Changes in fruit quality and volatile flavor compounds during on-tree maturation of longkong

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Abstract

Longkong (Aglaia dookkoo Griff.) is a non-climacteric fruit, juicy with typically aromatic smell and sweet but slightly sour taste. The optimal harvest period was started from 13 to 15 weeks after anthesis. The physical and chemical qualities of longkong at different stages of on-tree maturation were evaluated as follows: ripe (13 weeks after anthesis); medium ripe (14 weeks after anthesis); and full ripe (15 weeks after anthesis). It was found that longkong became light to bright yellow with the stages of maturation (p < 0.05). This can be indicated by the highest lightness ($L^* = 64.21$) and yellowness ($b^* = 34.37$) values shown in longkong at the full ripe stage. Moreover, the texture changed and softened with maturation (p < 0.05). The fruit size and weight increased with the stage of maturity but there was no significant difference (p < 0.05). The fruit were in the range of 3.17-3.30 cm in diameter and 19.94-21.36 g in weight. The moisture content was slightly increased with the stage of maturity (p < 0.05). During on-tree maturation, from the ripe to full ripe stages, acidity and organic acids (citric, maleic and malic acids) decreased while pH, TSS and total sugar content increased. The main sugars found in longkong were sucrose, glucose and fructose, respectively. From the ripe to medium ripe stages of longkong maturation, the sucrose content slowly increased from 9.88% to 11.44%. However, during the full ripe stage the sucrose content was 7.03% (p < 0.05). The glucose content significantly decreased from 1.98% to 1.46% during the ripe to medium ripe stages of the maturing longkong and then it increased again to 2.92% at the full ripe stage (p < 0.05). Similarly to fructose content, it also decreased from 2.76% to 2.20% during the ripe to medium ripe stages of the maturing longkong. After that it increased again to 3.39% at the full ripe stage (p < 0.05). The green aroma of 1-hexanol was the only volatile compound which was found in ripe longkong. From the ripe to full ripe stages, the longkong had more fruity and sweet characteristics. There were many terpenes and their derivatives. The key volatile flavor compounds were d-germacrene and 3-hydroxy-2-butanone. They had herbaceous and sweet attributes.

Introduction

Longkong (Aglaia dookkoo Griff.), is a well known and important commercial fruit grown in southern Thailand. It belongs to the Meliaceae family and has its origin in the South of Thailand, Indonesia, the Philippines and the Malau Islands (Paull, 2004). The demand for this fruit has increased tremendously. This is because this fruit is juicy, has a pleasant taste and contains a variety of nutrients. The Office of Agricultural Economics (2009) reported that farm values of longkong increased from 4,521 in the year 2000 to 5,092 million baht in the years 2007. However, the farm values of longkong have decreased to 3,000 million baht in the year 2013. It was probably due to the insurgency in the south of Thailand. Longkong is a non-climacteric tropical fruit, which comes in racemes. The fruit is round; roughly 3-4 cm across and soft. It has a smooth, thin and bright yellow skin. There are 15-20 fruits per raceme, and are almost seedless and free of latex. Longkong pulp is white, juicy with a typically aromatic smell and a sweet but slightly sour taste (Sabah, 2004; Paull, 2004). Normally, physical or chemical qualities such as color changes, acidity changes and total soluble solid (TSS) changes can be used as a harvesting index for longkong (Pantuvanid, 1985; Bumrugrak, 1992). The ripe stage (13-15 weeks after anthesis) is the optimal stage for harvesting. The fruit skin becomes a full bright yellow and its flesh becomes transparent and it has typical aromas and a sweet taste (Bumrugrak, 1992).

In addition, the National Bureau of Agricultural Commodity and Food Standards (2006) reported that the characteristics of longkong at the full ripe stage should be a full bright yellow skin color, soft
flesh and 15ºBrix of total soluble solid. The chemical constituents that affect the organoleptic profiles and characteristic of fruit flavors are the important quality standards for fresh fruit. Sweetness is related mainly to types and concentrations of sugars. Organic acids mainly contribute to the sourness. During maturation, sugars increase and organic acids decline. This makes the fruit much sweeter (Wills et al., 1998; Vendramini and Trugo, 2000; Sapii et al., 2000; Soares et al., 2007). Volatile flavor compounds mainly contribute to the fruity aroma and are synthesized during the maturation of the fruit. The unique flavor characteristic of longkong seems to directly influence the demand for this fruit. However, the formation of the flavor profile in longkong during on-tree maturation has not yet been investigated. Therefore, this study was undertaken to establish the changes in longkong quality, in physical or chemical qualities and volatile flavor compounds, during on-tree maturation.

Materials and Methods

Chemicals
The D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand).

Plant material
Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). It was harvested at different stages of on-tree maturation; at 13 (ripe), 14 (medium ripe) and 15 (full ripe) weeks after anthesis. Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis.

Fruit quality evaluation
Both physical (fruit color, fruit firmness, fruit size and fruit weight) and chemical (total soluble solid; TSS, sugars (reducing sugars, total sugars and type and concentration of sugars), titratable acidity; TA, pH, moisture content, type and concentration of organic acids and volatile flavor compounds) qualities were evaluated. For chemical quality analysis, the homogenate was prepared for analysis. It was prepared by flesh (de-seeded) blending at constant speed, using a blender for 3 min at 4°C. After that, it was filtered through the stainless steel sieve, yielding the homogenate. For physical quality analysis, the fruit was cut-off raceme forming of individual fruit. The 10 individual fruits were used to measure in each quality.

Fruit color
Color changes on two opposite sides of fruit skin were quantified in term of CIE Lightness (L*), Redness (a*) and Yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. CIE values were calculated in terms of Hue (h°) angle = arctangent b*/a* and Chroma (C* value) = (a*²+b*²)½ (Apai, 2010).

Fruit firmness
Fruit firmness on two opposite sides of fruit was measurement using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2) with penetrometric method. The results were expressed as gram force (Adapted from Sapii et al., 2000).

Fruit diameter
Fruit diameter was measured by using a SV-02 Stainless Steel Dial Vernier Calipers.

Fruit weight
Fruit weight was evaluated by weighing by using a Sartorius BP310S analytical balance.

Moisture content
Moisture content was determined gravimetrically by drying at 105°C (A.O.A.C., 2000).

TSS
TSS was determined using an Atago 1E (Japan) hand refractometer at 25°C and expressed as ºBrix.

Total and reducing sugars
Total sugar and reducing sugar content was quantified by titration with Fehling reagents according Lane and Eynon and Volumetric method (Adapted from Ong et al., 2006).

TA
TA was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthaleine as an indicator. The result was calculated as percentage of citric acid.
Types and concentrations of sugars

Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with Hypersil APS-2 column (Thermo Scientific, USA). Injection volume was 10 µl with an isocratic flow rate 1 ml/min and refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing retention time and peak area of the samples with known standards (Adapted from Ong et al., 2006).

Types and concentrations of organic acids

Organic acids (citric, maleic and malic acids) were determined by HPLC (Agilent 1100 series HPLC) with Hypersil ODS 4.0×250 mm, 5 µm reverse phase column (Thermo Scientific, USA). Injection volume was 10 µl with an isocratic flow rate 0.5 ml/min and diode array detector was set at 210 nm. Mobile phase was 0.05 disodium hydrogen phosphate, pH 2.5. Their concentrations were quantified by comparing retention time and peak area of the samples with known standards (Adapted from Chairgulprasert et al., 2006).

Volatile flavor compounds

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml dichloromethane. The mixture was extracted for 90 min and left equilibrates for 30 min at room temperature (~30°C). Solvent phase was collected and sediment was re-extracted twice. The combined solvent phase was dried on anhydrous Na$_2$SO$_4$, kept over-night at -20°C, cold- filtered and concentrated by purging of nitrogen gas to produce the concentrate. The concentrate was kept at -20°C prior to analysis. The volatile flavor compounds were identified by GC-MS. A chromatograph Hewlet-Packard 6890 (Palo Alto, CA, USA) was used with HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. MS was scanned in range m/z 40-350 at 1s intervals. The integration of peaks was done on HP chemstation software (Hewlett-Packard). The minimum peak area for detection level was 100,000 counts (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Statistical analysis

The experiment was performed by completely randomized design (CRD). Significant differences between means were estimated by Duncan’s new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 6.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

The physical qualities (fruit color, firmness, size and weight) of longkong at three different stages of on-tree maturation (13 weeks (ripe), 14 weeks (medium ripe) and 15 weeks (full ripe) were evaluated. The results were reported and discussed as follows.

Physical quality

Fruit color

The data on fruit skin color were recorded and quantified in terms of CIE (lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) values. A high $L^*$, $a^*$ and $b^*$ values refer to lighter, more reddish and more yellowish surface color of longkong. Fruit skin color was found to differ significantly with the different stages of on-tree maturation ($p < 0.05$). It was found that the fruit skin at the ripe stage was pale yellow with a tinge of green, and the $L^*$, $a^*$ and $b^*$ values were 43.16, 3.69 and 23.36, respectively (Figures 1A, 1B and 1C). The $L^*$ and $b^*$ values increased significantly with on-tree maturation ($p < 0.05$). The significant increase in $L^*$ value ($L^* = 58.43$) and $b^*$ value ($b^* = 23.95$) ($p < 0.05$) was found when the fruit reached to the medium ripe stage (Figures 1A and 1B). In the full ripe stage, longkong was lighter and more yellow indicated with sharpen increased in $L^*$ and $b^*$ values. The $L^*$ and $b^*$ values were 64.21 and 34.37, respectively (Figure 1A and 1B). Changes in fruit skin color from pale yellow with tinge of green to bright yellowness could be explained by degrade of chlorophyll (Sapii et al., 2000). However, the significant highest $a^*$ value of 5.21 was observed in full ripe longkong (Figure 1C). This color refers
to the brownish color that comes from enzymatic reaction. The enzymatic browning interacted with the substrate such as phenolic compounds and promoted fruit skin darkens (Sapii et al., 2000; Venkatachalam and Meenune, 2012). The results were in agreement with the study undertaken by Venkatachalam and Meenune (2012) who reported that a significant increase in a* value was observed during maturation stages. More appropriate measures of fruit skin color can be obtained from the calculation of the Hue angle (h°) and the Chroma (C* value). The fruit skin color of longkong at different stages of on-tree maturation in terms of h° and C* value are shown in Figure 2. Increases in the angle of h° and C* value at the mature stages of longkong were observed (p < 0.05). The angle of h° of the longkong at the ripe, medium ripe and full ripe stages was 80.74, 81.02 and 81.09. The angle of h° shown at nearly 90° indicates that the longkong skin becomes nearly yellow (h°, 90° = yellow). The results show a positive correlation with changes in CIE yellowness (b*) which increased with the maturation of the fruit (Figure 1B). The chroma C* value increased with the maturity of the longkong (p < 0.05). The C* values of ripe, medium ripe and full ripe longkong were 23.65, 23.98 and 34.82, respectively. The highest C* value was because of the high saturation from yellowness.

**Fruit firmness**

The loss of firmness in the fruit during fruit maturation is a major factor determining fruit quality. Changes in fruit firmness were associated with the stages of on-tree maturation. In ripe longkong, the fruit texture is firmer than that in the medium and full ripe stages. This can be indicated when using the highest force to penetrate into longkong at the ripe stage, which was 2,054.7 g. During the medium ripe to full ripe stages, fruit firmness decreased from 1,928.3 to 1,658.8 g (Figure 3). These phenomena could be explained by the degradation of the structural carbohydrates, cell wall disassembly and modifications to the pectin fraction by the role of pectin enzymes (White, 2002; Seymour and Gross, 1996).

**Fruit size and weight**

The size and weight of the longkong slightly increased from the ripe to full ripe stages (p < 0.05). The almost constant diameter of the longkong was observed in the ranges from 3.17 to 3.30 cm (ripe to full ripe stages) (Figure 4). This is in agreement with the standards of Tanyongmat the market in Narathiwat province. It prescribes that longkong grade A has a diameter of at least 3 cm or above. The fruit weight was found to be in the range from 19.94 g to 21.36 g (ripe to full ripe stages) (Figure 4). This is similar to the study of Bumrugrak (1992) which monitored the quality changes in longkong during its on-tree maturation (2 to 16 weeks after fruit set). It was found that at the ripe to full ripe stages (12
to 16 weeks after fruit set), the fruit weight and size remained almost constant. This is probably because a constant cell enlargement at these stages.

**Chemical quality**

Changes in chemical quality are important factors in determining the stage of fruit maturation. These are useful to determine the optimal harvesting time, such as the harvesting index. In this study, the chemical qualities (moisture content, pH, titrable acidity, total soluble solid and sugars) of longkong were evaluated during the edible stages of on-tree maturation (ripe, medium ripe and full ripe). The results were as follows.

**Moisture content**

The moisture content in the longkong varied significantly from the ripe to full-ripe stages during on-tree maturation (p < 0.05). The highest moisture content was observed at the full ripe stage. It was probably due to the movement of water from root throughout xylem into fruit cell during fruit maturation on-tree (Kays, 1991). In addition, an increase in the amount of the components such as sugars and acids in longkong during maturation might be affected on turgor pressure resulting in osmotic flow of water from area of low solute out into fruit cell (Campbell and Reece, 2008).

**pH and TA**

The pH increased significantly with on-tree maturation (p < 0.05). The pH values increased from 3.85 at the ripe stage to 4.27 at the full ripe stage (Table 1). The lowest acidity was found in longkong at the full ripe stage, which decreased significantly (p < 0.05) from 0.95% to 0.61%. This result was in agreement with the study undertaken by Sapii et al. (2000). They monitored the changes in the quality of longkong at different stages of ripeness (4, 7, 10 and 14 days after the fruit yellowed). It was found that the TA was significantly higher in the fruit harvested at the earlier stage. The acidity declined during ripening from 0.82% to 0.68%. The decreases of acidity during fruit maturation could be the main reason of pH increase, because acids were also used as a respiration substrate and produced the flavors (Wills et al., 1998).

**TSS and sugars**

There was a significant difference in the TSS, reducing and the total sugar contents in longkong at the different stages of on-tree maturation (p < 0.05). Table 1 shows that the TSS gradually increased significantly at the more mature stages of the ripening of the longkong (p < 0.05). The TSS increased significantly from 14.08 to 17.50°Brix. During maturation, reducing and the total sugar contents tended to increase (p < 0.05). The reducing sugar content increased from 4.76 to 5.38% during the ripe to full ripe stages. This finding indicated an increase in the total sugar content. The total sugar content increased from 12.78 to 15.59% during the ripe to full ripe stages (Table 1). The increase in the TSS could be attributed to the decomposition of the cell walls which caused the release of water-soluble components. In addition, increases in the TSS were probably due to the solubilization of neutral sugars from carbohydrate polymer residues (Beira-da-Costa et al., 2006). Increases in the TSS may be affected by the increase in the acids (Brady, 1987).

**Types and concentrations of sugars**

The main sugars were found in longkong were sucrose, fructose and glucose. The sucrose content increased slowly from 9.88 to 11.44%, during the ripe to medium ripe stages, and then gradually decreased to 7.03 at the full ripe stage (p < 0.05). The glucose content decreased from 1.98 to 1.46% from the ripe to medium ripe stages and increased to 2.92% in the full ripe stage. This was similar to the fructose content which decreased in the ripe to medium ripe stages, from 2.76 to 2.20%, and then increased to 3.39 at the full ripe stage (Figure 5). The decrease and increase in the glucose and fructose contents at the different stages of on-tree maturation could be explained by: the decrease was due to the rapid consumption of sugars in the respiration process to produce the energy required for the synthesis of the components. However, an increase in the glucose and

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Moisture content (%)</th>
<th>pH</th>
<th>TA (% as citric acid)</th>
<th>TSS (% Brix)</th>
<th>Reducing sugar (%)</th>
<th>Total sugar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ripe</td>
<td>81.64 ± 0.10</td>
<td>4.45 ± 0.01</td>
<td>0.77 ± 0.00</td>
<td>16.65 ± 0.06</td>
<td>5.05 ± 0.01</td>
<td>15.1 ± 0.01</td>
</tr>
<tr>
<td>Medium ripe</td>
<td>82.04 ± 0.14</td>
<td>4.27 ± 0.00</td>
<td>0.70 ± 0.00</td>
<td>17.50 ± 0.09</td>
<td>5.36 ± 0.00</td>
<td>15.9 ± 0.04</td>
</tr>
<tr>
<td>Full ripe</td>
<td>82.95 ± 0.12</td>
<td>3.85 ± 0.01</td>
<td>0.68 ± 0.00</td>
<td>15.59 ± 0.03</td>
<td>5.00 ± 0.00</td>
<td>15.3 ± 0.05</td>
</tr>
</tbody>
</table>

Note: Data are mean ± standard deviation (in four replicates). Means with different superscript letters indicate that there are significant differences between the variety (p < 0.05).
fructose content at the full ripe stage may be due to the higher inversion reaction of the sucrose to glucose and fructose molecules (Ong et al., 2006).

Types and concentrations of organic acids

Citic, maleic and malic acids were monitored by HPLC in accord with Chairgulprasert et al. (2006). A reduction in the concentration of organic acids was observed during on-tree maturation (p < 0.05). The concentration of citric acid in the ripe stage was 1.22% and this was reduced from 1.19 to 0.79% in the medium ripe to full ripe stages. The maleic acid also decreased with on-tree maturation (p < 0.05). It was 0.40% in the ripe stage and then decreased to 0.36 and 0.05% in the medium ripe and full ripe stages, respectively. This was similar to the decrease of malic acid which decreased from 0.20 to 0.13% from the ripe to the medium ripe stages. However, the malic acid was not detected at the full ripe stage. This was probably due to the quick consumption of malic acid by it being converted to pyruvate and being used as a substrate for the respiration process. In addition, the malic enzyme showed high activity in the late maturing fruit (Knee, 2002).

Volatiles flavor compounds

Fruit volatile flavor profile is directly influenced by fruit maturation. The formation of volatile flavor compounds in fruit is a dynamic process. Volatile flavor compounds are continuously synthesized and developed both qualitatively and quantitatively during fruit maturation. The volatile flavor compounds developed in longkong at the three different stages of on-tree maturation (ripe, medium ripe and full ripe) are shown in Table 3. The volatile flavor compounds included alcohols, terpenes, ketones and phenol. The terpenes and their derivatives were the major constituents. They promote the fruity, floral, flowery, herbaceous characteristics in longkong, which are normally found in a fruit aroma. In the ripe stage, the volatile flavor compounds which have fruity, floral, flowery, and herbaceous characteristics were not detected. Green aroma C6-alcohol (1-hexanol) was only the volatile flavor compound that was found in the ripe longkong. Thus it has a green aroma at the ripe stage. The longkong also showed some herbaceous and sweet characteristics by showing the lowest constituent levels of d-germacrene and 3-hydroxy-2-butane. When the fruit became more mature, more terpenes and their derivatives were detected. As a consequence, more fruity and sweet characteristics were found.

Conclusion

During on-tree maturation, the physical, chemical and volatile flavor compounds in longkong are still changing. This information could indicate suitable harvesting times and the edibility of the fruit. The fruit skin became lighter and yellower with a softer flesh at the full ripe stage. Furthermore, the fruit size and weight was almost constant during the ripe to full ripe stages. The TSS and sugars increased while the acidity decreased with on-tree maturation, and this makes the fully ripe longkong more palatable. Organic acids such as citric, maleic and malic acids declined during on-tree maturation. The volatile flavor compounds were synthesized during on-tree maturation. The green aroma of 1-hexanol was the only volatile compound found in ripe longkong. The d-germacrene and 3-hydroxy-2-butane were the major compounds in the medium and fully ripe longkong. These compounds were the components that gave the longkong more fruity and sweet characteristics.

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