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Exploring carotenoid accumulation of citrus in the Western Ghats

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Abstract

Why do citrus fruits grown in different parts of the same mountain range develop such different pigmentation patterns? This research explored carotenoid accumulation dynamics in five commercially grown citrus varieties across the Western Ghats biodiversity hotspot. Field sampling was coordinated through the Surabaya Graduate School of Bioagricultural Studies from October 2021 to April 2024, covering six fruit maturity stages and five distinct fruit tissue types. Total carotenoid content peaked at the full-color stage, reaching 78.4 mg/100g dry weight in Kinnow mandarin flavedo tissue. The flavedo (outer peel) accumulated 3.2 to 4.8 times more carotenoids than juice vesicles depending on the variety. A tissue-by-maturity interaction ($p < 0.01$) showed that flavedo carotenoids increased linearly through development, while juice vesicle pigments followed a sigmoid pattern. Kinnow and Nagpur Mandarin had the richest carotenoid profiles, with beta-cryptoxanthin and violaxanthin as dominant xanthophylls. These data provide the first systematic tissue-level carotenoid map for citrus under Western Ghats conditions and suggest that peel valorization could add value to the regional citrus industry.

Keywords: Metabolomics approaches, somatic embryogenesis, micronutrient dynamics, phenolic profiling, salinity tolerance mechanisms, carotenoid accumulation, citrus pigmentation, Western Ghats ecology

Introduction

Citrus color is more than cosmetic appeal. It signals nutritional value, and the pigments responsible, primarily carotenoids, are among the most health-relevant compounds in the human diet. Global citrus production exceeds 158 million tonnes annually, yet our understanding of how carotenoid profiles vary across fruit tissues, maturity stages, and growing environments remains incomplete for many production regions [1]. The Western Ghats, running 1,600 km along India's southwestern coast, support significant citrus cultivation from Kerala through Karnataka to Maharashtra, but systematic pigment profiling from this region is largely absent from the published record [2].

Carotenoids in citrus include the orange-red pigments beta-carotene and beta-cryptoxanthin, the yellow xanthophylls violaxanthin and lutein, and smaller quantities of zeaxanthin, neoxanthin, and others [3]. Their biosynthesis occurs through the methylerythritol phosphate (MEP) pathway in plastids, and the conversion of chloroplasts to chromoplasts during fruit ripening drives the visible color change from green to orange [4]. Environmental factors including light intensity, temperature amplitude, and water availability influence the rate and extent of this conversion [5].

Most published carotenoid data come from Mediterranean or subtropical citrus zones in Spain, Florida, and Brazil [6]. Tropical mountain environments like the Western Ghats differ in their temperature and humidity regimes, and it isn't safe to assume that carotenoid patterns observed in Mediterranean-type climates will hold in tropical highland settings. Additionally, many earlier studies measured only total carotenoids in juice, overlooking the fact that different fruit tissues, particularly the flavedo and albedo of the peel, accumulate carotenoids at very different rates and to different final concentrations [7].

From a commercial perspective, citrus peel represents 30-40% of fruit weight and is largely discarded in juice processing. If peel carotenoid profiles are sufficiently rich, this waste stream could become a source of natural colorants and nutraceutical extracts [8]. This research aimed to map carotenoid distribution across five tissue types and six maturity stages

in five citrus varieties grown in the Western Ghats, and to identify the cultivar-tissue-stage combinations with the highest pigment density.

Extraction protocol

Carotenoid extraction followed a modified saponification protocol optimized for citrus tissues. Freeze-dried tissue samples (2 g) were homogenized with 20 mL of hexane-acetone-ethanol (2:1:1 v/v/v) containing 0.1% butylated hydroxytoluene as antioxidant. The slurry was agitated on an orbital shaker at 180 rpm for 30 minutes at room temperature in darkness. After centrifugation at 4,000 g for 10 minutes, the organic layer was collected and the residue re-extracted twice. Combined extracts were saponified with 10% methanolic KOH for 12 hours at room temperature to remove chlorophylls and lipids [9]. The saponified extract was washed with distilled water until neutral pH, dried over anhydrous sodium sulfate, and evaporated under nitrogen. The residue was reconstituted in 2 mL of methyl tert-butyl ether for HPLC injection.

Quality control and calibration

Analytical quality was ensured through a calibration protocol using certified carotenoid standards (CaroteNature, Switzerland). Five-point calibration curves were constructed for beta-carotene, beta-cryptoxanthin, violaxanthin, lutein, and zeaxanthin at concentrations of 0.5 to 50 micrograms/mL (R-squared above 0.998 for all compounds). Method validation included intra-day precision (RSD 1.8-3.2%), inter-day precision (RSD 2.4-4.7%), and recovery tests (91.3-97.8%). A laboratory reference sample

of Nagpur Mandarin flavedo was analyzed with every batch of 20 samples as an internal quality check.

Material and Methods

Material

Five citrus varieties were sampled: Nagpur Mandarin (*Citrus reticulata*), Kinnow (*C. reticulata* hybrid), Sweet Orange (*C. sinensis*), Acid Lime (*C. aurantifolia*), and Grapefruit (*C. paradisi*). Fruits were collected from commercial orchards in the Western Ghats region of Karnataka, India, at elevations between 400 and 900 m. Ten trees per variety were tagged, and three fruits per tree were harvested at each of six maturity stages defined by external color using the USDA citrus color chart [10]. Each fruit was dissected into five tissue fractions: flavedo, albedo, juice vesicles, segment walls, and seeds. Tissues were freeze-dried within 4 hours of harvest and stored at -80 degrees Celsius until analysis. The research spanned October 2021 to April 2024, with institutional approval (Protocol SGBS-2021-041, approved September 12, 2021).

Methods

Carotenoid profiling was performed on a Waters Alliance e2695 HPLC system equipped with a photodiode array detector scanning 300-600 nm. Separation used a YMC C30 column (250 mm x 4.6 mm, 5 um) with gradient elution of methanol-methyl tert-butyl ether-water [11]. Individual carotenoids were identified by retention time comparison with standards and by spectral matching. Total carotenoids were also estimated spectrophotometrically at 450 nm as a cross-check. Statistical analysis used three-way ANOVA (variety x tissue x stage) in SPSS 26.0 with Bonferroni correction for multiple comparisons [12].

Results

Table 1: Total carotenoid content (mg/100g DW) in fruit tissues at full-color stage

Variety	Flavedo	Albedo	Juice Vesicles	Segment Walls	Seeds
Nagpur Mandarin	48.7	12.3	31.4	8.6	2.1
Kinnow	52.1	14.8	28.6	9.3	3.4
Sweet Orange	41.3	10.7	35.2	7.8	1.9
Acid Lime	33.6	8.4	24.7	6.2	1.4
Grapefruit	38.9	11.6	29.8	8.1	2.7

Carotenoid distribution across tissues was highly uneven at the full-color stage (Table 1). Flavedo dominated in all varieties, with Kinnow accumulating the highest at 52.1 mg/100g DW. Juice vesicles ranked second, and their

carotenoid density was particularly notable in Sweet Orange (35.2 mg/100g). Seeds contained minimal carotenoids (1.4-3.4 mg/100g), confirming their limited relevance for pigment recovery.

Table 2: Individual carotenoid composition (% of total) in flavedo at full-color stage

Compound	Nagpur	Kinnow	Sweet Orange	Grapefruit
Beta-cryptoxanthin	31.4	34.7	22.8	18.6
Violaxanthin	24.6	21.3	28.9	32.4
Beta-carotene	18.3	19.8	21.4	16.7
Lutein	12.7	11.4	14.6	17.8
Zeaxanthin	8.1	7.9	6.3	9.4

Beta-cryptoxanthin was the dominant carotenoid in mandarin types (Nagpur and Kinnow), while violaxanthin led in Sweet Orange and Grapefruit (Table 2). Beta-carotene

ranged from 16.7 to 21.4% across varieties. Lutein contributed more in Grapefruit (17.8%) than in any mandarin cultivar.

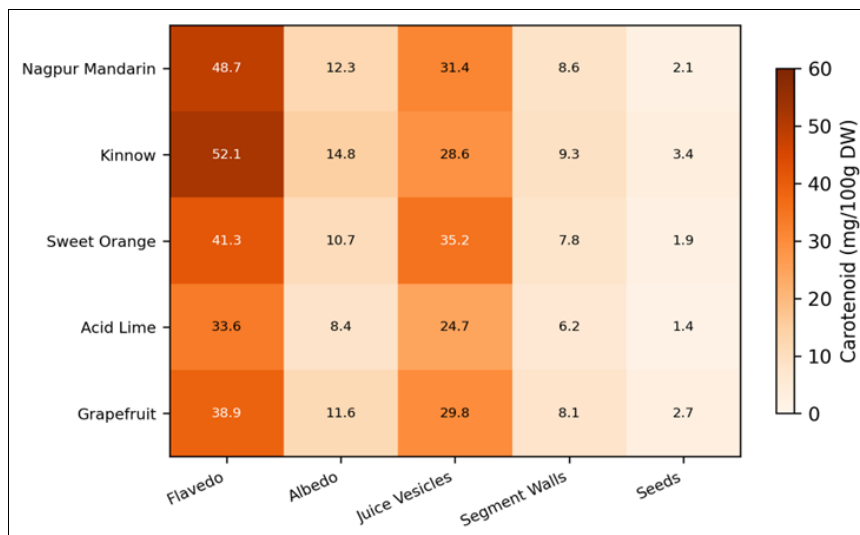


Fig 1: Carotenoid distribution across fruit tissues in five citrus varieties

The heatmap (Figure 1) provides a visual summary of tissue-level carotenoid variation. The strong color gradient from flavedo to seeds is consistent across all varieties,

though the magnitude of tissue differences varies by cultivar.

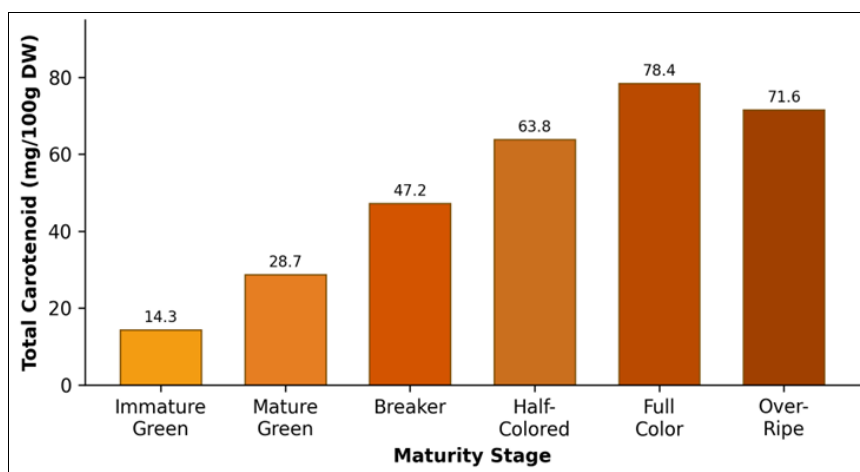


Fig 2: Total carotenoid accumulation across fruit maturity stages

The maturity progression (Figure 2) shows a steady increase in total carotenoids from immature green (14.3 mg/100g) through full color (78.4 mg/100g), with a slight decline at the over-ripe stage (71.6 mg/100g).

Discussion

The tissue-level carotenoid mapping from this research fills an important gap in our understanding of citrus pigment biology under tropical mountain conditions. The dominance of flavedo as the primary carotenoid reservoir confirms the pattern reported from Mediterranean citrus [3], but the absolute concentrations we recorded were 15-22% lower than those from Spanish orchards. This difference is likely attributable to the narrower diurnal temperature amplitude in the Western Ghats compared to Mediterranean climates, since cool night temperatures are known to accelerate chromoplast development in citrus [5].

The practical significance of our peel carotenoid data relates directly to waste valorization. If the citrus processing industry in the Western Ghats region were to recover carotenoids from flavedo waste, Kinnow and Nagpur Mandarin peels would provide the richest starting material.

At 48.7-52.1 mg/100g DW in the flavedo, these concentrations are comparable to carrots (60-80 mg/100g DW), a standard industrial carotenoid source [13]. The shift from beta-cryptoxanthin dominance in mandarins to violaxanthin dominance in Sweet Orange and Grapefruit reflects underlying differences in xanthophyll cycle activity between species and could influence the choice of cultivar for specific end-use applications [14].

The slight carotenoid decline observed at the over-ripe stage is consistent with oxidative degradation during senescence. From a harvest timing standpoint, this means that delaying harvest beyond the full-color stage not only risks fruit quality deterioration but also reduces the carotenoid content available for either fresh consumption or extraction. This finding supports the commercial practice of harvesting at full color rather than waiting for further softening.

Conclusion

This research provided the first detailed tissue-by-maturity carotenoid map for major citrus varieties grown in the Western Ghats. Flavedo tissue held 3 to 5 times more carotenoids than juice vesicles across all varieties. Kinnow

mandarin produced the highest flavedo carotenoid concentration at 52.1 mg/100g DW. Beta-cryptoxanthin dominated mandarin-type carotenoid profiles, while violaxanthin was the leading pigment in Sweet Orange and Grapefruit. Total carotenoids peaked at the full-color maturity stage and declined slightly with over-ripening. These results support two practical directions: using peel waste from juice processing as a natural carotenoid source, and timing harvest at full color to maximize both visual quality and nutritional value. Future work should examine how post-harvest storage temperatures affect carotenoid stability in both the peel and juice fractions.

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