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Sugar status at harvest and during postharvest storage of *Nephelium lappaceum* cv. 'Sri-Chompoo' fruit from different maturity stages

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Abstract

Nephelium lappaceum cv. 'Sri-Chompoo' (rambutan) fruit showing different colors at four different maturity stages were collected: green-yellow, orange-yellow, orange-pink, and red-pink rind. At harvest and during post-harvest storage at 25°C for 6 days, the glucose, fructose and sucrose contents in the arils of rambutan fruit of different maturity stages were investigated using high performance liquid chromatography and invertase activity was also determined. At harvest, glucose was the most abundant sugar in 'Sri-Chompoo' rambutan fruit except at the red-pink stage in which sucrose was the dominant sugar. During post-harvest storage of fruit from four stages of maturity, glucose content decreased slightly while fructose content increased during the first 4 days of storage in green-yellow and orange-yellow fruit. Orange-pink and red-pink fruit had a nearly similar trend of glucose and fructose contents which did not differ much from that at harvest. Besides, during storage changes in sugar components in the fruit of different maturity stages were not directly related with those in invertase activity.

Introduction

Nephelium lappaceum L. (rambutan), closely related to longan and lychee, is an evergreen, bushy, dioecious, tropical tree belonging to the family Sapindaceae. This plant species is indigenous to Malaysia in Southeastern Asia and its name has also arisen from the Malayan word "rambut" (means hairy) which reflects the characteristics of the fruit. The major part of this globose, ovoid or oblong fruit possesses an edible aril that is white or translucent, sweet, juicy and encloses a nut-like seed (Abbey, 2000; IPGRI, 2003; Kunz, 2007; Arenas, 2010). Rambutan fruit have future potentials in the export market place due to an increasing trend of demand. To meet the requirements of international quality, rambutan fruit should contain the following attributes: consistency; free of dirts, lesions, insects and diseases; weight more than 30 g; bright rind and spintern color; spintern less than 1 cm; thick, firm aril which is easily separable from the kernel, high in soluble solid content and low in titratable acidity (Ketsa and Klaewkasetkorn, 1992; Abbey, 2000; Arenas, 2010). Moreover, aril sweetness should also be the most significant standard feature for rambutan that fosters worth, approval and faithfulness to customer (Oude Ophuis and van Tripp, 1995).

In Thailand, 'Sri-Chompoo' is the most widely grown cultivar of rambutan in addition to 'Bang-Yeekhan' and 'Rongrien'. This cultivated variety is a mutant from 'Bang-Yeekhan' rambutan. The rambutan fruit shows a non-climacteric respiratory pattern (Lam and Kosiyachinda, 1987) and is large with thin pericarp that is pink to pinkish red when ripe. Its aril has a good flavor and total soluble solid diverges from 18-20% (Tindall *et al*., 1994). Since the sweetness of 'Sri-Chompoo' rambutan aril is the main satisfaction for customer, it is of great interest to determine the component and content of sugars in the aril but most previous reports showed only total solid contents in fruit. The objective of the present study is to increase our understanding of the sugar status and the possible changes due to sucrose hydrolysis by invertase activity that could affect the sweetness of 'Sri-Chompoo' rambutan aril at harvest and during a postharvest period before consumption. First, the glucose, fructose and sucrose contents and invertase activity in 'Sri-Chompoo' rambutan fruit harvested at different stages of maturity were determined. Additionally, sugar and invertase changes in 'Sri-

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Table 1. Sugar compositions and invertase activity at harvest of 'Sri-Chompoo' rambutan fruit of different stages of maturity

Harvest maturity	Sucrose (mg/gFW)	Glucose (mg/gFW)	Fructose (mg/gFW)	Invertase (unit/min/mg protein)
Green-yellow	643.88±21.18b	860.78±54.70b	632.10±12.86b	0.79±0.33b
Orange-yellow	631.24±32.98b	1013.48±55.48a	680.30±36.11b	1.57±0.14a
Orange-pink	105.36±12.18c	1121.26±48.15a	1043.98±59.34a	0.47±0.09b
Red-pink	1010.27±11.51a	872.34±54.78b	618.07±22.92b	0.38±0.05b

The same letter in a column indicates no statistically significant difference (ANOVA, $P < 0.05$)

Chompoo' rambutan fruit from different maturity stages during postharvest storage at 25°C for 6 days were also investigated.

Materials and Methods

Plant materials

Rambutan (*Nephelium lappaceum* cv. 'Sri-Chompoo') fruit were collected from a commercial orchard in Chanthaburi province, Thailand. The harvested fruit were at four maturity stages associated with the respective rind ripeness colors: green-yellow, orange-yellow, orange-pink, and red-pink (Figure 1). After collection, 'Sri-Chompoo' rambutan fruit were sorted out for size consistency without any defects, filled in rambutan cartons, and transported within two hours from harvest to the postharvest laboratory at King Mongkut's University of Technology North Bangkok, Bangkok, Thailand. Subsequently, the damage-free fruit of uniform color and size at each of the maturity stages were re-sorted and handled as samples. Sixty fruit of 'Sri-Chompoo' rambutan had been used per replication in each treatment and three replications were used for each treatment.

Condition for storage

'Sri-Chompoo' rambutan fruit were kept in a controlled room at 25±2°C and 60% RH for 6 days. During 6 days of storage, fruit were taken out for sugar assay at 2-day intervals. At each sampling period, 10 fruit per replicate were analyzed and there were three replicates.

Sugar components and invertase activity analysis

Glucose, fructose and sucrose contents were analyzed using high performance liquid chromatography (HPLC) as described in our previous study (Tongtao *et al*., 2014). The aril (20 g) from 10 sample fruit per replicate was mixed in a blender and extracted by squeezing out in two layers of thin cotton cloth. The extract was then centrifuged at 12,000 rpm for 20 min and later the supernatant was filtered through 0.45 µm filter membrane. The filtrate (20 µl) was injected into HPLC installed with Refractive Index detector and 25 mm x 4.6 mm APS-2 HYPERSIL column (Thermo Scientific) using deionized water:acetonitrile at 30:70 (v:v) as mobile phase at a flow rate at 0.6 ml/min (Conrad and Palmer, 1976). The assay was carried out in 3 replications.

Invertase extraction and activity assay in triplicates were also performed according to our prior study (Tongtao *et al*., 2014) based on the methods of Kubo *et al.* (2001) and Lee and Sturm (1996). The reaction mixture consisted of 0.3 ml of 100 mM sucrose, 0.6 ml of 100 mM sodium acetate buffer at pH 4.5 and 0.1 ml 'Sri-Chompoo' rambutan fruit extract which was replaced with sodium acetate buffer in the control reaction mixture. The reaction mixture was incubated for 1 h at 37°C before dinitrosalicylic acid (DNS) was added, and then placed in a hot water bath (100°C) for 10 min. Then the absorbance of the reaction mixture was measured at 540 nm using a spectrophotometer. A standard curve was constructed with different concentrations of glucose. The content of soluble protein in the 'Sri-Chompoo' rambutan fruit extract was determined following the method of Bradford (1976) using bovine serum albumin as standard. One unit of invertase activity was defined as one µmole of reducing sugar produced per min per mg protein.

Statistical analysis

The postharvest storage experiments were laid out in Completely Randomized Design (CRD). The data were subjected to analysis of variance (ANOVA) and treatment mean comparison by the least significant difference test (LSD) using the SAS

Green yellow Orange-yellow Orange-pink Red-pink

Figure 1. Different maturity stages of 'Sri-Chompoo' rambutan fruit based on color development (Kosiyachinda, 1988)

Statistical Software version 17.

Results and Discussion

The sweetness of fruit is an important fruit quality. Generally, sugar component has an actual influence on fruit sweetness since sugars vary in sweetness. The major sugars found in many commercial important fruits are glucose, fructose and sucrose. These sugars contribute to the fruit quality in term of both flavor: balance of sugars and acids, and an important part of total soluble solid content (Klann *et al*., 1993; Génard *et al.*, 2003). In the present study, differences in sugar components in the aril of 'Sichompoo' rambutan fruit at its four maturity stages were observed at harvest (Table 1). It was found that the sugar contents in the in the aril of 'Sichompoo' rambutan fruit was so high that it was impossible to measure using a hand-held refractometer. Nevertheless, attempts were made to quantify the sugar contents in the aril and some trends showing statistically significant differences (ANNOVA, $p<0.05$) among the different sugars at harvest were found. In green-yellow and orange-yellow fruit (the two earlier stages of maturity), the levels of glucose (860 and 1013 mg/gFW, respectively) were higher than those of fructose or sucrose. In the red-pink fruit (the most advanced stage of maturity), the level of sucrose (1010 mg/gFW) was higher than that of glucose or fructose coinciding with the fruit having the sweetest taste at this maturation stage. Among the four maturity stages, the orange-pink fruit had the lowest sucrose content (105 mg/gFW) but the highest glucose and fructose contents (1121 and 1043 mg/gFW).

It seemed that the sucrose content in 'Sri-Chompoo' rambutan fruit decreased throughout fruit maturation but only rose when ripe or at the most advanced maturation stage (red-pink fruit) (Table 1). In contrast, both the glucose and fructose contents in

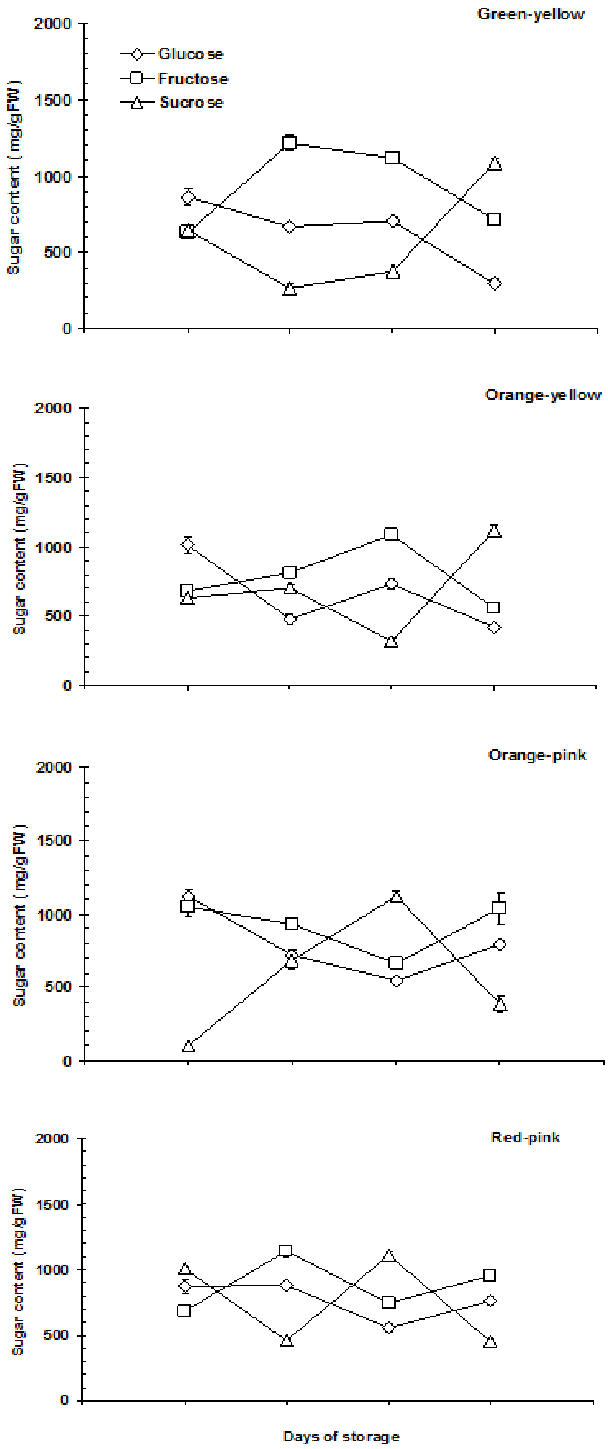

Figure 2. Changes in sugar components during storage at 25°C of 'Sri-Chompoo' rambutan fruit of different harvest maturities

'Sri-Chompoo' rambutan fruit exhibited the trend of similar levels in the first and last maturation stages, the green-yellow and red-pink fruit, respectively, which were lower than that in the third maturation stage, orange-pink fruit. These trends in the changes of different sugars during maturation of 'Sri-Chompoo' rambutan fruit suggested that particularly during the last two maturation stages, the orange-pink and red-pink fruit, there might be an inverse relationship between metabolism of sucrose and its two constituent monosaccharides, glucose and sucrose (Koch, 2004). Interestingly, the highest invertase activity was found in the orange-yellow 'Sri-Chompoo' rambutan fruit while the level of the enzyme activity was the same in the other maturation stages (Table 1), suggesting that the invertase activity studied might not have played a role in changes of the three sugars during maturation of 'Sri-Chompoo' rambutan fruit.

Changes in glucose, fructose and sucrose contents during fruit maturation usually depend on the fruit species and variety. Several fruits showed increasing glucose, fructose and sucrose levels with the more advanced stages of maturation (Shiow *et al*., 2009; Mahmood *et al*., 2012). The dominant sugar likewise differs with fruit species, such as sucrose in peach and fructose in plumcot (Bae *et al*., 2014). Moreover in peach, sucrose content increased with maturity while glucose and fructose contents decreased (Wang *et al*., 2008). Overall, the results obtained here and in a previous study (Tongtao *et al*., 2014) show that the characteristic sweet taste of freshly harvested rambutan fruit was due to the presence of the three sugars and that the more dominant sugar depends on the variety and stage of maturity. Sucrose was the dominant sugar in 'Rongrien' fruit regardless of the specific stages of maturity (Tongtao *et al*., 2014) but glucose was the dominant sugar in 'Sri-Chompoo fruit except at the most advanced stage of maturity (pink-red fruit).

Throughout 6 days of postharvest storage at 25°C of 'Sri-Chompoo' rambutan fruit from four different maturation stages, glucose content changed slightly (Figure 2). In the first 4 days of postharvest storage of the green-yellow and orange-yellow 'Sri-Chompoo' rambutan fruit, fructose content increased compared to that at harvest and peaked at 2 and 4 days, respectively, from the start of storage. At day 6 of storage, the fructose content in the fruit of these two maturation stages decreased back to the level before storage began. At day 4 of storage, the fructose content in the orange-pink fruit decreased before it rose back to a level similar to that at harvest. In the red-pink fruit, fructose content fluctuated during storage and was higher at day 6 than that at harvest. As far as changes in sucrose content are concerned, there was a noticeable increase in the green-yellow and orange-yellow fruit from day 4 of storage so that the level at day 6 was about doubled that at harvest. In contrast, the sucrose contents in the orange-pink and red-pink fruit peaked at day 4 and then declined to levels similar to the pre-storage levels.

In comparison with 'Rongrien' rambutan fruit in which sucrose content decreased during storage regardless of the maturity status of the fruit under study (Tongtao *et al*., 2014), 'Sri-Chompoo' rambutan fruit did not exhibit dramatic changes in glucose, fructose and sucrose contents after storage relative to those at harvest or before storage. These sugar components either decreased or increased during storage but the final levels after 6 days storage did not deviate much from the initial levels. Furthermore, in 'Sri-Chompoo' rambutan fruit, there was no marked shift in sugar components during storage. Earlier studies showed that sucrose content slightly increased during storage of rambutan fruit at 12°C (Paull and Chen, 1987). This was not observed in the present study that was carried out at a much higher storage temperature of 25°C. Yamaki (2010) further noted that there was no net increase in glucose content probably due to respiratory utilization while fructose content increased. Both results were observed in 'Sri-Chompoo' rambutan fruit but only in the green-yellow and orange-yellow fruit. In orange-pink and red-pink fruit, the increase in both glucose and fructose contents seemed to be reflective of their conversion from sucrose. Sucrose degradation with concomitant increase in glucose and fructose has been obtained in both climacteric and non-climacteric fruit such as peach (Borsani *et al*., 2009) and citrus (Echeverria and Burns, 1989).

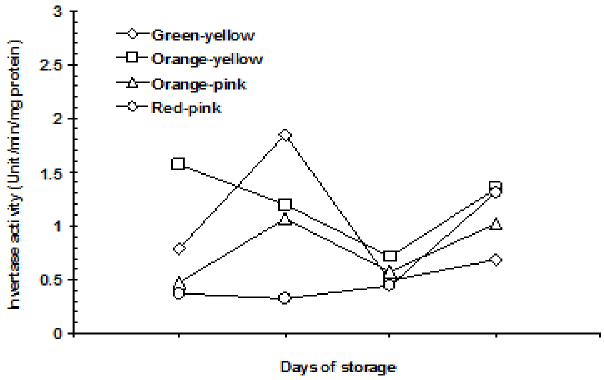

Figure 3. Activity of invertase during storage at 25°C of 'Sri-Chompoo' rambutan fruit of different harvest maturities

After two days of storage, invertase activity increased in green-yellow and orange-pink fruit (Figure 3). Then the enzyme activity decreased at day 4 before rising at day 6 to a level lower than the peak level but still higher than the pre-storage level. In orange-yellow and red-pink fruit, the changes in invertase activity during storage were diametric as in the orange-yellow fruit invertase activity decreased in the first 4 days of storage before rising to a higher level while it increased gradually throughout storage in the red-pink fruit. Comparing the trends in changes of sugars exhibited during storage of fruit of different maturity status in Figure 2, changes in the invertase activity did not correlate with the changes in sucrose, glucose and fructose contents. These results corroborate previous findings that invertase activity may not be directly associated with changes in sucrose, glucose and fructose contents (Sugiyama *et al*., 1992; Yamaki, 2010). In citrus, the increase in sucrose breakdown during storage was not accompanied by increases in enzymes of sucrose catabolism, in particular sucrose synthase and invertase (Echeverria and Burns, 1989). Similarly, changes in sucrose contents in peach during ripening were not associated with changes in invertase activity and expression of specific transcripts encoding invertase (Borsani *et al.*, 2009).

In conclusion, it was found in this study that the juice obtained from the 'Sri-Chompoo' rambutan fruit was essentially a sugar syrup with high sugar contents that pose difficulties in more precise determination of sugar contents. Within the postharvest period (up to six days) without refrigeration before consumption, it would be important to maintain or enhance sweetness and the nutritive value of 'Sri-Chompoo' rambutan fruit. Treatments that promote sucrose accumulation and/or minimize sucrose degradation would be useful.

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