The Morphology Associated with Harvesting Stages of Siam Red Ruby Pumelo (*Citrus grandis*)

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Abstract— **This study aims to evaluate fruit morphological characteristics and potential production of 'Siam Red Ruby' pumelo from Pak Panang district, Nakhon Si Thammarat Province compared between normal and senescence fruit peel. A three-year-old tree was selected to evaluate fruit morphological characteristics and a harvesting date or time at 90, 120, 150, 180, and 210 Days After Fruit Set (DAFS). The result showed anatomical association to the senescence of 'Siam Red Ruby' pummelo using the peel of normal and senescence zones using compound microscopy. The 'Siam Red Ruby' pummelo was shown a green color inside a peel-90 days after fruit setting and in a peel showed a trichome or hair covering all the fruit (100%) then decreased during fruit development until 210 days after fruit setting showed the hair cover 20% in all fruit compared with others stage. During the maturity stage, the pulp was yellow-colored from 90 to 120 days after the fruit set. From 150 days after the fruit set, the pulp turned red gradually. In addition, the structure of the peel was changed during senescence. The first day of the peel or normal zone showed high firmness and peel green color. In the senescence zone, the peel changed to a yellow color. In addition, Titratable Acidity (TA) decreased from 90 to 210 days after fruit setting and related with Total Soluble Solid (TSS) increased from mature fruit (90 days after fruit setting) to ripening fruit (210 days after fruit setting). In reflective sheet treatment, clouds induce the red color in the pulp of 'Siam Red Ruby'** ***pumelo.***

Index Terms— Siam Red Ruby Pumelo, Senescence, Morphology

I. Introduction

'Siam Red Ruby' pumelo (*Citrus grandis*) is plantation the Pakpanang district, Nakhon Si Thammarat province, Thailand. It is a Geographical Indication (GI) product of Thailand and is a popular pumelo cultivar in the premium fruit marketplace. The external appearance of the 'Siam Red Ruby' pumelo fruit is characterized by a dark green colored peel with soft hair and clear oil glands. The internal appearance of the 'Siam Red Ruby' pumelo fruit is characterized by a dark pink to red color and a sour-sweet taste [1]. Recently, the demand for 'Siam Red Ruby' fruit has increased by 40% in domestic and 60% in international markets, especially markets in China, Taiwan, Hong Kong, Malaysia, Singapore, and Brunei. Today, the cultivation site of 'Siam Red Ruby' pumelo is expanding to commercial, continuous plantation operations due to the high value and marketing needs [2]. The 'Siam Red Ruby' pumelo is rich in bioactive compounds such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), and flavonoids (i.e., naringin, narirutin, hesperidin, and neohesperidin). In addition, 'Siam Red Ruby' pumelo juice has high amounts of fructose, glucose, and sucrose, at 6.07, 4.52, and 2.09 g L^{-1}, respectively [3]. Although fruit growers had good experience in pumelo orchard management, they lacked relevant information on the harvesting index of 'Siam Red Ruby' pumelo in these areas. The harvesting index information of "Tabtim Siam" pumelo is limited as most research has only focused on the effect of tree age and fruit age on fruit quality in Pakpanang district, Nakhon Si Thammarat province. For pumelo var. Tabtim Siam, fruit quality of 6 to 8-year-old trees was suitable for harvesting at 210 days after fruit set, reported [4] that the harvesting index of 4 and 7 years old of pumelo were 160 and 220 days after fruit set, respectively. In addition, the fruit quality of pumelo depends on the planting area such as soil nutrients, microclimate, and cultural practices of the grower. Flavors and some morphological characteristics of pumelo fruit in each area were different [5] Thus, the objective of this research was to evaluate fruit morphological characteristics and potential production of 'Siam Red Ruby' pumelo in Pak Panang district, Nakhon Si Thammarat provinces.

II. Materials and Methods

A. Plant Material

The experiment was conducted in Pakpanang district, Nakhon Si Thammarat province. The 'Siam Red Ruby' (*Citrus grandis*) at the harvesting stages (90, 120, 150, 180, and 210 days), was harvested and transported to the laboratory of Plant Science, Faculty of Technology and Community Development, Thaksin University, Phatthalung campus. Fruit samples were selected for uniformity of color and size and did not show the disease. These fruits of normal and senescence are to be determined by morphology using a microscope.

B. Study on the Morphology Using Compound Microscopy

In this study, we are using the peel of normal and senescence zones (210 days after fruit set) to observe changes in the physical morphology surface of the 'Siam Red Ruby' pumelo during senescence (storage at 25°C for 1 month). Cutting the tissue area to be studied for 5×5 cm. Then a sample on a microscope [6] the software for recording pictures using LASV 4.5.

C. Study on the Morphology Using Scanning Electron Microscope

The peel of 'Siam Red Ruby' pumelo has been cut to size 1×1 cm for normal and senescence zones. In this study, we have conducted fixation of the sample with Formaldehyde Acetic Acid (FAA) for SEM, components of FAA include Ethanol 25 ml and acetic acid 5 ml FAA 5 ml distilled water 5 ml Remove FAA, then dehydration of sample with T-Butanol (TBA) at different concentrations. The concentration TBA is 10, 20, 35, 55, 75, and 100%, these concentrations for 2 hr. After dehydration, keep the sample at -20°C for 24 hr before SEM analysis.

Color measurement with a colorimeter (Model CR300, Minolta, Japan). The CIE 1976 L* a* b* color scale was adopted. The hue angle (Ho) was calculated as Ho = arctangent (b*/ a*). Color readings were taken three times at the equatorial region of each fruit.

The Total Soluble Solids (TSS) of fruit juice were determined by using a Hand refractometer (Model N; Atago Co., Tokyo, Japan) and reported as percent soluble solids in fruit juice. The Titratable Acidity (TA) of fruit juice was determined by titrating 1 mL of fruit juice diluted 10 times with distilled water against 0.1 mol L-1 NaOH, using 1-2 drops of 1% (v/v) phenolphthalein as an indicator of the reaction end-point. TA was expressed as percent citric acid (meq. citric acid = 0.064).

Estimation of Chlorophyll: Five hundred mg of fresh leaf material was taken and ground with the help of a pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes the supernatant was stored. The residue was re-extracted with 5 ml of 80% acetone. The extract was utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in the UV-spectrophotometer.

Texture Analyzer (TA-XT2) was used to measure the penetration force needed to press a plunger with a diameter of 4 mm 5 mm into the fruit at a speed of 1.7 mm s^{-1}. The fruit was cut into halves and the firmness was tested for both halves. The readings were in Newton (N).

D. Statistical Analysis

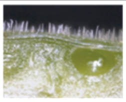
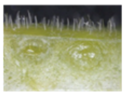
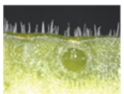
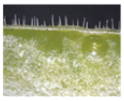
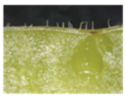
All values were shown in the present study as the mean ± Standard Error (SE) for three replicates. The data were analyzed, and Tukey's Honest Significant Difference (HSD) test was used to compare the means at $P < 0.05$. Calculations were performed using JMP software (SAS Institute, Cary, NC).

III. Results

A. The Morphology Associated Change with the Senescence of 'Siam Red Ruby' Pumelo

All Anatomical associated with the senescence of 'Siam Red Ruby' pumelo using the peel of normal and senescence zone using compound microscopy. The 'Siam Red Ruby' pumelo was shown a green color inside a peel 90 days after fruit setting and a peel showed a trichome or hair covering all the fruit (100%) then decreased during fruit development until 210 days after fruit setting showed the hair cover 20% in all fruit compared with immature stage. During the development or maturity stage, the pulp was yellow-colored from 90 to 120 days after the fruit set. From 150 days after the fruit set, the pulp gradually turned red. In addition, the structure of the peel was changed during senescence. The first day of the peel or normal zone showed high firmness and peel green color. In the senescence zone, the peel changes to the yellow color (Table I). While the changes of hue angle were in parallel with color development in the pulp. In addition, the Titratable Acidity (TA) was constant from 90 to 150 days after the fruit setting and then decreased significantly from 180 days after the fruit set. On the other hand, the Total Soluble Solid (TSS) value of the fruit juice increased significantly during the fruit process. Among them, the carotenoid in the pulp of 'Siam Red Ruby' pumelo, increased rapidly during the stage and accounted for the maturation stage (210 days after the fruit setting) (Table I).

TABLE I
CHANGE OF PHYSICAL MORPHOLOGY AND INTERNAL QUALITY ON PEEL AND PULP COLOR CHANGE DURING THE 'SIAM RED RUBY' PUMELO FRUIT DEVELOPMENT STAGE

Fruit stage	Outer pericarp section	Time (d)	Firmness (N)		Hue angle		Total Chlorophyll (mg/100 FW)		Total Carotenoid (mg/kg^{-1} FW)		%TA	TSS (°Brix)
			Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp		
		90	43.64	11.39	110.26	65.37	18.55	4.10	1.21	2.84	0.56	9.2
		120	43.75	10.75	109.12	65.59	18.21	3.22	2.35	4.55	0.58	9.8
		150	42.77	10.80	109.2	55.44	17.86	3.54	2.33	4.87	0.55	9.7
		180	41.38	9.32	108.35	45.03	17.23	3.21	2.89	5.34	0.34	10.35
		210	39.42	8.12	104.67	38.90	15.23	3.45	3.01	5.79	0.28	12.54

The data is not statistical and SE or SD (±)

B. Anatomical of 'Siam Red Ruby' Pumelo During Senescence

The anatomical and surface of the peel normal cell on the upper show differences with senescence cells. The peel normal cell of 'Siam Red Ruby' pumelo showed freshness and structure, and the shape of the cells was round and turgid of cells. , we found stomata were turgid cells, especially guard cells, these show close stomata (Fig. 1). While the peel senescence cell of 'Siam Red Ruby' pumelo as shown in Fig. 1 and Fig. 2, The peel senescence cell collapsed of surface cells and the arrangement of cells was incomplete, the peel cell found loss of turgid, show stomata of petal blackening cell were opening more than peel normal cell and lost turgid of guard cell (Fig. 2).

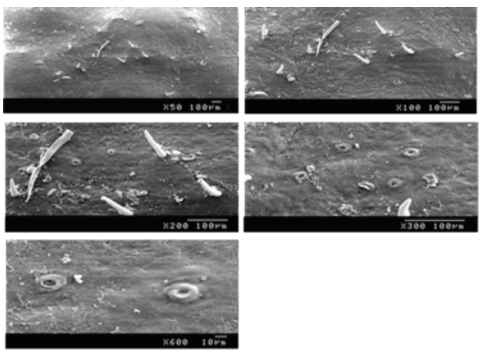


Fig. 1. Change of anatomical in the peel of 'Siam red ruby' pumelo by using scanning electron microscope; Sem on the normal fruit.

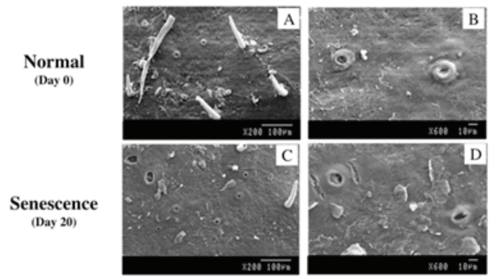


Fig. 2. Change of anatomical in the peel of 'Siam Red Ruby' pumelo by using scanning electron microscope; Sem on the senescence of the fruit (A, B, C, & D).

IV. DISCUSSION

The results of this study showed significant differences in the pulp color, total carotenoid content, and carotenoid composition in 'Siam Red Ruby' pumelo during the development stage. During fruit development, there was a difference in pulp color from 60 to 210 days after fruit setting. The hue angle decreased sharply during this time. In addition, color development in the pulp was related to the carotenoid content, with the yellow color generally being greater in immature fruit and reddening occurring during the development stages due to changes in the carotenoid composition. There were differences during fruit development in TSS and TA values (Table I). TSS increased significantly during fruit development. During fruit development, sugar, and pigment content accumulated while acid content decreased in the pulp of fruit. Determining the different sugar/acid ratios during ripening is often used as a major postharvest maturity. As TSS content increases, this parameter is a very practical index of internal fruit quality. The understanding of the physiological and biochemical determinants for TSS and TA content in fruits will allow the enhancement of fruit quality during growth and pre and post-harvest practices, the improvement of citrus fruit sweetness and fruit acidity, the characterization of physiological disorders that depend on TSS and TA fruit content and physicochemical composition of the fruits, especially ascorbic acid, TSS and TA depends on the maturity stage. Moreover, a high correlation between TSS and TA has been described Obenland et al. [7].

Stomata are pores in the gastight waxy cuticle that cover the outer surface of aerial parts of plants. They make the uptake of CO2 possible, which is needed for photosynthesis. At the same time, water vapor will leave the plant via the stomata [8]. To optimize photosynthesis, while at the same time preventing excess water loss, stomata control their opening by a signaling network of pathways that respond to environmental conditions such as light and darkness, water Vapor Pressure Deficit (VPD), temperature, CO2, and ethylene. Water loss is one of the most obvious changes in harvested vegetables, often limiting marketing life, and a negative water balance (uptake of water is insufficient to compensate for transpiration) is one of the most important reasons for the end of the vase life of cut flowers [9]. In addition, the postharvest quality of some fruits was negatively affected by water loss. In leafy vegetables, stomata are portals that make the invasion of bacteria into the inner tissue possible and protect in that way bacteria from sanitizers in washing solutions with the risk of food-borne bacterial diseases. This chapter discusses how several environmental factors, during preharvest cultivation and postharvest storage, influence stomata closing control in harvested fruits. Also, the role of the number of stomata and their variability between genotypes and due to cultivation conditions are discussed in relation to postharvest life. One of the most striking factors is low VPD (high humidity) during the growth of plants: after exposure for several days to low VPD, the control of stomata closure is largely disturbed; stomata do not respond anymore to stimuli that normally induce closure. This malfunctioning is persistent and results in high water loss afterward in the harvested products. Fast cooling of produce can close the stomata of some crops, while in others, the stomata stay open until wilting [10]

Plant stomata, consisting of a pair of guard cells, are dynamic structures that open or close to modulate gas exchange and water loss and allow plants to respond appropriately to diverse pathogens invasion. Therefore, stomata play essential roles in abiotic and biotic stress responses [11]. The regulation of stomata movement is complex, and as time progresses, researchers discover new signaling elements that make the signaling networks of stomata movement more complex [12]. In recent years, in addition to plant growth and development, plant small signaling peptides have been implicated in stomata aperture regulation [13].

V. Conclusion

The specific fruit shape of this cultivar was obovoid and piriform. The fruit peel color was light green to dark green with a hairy cover. Peel color development of ‘Siam Red Ruby’ pumelo developed faster during fruit development. The pulp color was dark red similar to a ruby. The harvesting index of the 5-6-year-old pomelo tree in these production regions was at 6 months after the fruit set presenting the highest overall acceptance. In ‘Siam Red Ruby’ pumelo, Total Soluble Solid (TSS) and Titratable Acidity (TA) were 10.4 ^{o}Brix, 0.56% and 20.3, respectively.

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