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Fruit colour progression in grapefruit with relation to carotenoid and Brix-acid ratio

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ABSTRACT

In citrus, fruit colouration is considered one of the criteria to assess the optimum harvest maturity. The present study the fruit colour variations, *β*-carotene and its relation to soluble Brix/acid ratio (B/A ratio) during development in four grapefruit varieties viz. Marsh Seedless, Flame, Rio Red and Redblush were investigated. Among different grapefruit varieties, the highest peel colour coordinate *a value and β-carotene content were recorded in cv. Flame at 270 days after full bloom (DAFS). The peel β-carotene concentration varied from 3.61 to 61.42 μg.g^{-1} during fruit development. *L**coordinate showed a declining pattern in the pulp, while it increased in peel till 270 DAFS. Likewise, *b** values also increased in both peel and pulp. Chroma (*C**) and B/A ratio values of fruit increased with maturity, while the hue angle (*h*°) decreased. *L*a*b**colour coordinates showed a strong correlation with B/A ratio compared to β-carotene content. At the final fruit harvest, the higher value for *L** coordinate in peel and pulp was noted in 'Redblush' and 'Marsh Seedless'. In contrast, the highest *b** coordinate was observed in the peel and pulp of the Marsh Seedless variety. Overall, a* coordinate was observed to be the most reliable colour parameter to determine the maturity index in the studied varieties of grapefruit.**

Key words: *Citrus paradisi*, β-carotene, Flame, Marsh Seedless, Redblush, Rio Red

INTRODUCTION

In the marketing of citrus fruits for fresh consumption, the appearance of the colour is likely to be the consumer's first preference and the deciding factor for their adoption. Grapefruit is a high-value citrus crop grown worldwide and known for its nutraceutical properties. Pigments such as β-carotene and lycopene in red varieties are responsible for colouration in grapefruits (Khan and Mackinney, 7). Carotenoids are the precursor of vitamin A, and their compounds are known for their antioxidant capacities which are beneficial against cardiovascular diseases, age-related disorders, carcinogenesis and other degenerative processes (Rao and Rao, 14). Fruit colour is a crucial maturity index in indicating the optimum harvesting stage. Carotenoids are compounds that impart yellow, red or orange colour to the fruits. These are primarily present in citrus fruit peel, and comparatively low content was observed in pulp and juice (Liu *et al.*, 8). The colour of citrus fruit begins to change in response to a fall in ambient temperature resulting in the synthesis of carotenoid pigments and the development of yellow colour (Casas and Mallent, 3). Previous reports explained that the colour of citrus fruits varies qualitatively or quantitatively within different varieties and species with the advancement of maturity (Gross, 5).

Earlier studies have shown the accumulation of β-carotene content during fruit development in mandarins (Singh *et al.*, 16) and Star Ruby grapefruit (Singh *et al.*, 15). The sugar-acid ratio is the key index for developing good organoleptic quality in fruits, and the low sugar-acid ratio provides an acidic taste and represents immaturity for harvest (Barragan-Iglesias, 2). However, there is no information on fruit colour development concerning the Brix-acid (B/A) ratio and β-carotene content in grapefruit varieties (Marsh Seedless, Flame, Rio Red and Redblush). Hence, this experiment was conducted to study variations in the fruit colour of grapefruit varieties in relation to β-carotene content and B/A ratio during fruit development.

MATERIALS AND METHODS

Fruits of one white-fleshed variety Marsh Seedless and three red-fleshed grapefruit varieties, *viz.*, Flame Seedless, Rio Red and Redblush (Fig. 1), were harvested from five-years-old trees budded on *Citrus jambhiri* rootstock during 2019. Experimental trees were spaced at 6m × 3m at Punjab Agricultural University Research Station, Jallowal-Lesriwal, Jalandhar, India and maintained through uniform cultural and plant protection practices. For the collection of fruit samples, 2 plants per replication were tagged, and four replications were under each cultivar. Fruits were harvested with the help of fruit clippers from marked trees from 120 to 270 DAFS

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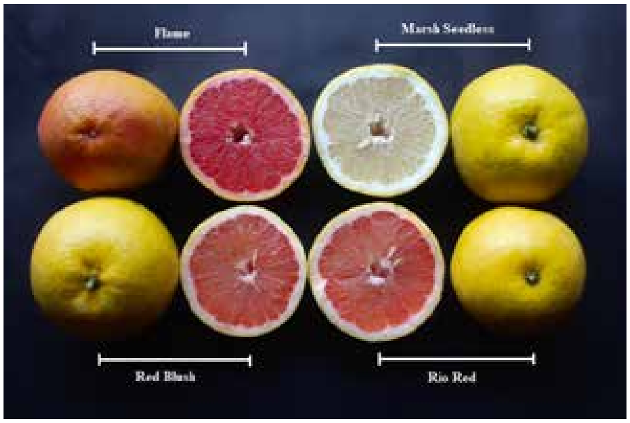

Fig. 1. External and internal colour appearance of four grapefruit varieties at 270 DAFS.

at 30 days interval for further analysis. Fruits were randomly picked and cleaned with distilled water and wiped dry at room temperature. Samples were transferred to PAU Ludhiana for biochemical and colour analysis.

The carotenoids were determined in the form of β-carotene content from the peel of four grapefruit varieties as per the method given by Ranganna (19). The fruit peel colour of 10 fruits per replication at each sampling interval was measured from four equatorial faces of fruit by using Colour Flex meter (Colour Flex, Hunter Associates Inc., Reston, VA, U.S.A.) by the method of Hunter (11). Similarly, the fruit juice colour of composite samples was recorded after extracting it from fruits. The colour coordinates C.I.E. *L** (brightness) varies from 0 (black) to 100 (white), *a** varies from negative value (green colour) to positive value (red colour) and *b**(negative value with blue and positive with yellow). Chroma (*C**) and hue angle (*h*°), the more spontaneous and understandable colour variables, were calculated by $(a^{*2} + b^{*2})^{1/2}$ and $\tan^{-1}(b^*/a^*)$, respectively, given by McGuire (17). T.S.B./Acid (B/A) ratio of fruit juice was calculated from T.S.S., which was measured with a digital refractometer (0-53% range) at 20 °C (A.T.A.G.O., PAL-1, Japan) and acid content was determined by titrating the fruit juice with 0.1 NaOH using phenolphthalein as indicator (A.O.A.C., 3).

All values for β-carotene, colour parameters and B/A ratio were recorded in triplicate and presented in terms of their means ± standard deviations. The statistical difference among time intervals and varieties was identified from a one-way analysis of variance (ANOVA) using the least significant difference ($p<0.05$). The correlation coefficients among β-carotene, fruit colour coordinates and B/A ratio were evaluated through Pearson's correlation coefficients ($p\leq0.05$) and linear, cubic and quadratic regressions of β-carotene equivalent and B/A ratio content with colour parameters were calculated by using SAS 9.3 (The S.A.S. system for Windows, Version 9.3, S.A.S. Institute, Cary, NC).

RESULTS AND DISCUSSION

The variations in peel and pulp colour of different grapefruit varieties with the progression of fruit maturation are presented as C.I.E. *L*, a*, b*, C** and *h*° colour parameter (Fig. 2). The fruit peel *L** coordinate increased significantly with fruit development. In contrast, the pulp *L** decreased, indicating lower luminosity. The changes in *L** coordinate with fruit development in the present study were in accordance with the findings of Soares *et al*. (17), who showed an

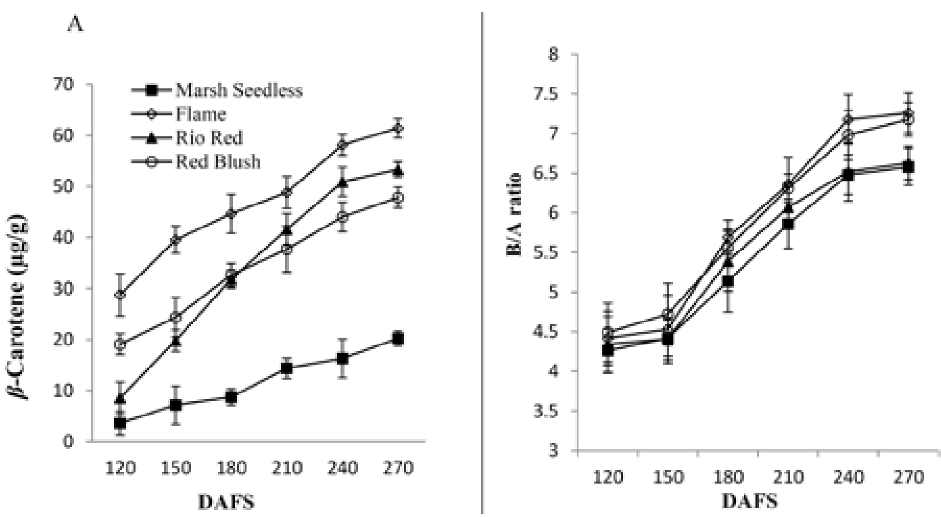

Fig. 2. Change in peel *L** (A_1), *a** (B_1), *b** (C_1), *C** (D_1) and *h*° (E_1) and in pulp *L** (A_2), *a** (B_2), *b** (C_2), *C** (D_2) and *h*° (E_2) of four grapefruit varieties during fruit development from 120 to 270 DAFS.

increasing trend of *L** from 53.83 to 71.87 in guava fruit. A similar inclining pattern of *L** coordinate in the peel of 'Star Ruby' grapefruit with fruit development was also reported by Singh *et al*.(15). In evaluated grapefruit varieties, the peel *L** coordinate ranged from 47.69 (120 DAFS) to 76.61 (270 DAFS) even though in the pulp, it varied from 79.69 (120 DAFS) to 37.11 (270 DAFS). Likewise, peel and pulp *a** colour coordinate value changed from negative to positive scale with the advancement of fruit development, indicating a transition of colour from green to red or yellow pigmentation with fruit maturity. The peel *a** colour coordinate in different grapefruit varieties did not vary initially, while in the pulp, a significant difference was noted between 'Marsh Seedless' and 'Flame'. At 270 DAFS, the highest colour value of coordinate *a** was recorded in both the peel and pulp of the Flame variety, while the Marsh Seedless variety displayed the lowest value.

Similarly, all the grapefruit varieties exhibited a comparable range of *b** coordinate during the initial sampling. Afterwards, a significant variation in colour development was noted till the final fruit sampling. The highest value of *b** colour parameter was observed in the Marsh Seedless variety, while Flame exhibited the lowest *b** as opposed to *a** coordinate. The increased *a** and *b** values during fruit development indicate loss of green colour with concurrent yellow and red colour development in the peel of grapefruit varieties.

Colour parameter *C** explained the colour inundation of fruit, and *h°* showed colour dubiousness. Both these characters were used by Manera *et al*. (10) to describe the evolution of colour during fruit development. In the present studies, an increase in *C** coordinate with a decrease of peel and pulp hue angle during fruit development was observed in grapefruit varieties. In peel, *C** values varied from 21.16 to 59.43, while in pulp, they ranged between 13.83 to 30.97, which were lower than observed in peel during fruit development in all studied grapefruits. Likewise, the *h°* value for the pulp of Flame, Rio Red and Redblush varieties was lower than those of peel as opposite to Marsh Seedless, in which pulp (77.41) exhibited a slightly greater hue angle than its peel (75.84). At 270 DAFS, *C** was calculated in both peel and pulp in the order of Marsh Seedless (59.43)>Redblush (57.98)>Rio Red (52.59)>Flame (51.64) and Flame (32.21)>Redblush (30.97)>Rio Red (30.08)>Marsh Seedless (29.11), respectively, whereas *h°* was recorded in the sequence of Marsh Seedless>Redblush>Rio Red>Flame. An increasing pattern of *L*, a*, b** and *C** and a decline in *h°* coordinates was also reported by Chahal and Singh (4) in 'Daisy' tangerine fruit with the intensification of colour from green to orange till maturity.

During maturation of the grapefruit varieties, the β-carotene concentration in the peel steadily increased with fruit development (Fig. 3). 'β-carotene' is the most dominant pigment in fruits, and total carotenoid content in fruits increased mainly due to an increase in 'β-carotene' (Ma *et al*., 9). Citrus fruit peel endures a "colour break" during fruit maturation which includes chlorophyll degradation and a gradual increase of carotenoids with peel colour changes from green to yellow at maturity (Porras *et al*., 12). β-carotene concentration at the final stage of fruit sampling was 2.14 to 6.25 times more than the initial sampling. A minimum increase in β-carotene was observed in cv. Flame. It might be due to slight colour variation in different grapefruit varieties throughout the fruit development period. The β-carotene content varied in different grapefruit varieties from 3.61-28.75 μg g^{-1} at initial sampling and 20.23- 61.42μg.g^{-1} at 270 DAFS. The highest β-carotene content was observed in the deep red-coloured flesh cv. Flame and the lowest in white-fleshed 'Marsh Seedless' during the fruit development. The red-fleshed cv. Flame had 3.03 times more β-carotene concentration than white-fleshed Marsh Seedless grapefruit. A significant increase in β-carotene content was observed till 240 DAFS. Afterwards, no significant variations were recorded in grapefruit varieties examined during the present study. However, these four grapefruit varieties exhibited a significant variation in β-carotene content with the approach of commercial fruit harvesting. Yoo and Moon (18) observed higher total carotenoids content in the dark yellow peel lemon as compared to its green and light-yellow peel varieties.

As the grapefruits matured, the juice B/A ratio increased linearly and recorded maximum at final fruit maturity (Fig. 3). The B/A ratio in the juice of four grapefruit varieties ranged between 4.26 (120 DAFS) to 7.26 (270 DAFS). The B/A ratio in grapefruit increased from 54.46 to 64.25 per cent during fruit development with the sequence of Flame (7.26)>Redblush (7.18)>Rio Red (6.63)>Marsh Seedless (6.58) variety. At initial fruit sampling, the differences were non-significant. However, towards the end of fruit maturity Flame and Redblush varieties showed significant ($p<0.05$) differences with the rest of the varieties, exhibiting 10.3% more B/A ratio than white fleshed 'Marsh Seedless'. Similarly, Singh *et al*. (15) also observed a significant T.S.B./Acid ratio increase in 'Star Ruby' grapefruit from 120 to 210 DAFS.

To evaluate the relation of grapefruit peel colour parameters *L*, a*, b**, *C** and *h°* with β-carotene and B/A ratio, correlation coefficients were analyzed and presented in Table 1. The correlation coefficients

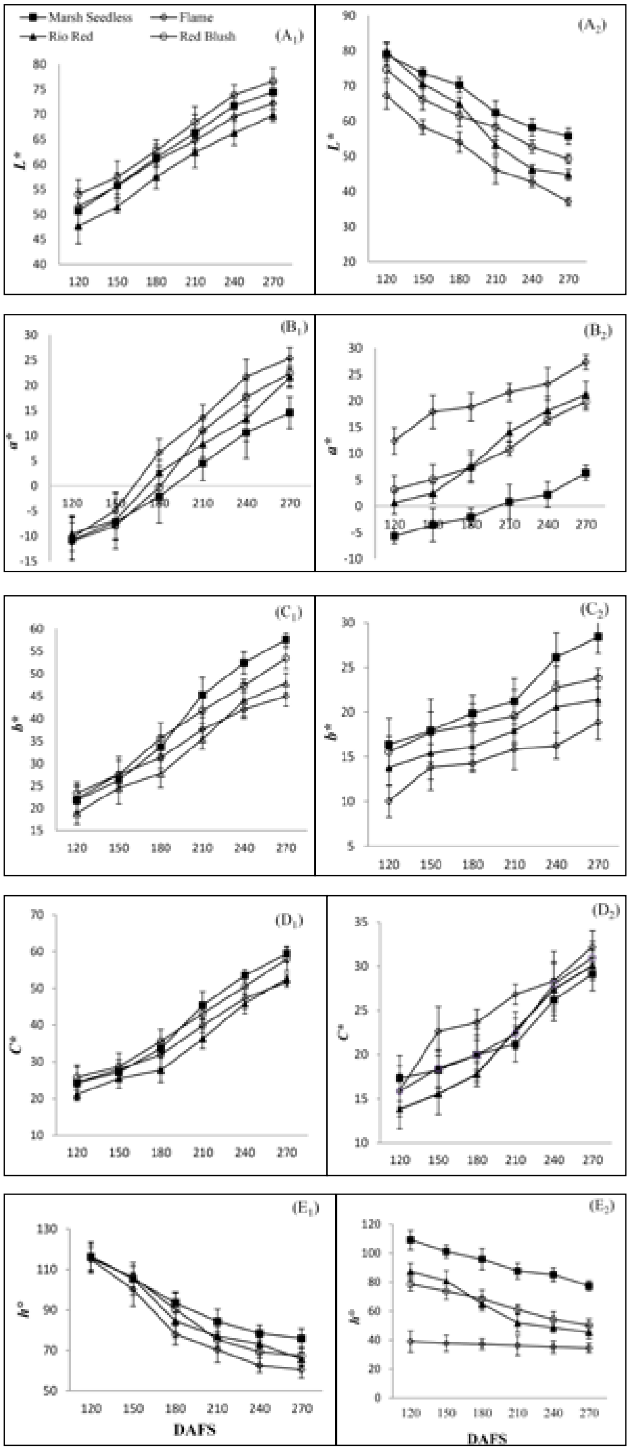

Fig. 3. Change in β-carotene content and B/A ratio in four grapefruit varieties during fruit development from 120 to 270 DAFS.

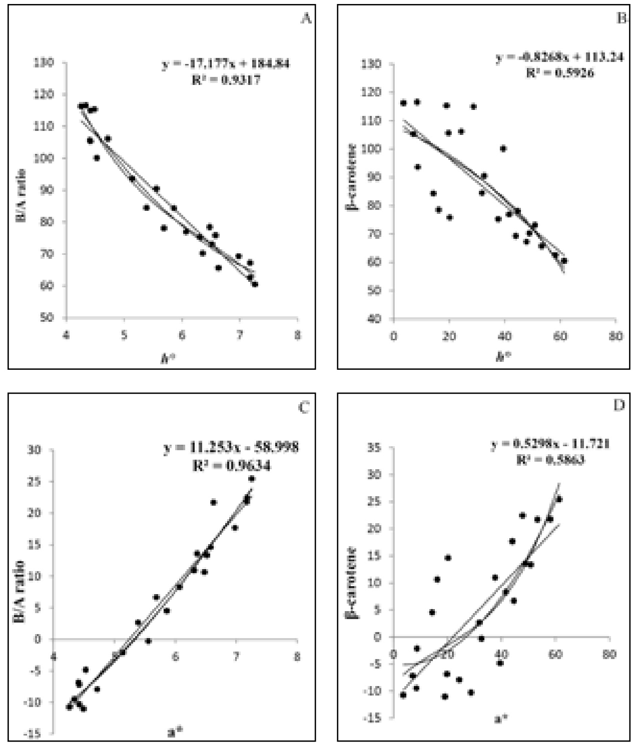

Fig. 4. Linear, cubic and quadraticregression between mean values of B/A ratio-$h°$ (A), β-carotene-$h°$ (B), B/A ratio-a^* (C) and β-carotene-a^* (D) in four grapefruit varieties during fruit development from 120 to 270 DAFS.

measured for changes in colour coordinates, carotenoid and B/A ratio with maturity were significant ($p<0.05$) and strongly correlated mutually. The colour value L* correlated weakly with β-carotene (r = 0.541). A weak correlation between L* and certain carotenoids would be expected because any increase in pigment would increase the darkness and thereby decrease L*. A stronger correlation was recorded between colour coordinates and B/A ratio than those with β-carotene content. Previous studies by Barragán-Iglesias *et al*. (2) explained that the changes in the fruit colour had a relationship with the sugar content of the fruit and recorded a comparable correlation value for colour coordinates with T.S.S. content. A strong correlation value of a^* and $h°$ colour parameters with B/A ratio expressed a steady rise of B/A ratio with a change in fruit colour from green to red till maturity. Similarly, higher correlation values were observed between β-carotene and hue angle (-0.741) followed by a^* (0.734) and b^* (0.439) coordinate values. Colour parameter C^* had a strong correlation with b^* coordinate (r = 0.984) followed by L^* (r = 0.914) and a^* (r = 0.847), and with hue angle, it was negatively correlated (r = -0.808) whereas hue angle itself correlated negatively with other colour coordinates but strongly correlated with a^* (r = -0.943). Linear, quadratic and cubic regression of β-carotene and B/A ratio with hue angle and a^*

Table 1. Pearson's correlation coefficient comparing relationship of peel colour coordinates with carotenoids and B/A ratio.

Y	X						
	*L**	*a**	*b**	*C**	*h°*	Carotenoid	B/A Ratio
*L**	-						
*a**	0.876*	-					
*b**	0.899*	0.813*	-				
*C**	0.914*	0.847*	0.984*	-			
h°	-0.844*	-0.943*	-0.798*	-0.808*	-		
β-carotene	0.541*	0.734*	0.439*	0.494*	-0.741*	-	
B/A Ratio	0.869*	0.925*	0.875*	0.890*	-0.915*	0.694*	-

*Correlation coefficients are significant at $p<0.05$.

coordinate was determined as hue angle and *a** are important colour parameters for red-fleshed grapefruit varieties. R^2 value obtained from hue and *a** with B/A ratio were higher than with β-carotene (Fig. 4). The most stable regression to the value of R^2=0.9634 was observed between B/A ratio and a* coordinate across all the four grapefruit varieties during fruit development from 120 to 270 DAFS. A significant correlation ($p<0.05$) of hue and chroma with fruit development stages was also defined by the regression equation in 'Daisy' tangerine fruit (Chahal and Singh, 4).

Significant fruit colour variations were observed in peel and pulp and β–carotene with fruit development in different grapefruit varieties. All colour coordinates, except hue angle, increased with fruit development. Among studied grapefruit varieties, the highest β–carotene, B/A ratio and *a**coordinate were recorded in the Flame variety due to the development of dark red colour. A significant relation of *L*a*b**colour coordinates with the B/A ratio followed by β–carotene was observed during fruit development. It can be summarized that a* coordinate was the most reliable parameter to judge the maturity indices in grapefruits.

AUTHORS' CONTRIBUTION

Conceptualization of research (T.S.C., P.P.S.G.); Designing of the experiments (T.S.C., VS); Contribution of experimental materials (VS, T.S.C., S.K.J.); Execution of field/ lab experiments and data collection (VS); Analysis of data and interpretation (VS); Preparation of manuscript (T.S.C., P.P.S.G.)

DECLARATION

The authors declare no conflict of interest.

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