Title Physiological and Biochemical Changes during Chlorophyll Degradation in Lime (*Citrus aurantifolia,* Swingle, cv. 'Paan')

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Abstract

Study on the mechanism of chlorophyll degradation is a novel control point to prevent peel yellowing in lime (*Citrus aurantifolia,* Swingle cv. 'Paan'). Physiological and biochemical changes occurring in the flavedo tissue of lime peel during chlorophyll degradation were explored with seven parts of investigations in this study. A colour index chart for lime fruit was developed with maturity stages 1 – 4 based on peel colour change from mature – green to full – yellow and its correlation with chlorophyll content and fruit quality attributes. Chlorophyll content decreased 53.9% of its initial level within 7 days in ambient storage but it decreased only 6.39% within 7 days at cold storage. To find out the natural biochemical and physiological changes during chlorophyll degradation, most the experiments were conducted under room temperature conditions. Chlorophyllase functions in early step of chlorophyll degradation and chlorophyll degrading peroxidase (POD) participates in the later part of chlorophyll catabolic routes. To test the effects of ethylene on chlorophyll degradation, exogenous ethylene was applied. The results indicated that ethylene response in lime fruit was not concentration dependent, but it was a sensitivity response by flavedo tissue. To control chlorophyll degradation in lime peel, 1-methycyclopropene (1-MCP), plant growth regulators-N_6-benzylaminopurine (BAP), gibberellic acid (GA_3), 2, 4-dichlorophynoxyacetic acid (2, 4-D), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were applied at ambient conditions. It was clearly noted that 1-MCP in low concentrations (250 or 500 hl l^{-1}) suppressed chlorophyll break – down, chlorophyllase, peroxidase and endogenous ethylene production. BAP (20 µl l^{-1}) was the most effective plant growth regulator to prevent yellowing until 42 days even under ambient storage. Conversely, IAA and IBA promoted yellowing, enhanced chlorophyllase, ACC oxidase and chlorophyll degrading peroxidase and endogenous ethylene production. In low oxygen condition, fruit stored with 5% and 10% O_2 retarded yellowing for 60 days, suppressing chlorophyllase, Mg-dechelatase, ACC-

oxidase and chlorophyll degrading peroxidase than those of regular air (RA). Fruit harvested in mature green stage was significantly delayed in chlorophyll break down than premature and full mature harvested fruits. Based on the results of this entire study, chlorophyllase was the key limiting factor in the chlorophyll degradation mechanism for *Citrus aurantifolia,* Swingle cv. 'Paan'. Mg-dechelation and chlorophyll degrading peroxidase functioned in the later part of chlorophyll degradation processes. Degradation of chlorophylls to chlorophyllides pigments was highly related to chlorophyllase. Formation of pheophytin pigments was not significantly decreased in fruit treated with BAP, GA_3 and low oxygen (5-10%) treatments until the end of storage period.