during the late ripening stages. During the ripening, the chloroplasts transform into chromoplasts which express various color (Chiang, 1973). During the transformation of plastids, the biosynthesis of Chl and carotenoid may be interrelated (Thomson and Whatley, 1980). It has been reported that a deficiency in carotenoids can cause a decreased Chl content (Mayfield and Taylor, 1984), and that carotenoid accumulation is exerted by the Chl accumulation (Oelmuller and Mohr, 1985). However, results from a sweetclover (Markwell *et al.*, 1986) and *Arabidopsis thaliana* (Lu *et al.*, 1995) are contrary. Little information are available for the biosynthesis of intermediates prior to Chl during the ripening of fruit.

The aim of this research is to examine the interrelation of Chl and carotenoid biosynthesis, and the biosynthesis or degradation of these pigments during the late ripening stages of *Citrus* fruit.

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MATERIALS AND METHODS

Fruit

The fresh fruit of orange (*Citrus ponki* (Hayata) Hort. *ex* Tanaka) was purchased from local market and categorized into five groups, indicating five stages in the late ripening process after harvesting, according to their homogeneous color. The first group with Chl a/b ratio approximately 2.5 is totally green and the fifth group with Chl a/b ratio approximately 4.5 is totally yellow. The Chl a/b ratios of other three groups are approximately 3.0, 3.5, and 4.0, respectively. Only the exocarp was sampled for extraction of pigments and porphyrins.

Pigment determination

Chl concentrations and a/b ratios were determined using the spectrophotometric method of Porra *et al.* (1989) following extraction of liquid nitrogen-dried experimental materials with 80% acetone. The proportion of absorbance at 480 nm due to carotenoid was estimated as described by Kirk and Allen (1965) following extraction of liquid nitrogen-dried exocarp with 80% acetone. Room temperature absorbance was determined with a Hitachi U2000 UV-visible spectrophtometer.

Pchlide assay

Anderson and Boardman's (1964) equation was used to eliminate the interference of Chl on the spectrophotometric assessment of Pchlide. Absorbance at 663, 645 and 626 nm due to Chl *a*, Chl *b* and Pchlide, respectively, were obtained with a Hitachi U2000 UV-visible spectrophotometer following 80% acetone extraction of exocarp. The relative Pchlide/Chl ratio was herein calculated. The concentrations of Chl determined by Porra *et al.* (1989) equation and of Pchlide determined by Kahn *et al.* (1976) were not used to calculate the ratio of Pchlide/Chl.

Porphyrin assay

The concentrations of PPIX, MGPP and Pchlide determined using the spectrophotometric method of Kahn *et al.* (1976). The 80% ammoniacal acetone (ammonia:acetone, 20: 80) extract was treated with hexane to remove the majority of the Chl pigments before measuring the porphyrin contents. The room temperature absorbance at 575, 590 and 628 nm due to PPIX, MGPP and Pchlide, respectively, were determined with a Hitachi U2000 UV-visible spectrophotometer. The mole percent of each porphyrin was herein calculated.

RESULTS AND DISCUSSION

Pigments

During the late ripening, the orange fruit can be randomized into five groups according to their exocarp color of which the Chl a/b ratio were approximately 2.5, 3.0, 3.5, 4.0 and 4.5, respectively. While the Chl content decreases from 659 at the first stage to 50 μ g g^{-1} at the fifth stage, the carotenoid content always remains similar level flutuating between 86 and 165 μ g g^{-1} fresh weight (Fig. 1). The more ripening, the less Chl. The

sharp decline of Chl reflects the quick degradation of this pigment; the remaining at similar level of carotenoid reflects the balance between synthesis and degradation of this pigment. The data is in contrast to the finding that the deficiency of Chl is resulted from a loss of caro-tenoid (Mayfield and Taylor, 1984), but is consistent with the results from a sweetclover (Markwell *et al.*, 1986) and an *Arabidopsis thaliana* (Lu *et al.*, 1995).

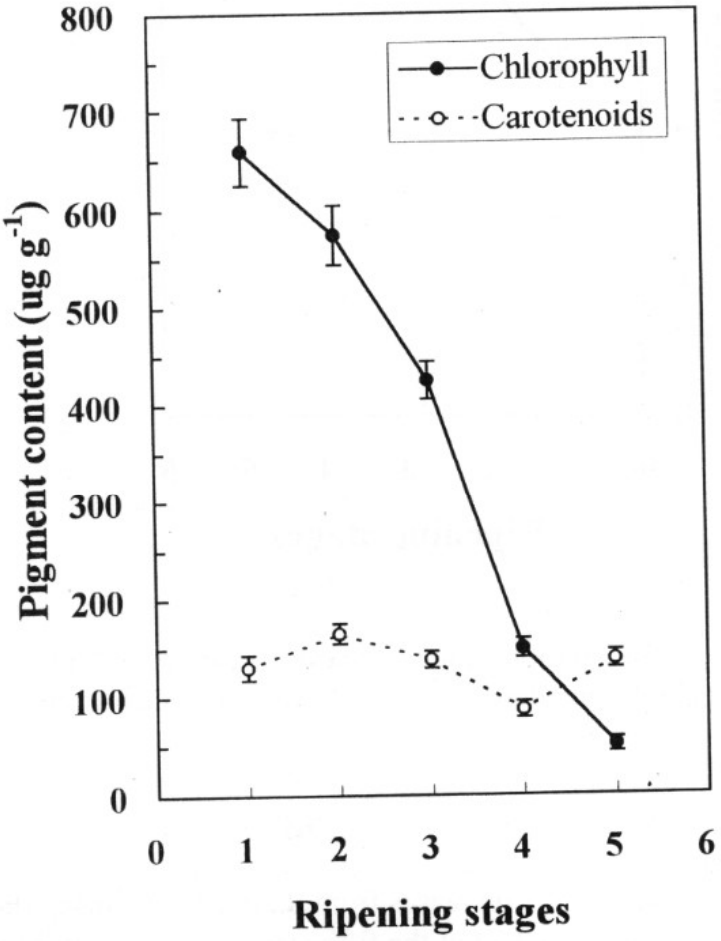

Fig. 1. The alteration of Chl and carotenoid content in the exocarp of orange fruit during its late ripening. Error bars indicate the standard deviation for triplicate determinations.

Chl a/b ratio

As the content of Chl declines by 12 folds, the a/b ratio of this pigment gradually increases from 2.51 to 4.42 (Fig. 2), strongly suggesting that the accumulation rate of Chl *b* is slower than that of Chl *a* or the degradation rate of Chl *b* is faster than that of Chl *a*, causing the increase of a/b ratio, during the late ripening of orange fruit.

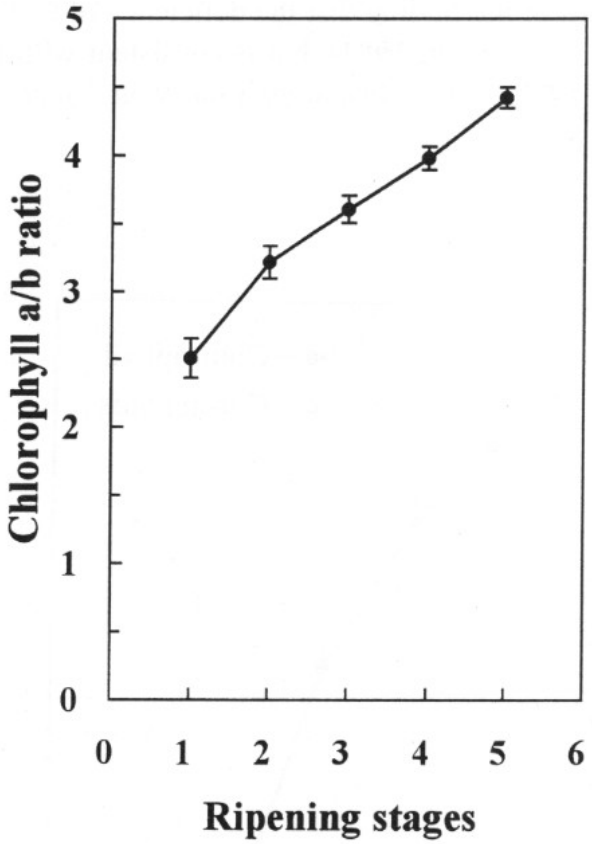

Fig. 2. The alteration of Chl a/b ratio in the exocarp of orange fruit during its late ripening. Error bars indicate the standard deviation for triplicate determinations.

Carotenoid/Chl ratio

The ratio of carotenoid/Chl just increases from 0.20 to 0.58 during the first four stages, but sharply increases from 0.58 to 2.74 at the fifth stage (Fig. 3). Combination of the three data indicates that a deficiency of carotenoid is not directly accompanied by the loss of Chl.

Porphyrins

As well as the decline of Chl, the amount of three Chl biosynthetic intermediates also gradually decreases; from 352 (100%) to 71 (20%) for PPIX, from 292 (100%) to 27 (9%) for MGPP, and from 182 (100%) to 7 (4%) μ g g^{-1} fresh weight for Pchlide (Table 1). During the first three stages, the mole percent of total porphyrins still remain similar level. MGPP and Pchlide, however, begin the acceleration of their degradation, resulting in the increase of mole of PPIX and the decrease of that of MGPP and Pchlide (Fig. 4). This further indicates that during the final stage of ripening, the degradation rate of MGPP and Pchlide is much faster than that of PPIX.

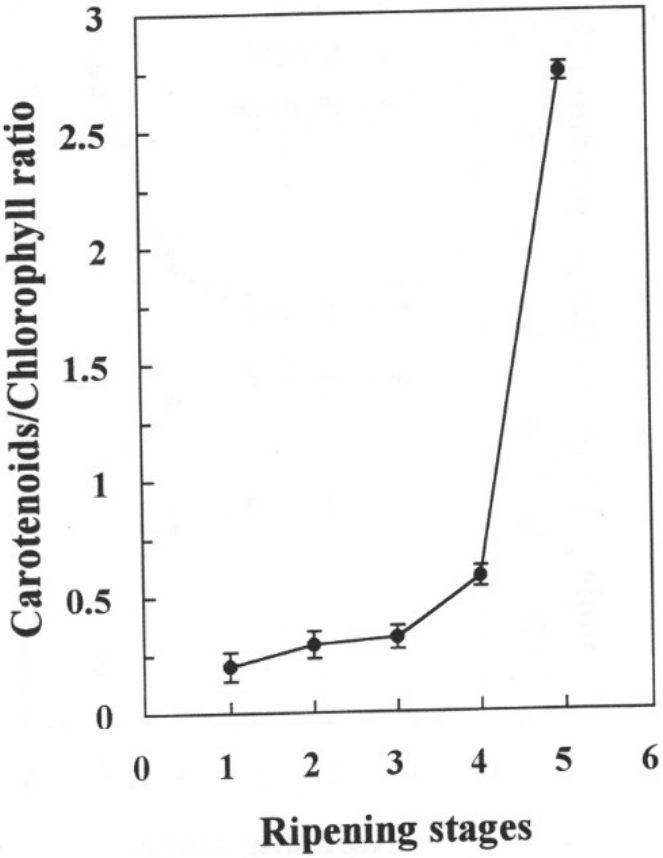

Fig. 3. The alteration of carotenoid/Chl ratio in the exocarp of orange fruit during its late ripening. Error bars indicate the standard deviation for triplicate determinations.

Table 1. The accumulation of porphyrins in the exocarp orange fruit during its late ripening stages. The method of Kahn *et al.* (1976) was used to obtain the concentration of porphyrins. The numbers in parentheses represent percentage. Results are the mean of triplicate determinations.

Stages	Porphyrins (μ g g^{-1} fresh weight)		
	PPIX	MGPP	Pchlide
1	351.6±17.6(100)	291.8±9.8(100)	182.3±11.6(100)
2	304.4±18.4(87)	250.3±11.7(86)	156.7±10.5(86)
3	208.8±11.2(59)	173.6±8.7(59)	112.1±5.7(61)
4	88.4±6.7(25)	63.2±4.4(22)	38.0±2.8(21)
5	70.5±4.9(20)	27.1±3.1(9)	6.7±1.1(4)

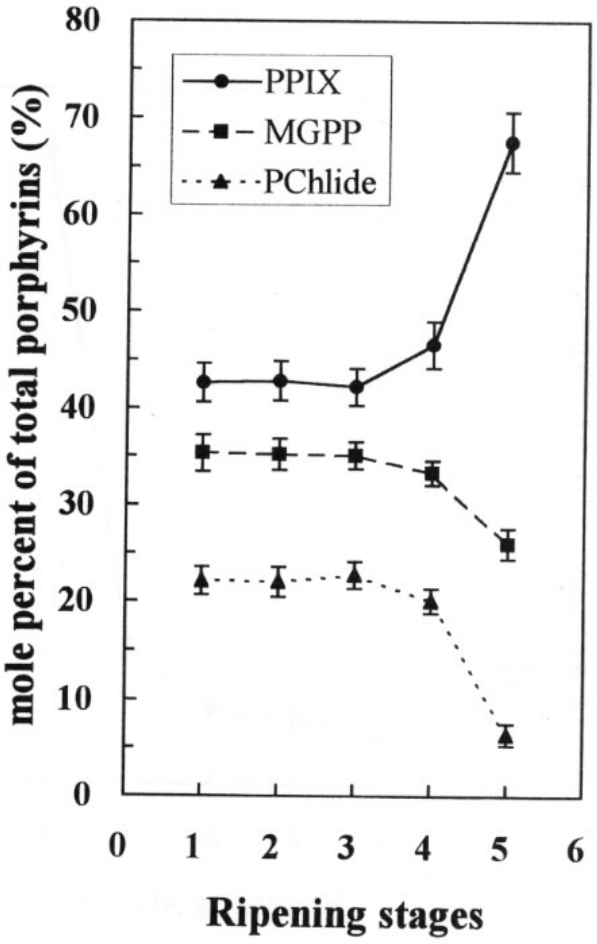

Fig. 4. The alteration of mole percent of porphyrins in the exocarp of orange fruit during its late ripening. Error bars indicate the standard deviation for triplicate determinations.

Pchlide/Chl ratio

During the time course of ripening, the ratios of Chl a/b and carotenoid/Chl gradually increase (Fig. 2 and 3). In contrast to this, the ratio of Pchlide/Chl sharply declines from 100% to only 7% (Fig. 5). The ratio of Pchlide/Chl seems could be used as indicator of the forward from Pchlide to Chl—the higher the ratio, the more difficult the forward to Chl. Even the contents of both Chl and Pchlide decrease during the ripening process, the Pchlide/Chl ratio sharply declines to almost zero, indicating that Pchlide is more easily forwarded to chl as ripening processes, or that the photoreduction of Pchlide to Chl is faster than the degradation of Pchlide as ripening processes. It seems reasonable that at the final ripening stage most of remaining or residual Pchlide are photoreduced to Chl.

It seems that the carotenoid concentration almost remains constant whereas the Chl dramatically degrades until the latter completely disappears during the late ripening stage, leading to the green exocarp turn yellow. It remains further study if most of residual Pchlide are photoreduced to Chl at the final ripening stage.

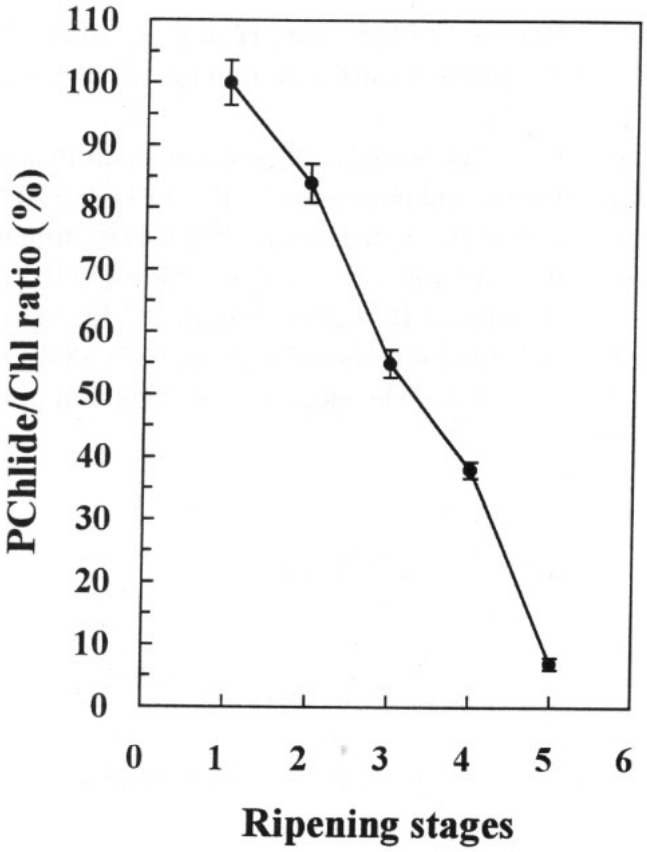

Fig. 5. The alteration of relative Pchlide/Chl ratio in the exocarp of orange fruit during its late ripening. Error bars indicate the standard deviation for triplicate determinations.

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橘皮色素的分析

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摘　　要

本研究檢測橘皮後熟階段葉綠素和類胡蘿蔔素，及三種葉綠素生合成途徑的中間代謝物—protoporphyrin IX (PPIX)， magnesium protoporphyrin IX (MGPP) 和 protochlorophyllide (Pchlide)，的含量變化。當葉綠素含量下降 12 倍，類胡蘿蔔素都保持相似的含量。同期間，葉綠素 a/b 比，類胡蘿蔔素/葉綠素比和 Pchlide/葉綠素比分別自 2.51，0.22 和 1.00 改變爲 4.42，2.74 和 0.07 。因此，類胡蘿蔔素的缺失並不直接伴隨著葉綠素的缺失；葉綠素 b 的崩解比葉綠素 a 快速；PPIX 的崩解比MGPP和Pchlide緩慢；在後熟的最後階段，殘餘的Pchlide似更容易轉化爲葉綠素而非崩解。

關鍵詞：葉綠素，類胡蘿蔔素，原吡啉鎂，色素，葉綠原素，原吡啉IX。

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