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Original Article

Physicochemical quality and antioxidant changes in 'Leb Mue Nang' banana fruit during ripening



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ABSTRACT

The physicochemical and antioxidant changes of 'Kluai Leb Mue Nang' banana fruit (*Musa* AA group) were investigated during ripening. The visual appearance, peel and pulp color, firmness, total soluble solids concentration (TSS), total acidity (TA) and bioactive compounds of the fruit at three stages of ripening (mature green, ripe and overripe) were monitored. Changes in both the peel and pulp color, texture, TSS and TA contents during banana ripening were similar to those of other banana fruits. Interestingly, the highest total antioxidants capacity and total phenols concentration were found in the ripe banana fruit. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity remained constant and the highest total flavonoids concentration was found in the mature green fruit.

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Introduction

Banana (*Musaceae*) is an economically important climacteric fruit for local and export markets worldwide (Abdullah and Pantastico, 1990). Banana fruit is considered to be a good source of nutrients including bioactive phenols, antioxidants and potassium (Williams, 1995). In Thailand, banana is a commercial fruit following mango, mangosteen, durian and longan in importance and there are many commercial cultivars such as 'Kluai Hom Thong' (*Musa* AAA group), 'Kluai Khai' (*Musa* AA group), 'Kluai Namwa' (*Musa* ABB group) and 'Kluai Leb Mue Nang' (*Musa* AA group) (Valmayor et al., 1999). As a climacteric fruit, the ripening process of banana fruit is related to various aspects, including a burst in ethylene production and an increase in respiration (Siriboon and Banluisilp, 2004), coincident with the onset of fruit softening, starch degradation, sugar accumulation, and changes in the organic acids content (Abdullah et al., 1985). The production of volatile compounds and bioactive compounds beneficial to health, including total phenols, total flavonoids and antioxidant activity, also increase (Ummarat et al., 2011). Li et al. (2011) reported that a loss of pulp firmness was related to an increase in reducing sugar and disease incidence and a reduction in the starch content in 'Baxi' (*Musa* AAA group) banana fruit during ripening. Previous research

has reported that the peak of ethylene production, subsequent fruit softening, increases in the moisture content, total acidity and total soluble solids concentration and the occurrence of fruit drop and senescence spot, were detected during ripening of Thai banana fruit such as 'Kluai Namwa' (Siriboon and Banluisilp, 2004) and 'Kluai Hom Thong' (Imsabai et al., 2006). Moreover, Ummarat et al. (2011) reported increases in certain bioactive compounds (such as ascorbic acid, free phenolic compounds and free flavonoids) in 'Kluai Hom Thong' banana fruit during ripening.

Most of these previous studies with commercial Thai banana fruits have investigated physiological changes in the cultivars 'Kluai Hom Thong' (Nguyen et al., 2003), 'Kluai Khai' (Nguyen et al., 2003, 2004) or 'Kluai Namwa' (Siriboon and Banluisilp, 2004; Imsabai et al., 2006), during storage and ripening. However, a study of the physicochemical changes that occur in 'Kluai Leb Mue Nang' banana fruit during ripening has not previously been conducted. 'Kluai Leb Mue Nang' is from the south of Thailand and it has recently become distributed widely across the country (Srangsam and Kanchanapoom, 2007). As a small finger banana with a shape like a lady's finger, and having firm texture, a sweet taste, yellow flesh and a desirable odor, the demand for the fruit in the market has recently increased and the price per bunch is higher than for 'Kluai Namwa' banana. Thus, the current study investigated physicochemical changes including physical quality attributes, certain chemical quality attributes and bioactive compounds in 'Kluai Leb Mue Nang' banana fruit during ripening.

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Materials and methods

Plant materials

'Kluai Leb Mue Nang' (*Musa AA* group) banana fruit samples at the full mature green stage (2 mth after full bloom), the ripe stage (left for 4 d at room temperature (27 ± 2 °C) after harvest) and the overripe stage (left for 8 d at room temperature after harvest) were obtained from a local banana orchard in Prateaw district, Chomphon province, Thailand. Ten hands of bananas at each stage were selected for uniformity of skin color and being free from any defects, including physical damage and diseases. The fruit hands were cleaned by dipping in circulated tap-water and dried at room temperature before physicochemical quality attributes (peel and pulp color, firmness, total soluble solids concentration (TSS), total acidity content (TA) content and selected bioactive compounds) were investigated.

Color measurement

Peel and pulp color of the fruit were measured in the middle section of each fruit of 10 randomly selected fruit using a HunterLab photometer (MiniScan@ XE Plus; Hunter Associates Laboratory Inc.; Reston, VA, USA). The brightness (L^*), greenness ($-a^*$), redness ($+a^*$), and yellowness (b^*) values were recorded and the whiteness index (WI) of the pulp was calculated according Equation (1) (Bolin and Huxsoll, 1991):

$$WI = 100 - \left([(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5} \right) \quad (1)$$

Firmness measurement

Ten fingers from each hand of bananas were randomly sampled for firmness measurement. Each fruit was peeled and the measurement was taken in the middle part of each fruit using a Texture Analyzer (TA Plus; Lloyd Limited; Fareham, UK) with a 6 mm cylindrical probe. The result was expressed as the maximum force measured in newtons.

Total soluble solids and total acidity measurements

Ten fruits per hand were selected for these measurements. The TSS concentration of the fruit pulp was measured using a hand-held refractometer (MNL-1125; ATAGO Co. Ltd.; Tokyo, Japan). The data were expressed as °Brix. The TA of the fruit pulp was determined using the standard method of Association of Official Analytical Chemists (1990). A 10 g sample of the banana pulp was homogenized with 20 mL of distilled water and filtered through a cloth sheet. A 5 mL aliquot of the extract was titrated with 0.1 N NaOH using 1% (w/v) phenolphthalein as the indicator. The volume of 0.1 N NaOH used in the titration was recorded. Total acidity was defined as the percentage of titratable acidity (% malic acid).

Total antioxidant capacity and 2,2-diphenyl-2-picrylhydrazyl scavenging activity measurements

Ten fruits per hand at each stage of maturity were selected. The fruit were peeled and then blended together. A 5 g sample of the banana pulp was homogenized with 50 mL 80% methanol and stirred at room temperature for 15 min before filtration using a cloth sheet. The filtrate was collected and centrifuged at $10,000 \times g$ for 15 min. The supernatant was used to assay bioactive

compounds. The total antioxidant capacity of the fruit pulp was assayed using the ferric reducing antioxidant potential (FRAP) method (Benzie and Strain, 1996). The FRAP reagent was a mixture of acetate buffer pH 3.0, 10 mM 2,4,6-tripyridyl-1,3,5-triazine and 20 mM ferric chloride hexahydrate (10:1:1). The supernatant was diluted with distilled water in a ratio of 1:1 (volume per volume; v/v). The reaction was started when 0.3 mL of the diluted supernatant was added into 3 mL of the FRAP solution and then incubated at room temperature for at least 30 min before measuring absorbance at 630 nm. The total antioxidant capacity was expressed as micromoles of Trolox equivalents per gram fresh weight ($\mu\text{mol TE/g FW}$). The 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was determined following Brand-Williams et al. (1995) with slight modification. The reaction was started when 5 mL of diluted supernatant was mixed with 0.5 mL of 1 mM DPPH in methanol. The absorbance at 517 nm was immediately recorded at 0 min and the mixture was then held in the dark for 5 min. The capability to scavenge the DPPH free radical was calculated using Equation (2):

$$\text{DPPH free radical scavenging activity(\%)} = \left[\frac{(A_0 - A_{10})}{A_0} \right] \times 100 \quad (2)$$

where A_0 is the absorbance of the sample at 0 min and A_{10} is the absorbance of the sample at 10 min.

Total phenols and total flavonoids measurements

The same supernatant from the total antioxidant assay was used to determine the total phenols (TP) and total flavonoids (TF) contents in the banana pulp. The TP content was determined using the method described by Slinkard and Singleton (1977). The reaction was started when 1 mL of the supernatant was added into 1 mL of 50% (v/v) Folin–Ciocalteu reagent solution and 2 mL of saturated Na_2CO_3 solution. The mixture was incubated at room temperature for at least 30 min before measuring the absorbance at 750 nm. The data were expressed in terms of micrograms of gallic acid per gram fresh weight ($\mu\text{g GA/g FW}$). The TF content was assayed using a method described by Zhishen et al. (1999). The reaction began when 0.25 mL of the supernatant was added into a mixture of 1.25 mL of distilled water and 75 μL of 0.5% NaNO_2 and then left for 6 min at room temperature. A 150 μL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added into the mixture and then allowed to stand for 5 min. A 0.5 mL aliquot of 1 M NaOH was then added and absorbance at 510 nm was recorded. The data were expressed as micrograms of catechin per gram fresh weight ($\mu\text{g catechin/g FW}$).

Statistical analysis

The data were shown as the mean of ten replications \pm SD. Statistical analysis was carried out using ANOVA and the means were compared using the least significant difference test at the significance level $p < 0.05$ using the SPSS software program (SPSS Inc.; Chicago, IL, USA).

Results and discussion

Visual appearance and fruit color

The visual appearance of the fruit was related to the peel color which was green in the mature green fingers while the ripe fingers were yellow with a green stalk and the overripe fingers had a few senescent spots and a black tip and stalk (Fig. 1). The senescent spots that generally appeared on the banana fingers were caused by a typical physiological disorder that occurs in the latter phase of

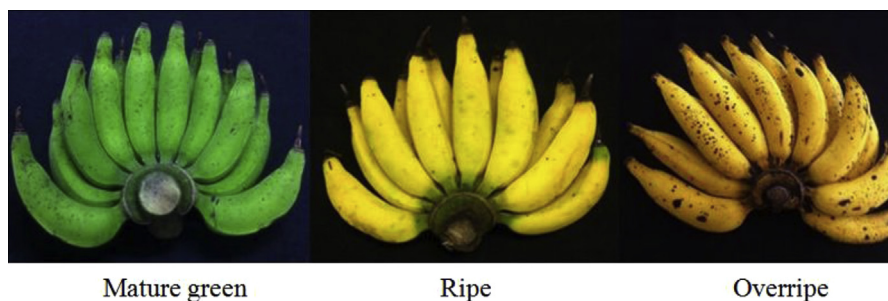


Fig. 1. Appearance of mature green, ripe and overripe 'Kluai Leb Mue Nang' banana fruit.

fruit ripening and these spots gradually increase in size and number as the fruit advance during the ripening process (Ketsa, 2000).

The peel and pulp color of 'Kluai Leb Mue Nang' banana fruit was assessed in terms of lightness, redness to greenness, yellowness and WI (Table 1). The change in peel color was similar to that in other banana fruits such as *Musa cavendishii* (Abdullah and Pantastico, 1990), *Musa acuminata* (Bugaud et al., 2009) and *Musa sapientum* (Mustaffa et al., 1998). The L^* value of the banana peel increased markedly from the mature green stage to the ripe stage and then decreased at the overripe stage, which was similar to the change in the L^* value of *M. cavendishii* cv. 'Montel' fruit and *M. sapientum* cv. 'Embun' fruit during ripening where the continuous increase in the L^* value was concomitant with a reduction of greenness and an increase in yellowness (Abdullah et al., 1985; Abdullah and Pantastico, 1990; Mustaffa et al., 1998). The a^* value of the fruit peel changed from -19.06 (green) with mature green fruit to 3.11 and 9.09 (red) of ripe and overripe fruit, respectively. The b^* value of the fruit peel increased markedly from the mature green stage to the ripe stage and then remained constant (Table 1). The L^* and WI values of the pulp color both decreased as the fruit progressively ripened. In contrast, the b^* values of both the mature green and ripe stages were similar but b^* markedly increased at the overripe stage. This increase in the b^* value during ripening has been reported previously with 'Grande Naine' banana fruit (Bugaud et al., 2009).

Firmness, total soluble solids and total acidity

Fruit softening and the TSS concentration and TA content are key factors indicating fruit maturity and quality (Abdullah et al., 1985). Typical changes in banana fruit firmness and TSS concentration are shown in Fig. 2A and B, respectively. The firmness of the fruit decreased from 40 N at the mature green stage to less than 10 N at the ripe and overripe stages. There was no significant difference in firmness between ripe and overripe fruit. Similarly, a rapid decrease in firmness with mature green 'Baxi' banana (*Musa* spp. AAA group) during ripening has been reported (Li et al., 2006). The softening of

banana fruit is associated with a degradation of cell wall compounds, a reduction of starch and an increase in the sugar content (Srivastava and Dwivedi, 2000; Li et al., 2006). The current study also found that the TSS concentration in 'Kluai Leb Mue Nang' banana fruit increased significantly from 3.6 °Brix at the mature green stage to 27.8 and 29.4 °Brix at the ripe and overripe stages, respectively (Fig. 2B). Li et al. (2006) reported that the increase in the total sugar content of 'Baxi' banana (*Musa* spp. AAA Group) fruit was positively related to the increase in sucrose phosphate synthase, sucrose synthase and invertase activities during ripening. The current study found that the TA contents of the ripe and overripe banana fruits were significantly higher than that of mature green fruit with the highest TA content being detected in the ripe fruit (Fig. 2C). Similarly, an increase in the TA content of 'Kluai Namwa' banana fruit during ripening has been reported where the increase coincided with the peak of ethylene production following which it progressively declined (Siriboon and Banluisilp, 2004). Wills et al. (1982) reported that the TA content of *Musa* sp. AAA group 'William' banana fruit at the mature green stage was markedly lower than that of ripe fruit and that this was associated with a reduction in the pH. However, in contrast, in 'Zhonggang' (*Musa* AAA group) banana fruit (Jiang et al., 2004) and 'Gross Michel' (*Musa acuminata*, AAA group) banana fruit (Thaiphanit and Anprung, 2010), the TA content decreased continuously during ripening.

Total antioxidant capacity and 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity

The total antioxidant capacity and DPPH radical scavenging activity of 'Kluai Leb Mue Nang' banana fruit during ripening are shown in Fig. 3. The total antioxidant capacity of the ripe fruit was significantly higher than that of mature green and overripe fruit (Fig. 3A) whilst the opposite was found with the DPPH radical scavenging activity (Fig. 3B). The amounts of the total antioxidant capacity and the DPPH radical scavenging activity of mature green fruit were similar to those of overripe fruit. The changes in the DPPH radical scavenging activity in this study were similar to those reported for 'Gross Michel' banana fruit (Thaiphanit and Anprung, 2010). Macheix et al. (1990) reported that the antioxidant capacity of banana pulp may be due to the flavonoids and total phenolic contents. Someya et al. (2002) identified gallic acid (a phenol) in banana fruit and indicated that the antioxidant capacity of the fruit may be attributed to the gallic acid content.

Total phenols and total flavonoids content

Following the findings of Macheix et al. (1990), changes in the TP and TF contents of 'Kluai Leb Mue Nang' banana fruit at the three stages of ripening were investigated. The TP content of the mature green fruit was significantly lower than that of both ripe and overripe fruit (Fig. 4A) but no difference in the TP content between

Table 1

Mean \pm SD values for peel and pulp color of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit.

Color†	Mature green	Ripe	Overripe
Peel			
L^*	47.85 ± 3.37^{ct}	72.65 ± 2.66^a	65.62 ± 4.55^b
a^*	-19.06 ± 0.77^c	3.11 ± 0.72^b	34.50 ± 1.74^a
b^*	9.09 ± 1.35^a	58.20 ± 3.25^a	59.47 ± 4.29^a
Pulp			
L^*	82.72 ± 2.42^a	78.81 ± 7.43^{ab}	75.45 ± 3.17^b
WI	68.58 ± 2.84^a	65.44 ± 5.68^a	58.11 ± 3.83^b
b^*	26.06 ± 0.90^b	26.75 ± 2.52^b	33.76 ± 4.09^a

† L^* = brightness, $-a^*$ = greenness, $+a^*$ = redness, b^* = yellowness, WI = whiteness index.

‡ Values followed by the same lowercase letter within a row are not significantly different at $p \leq 0.05$ level.

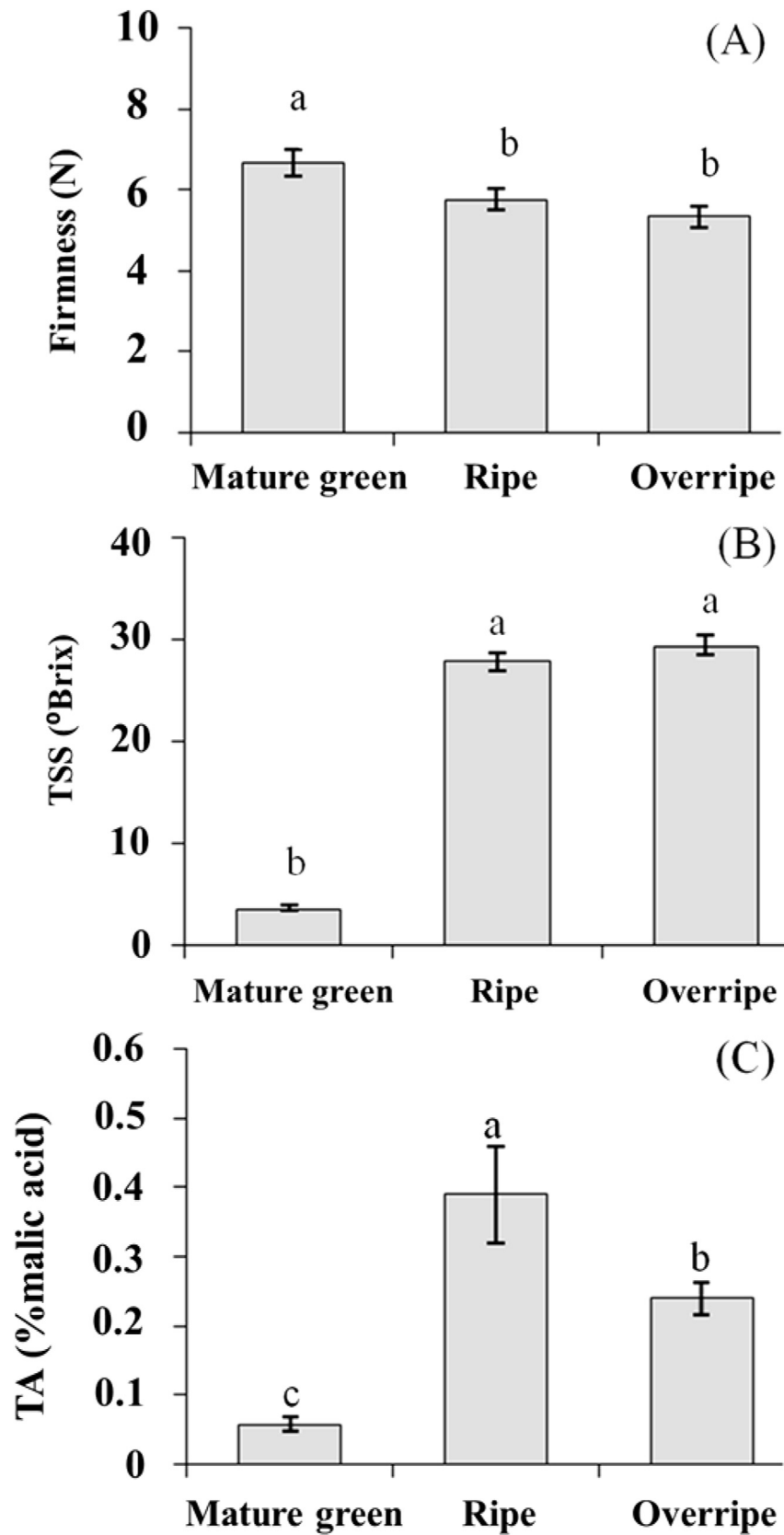


Fig. 2. Firmness (A), total soluble solids (TSS) (B) and total acidity (TA) (C) of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean ($n = 10$) \pm SD. Bars in the same subfigure with the same lowercase letter are not significantly different ($p \leq 0.05$).

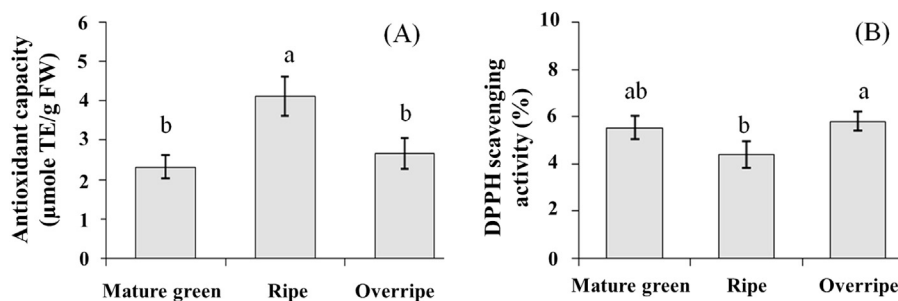


Fig. 3. Total antioxidant capacity (A) and DPPH scavenging activity (B) of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean ($n = 10$) \pm SD. Bars in the same subfigure with the same lowercase letter are not significantly different ($p \leq 0.05$). TE = Trolox equivalents; FW = fresh weight; DPPH = 2,2-diphenyl-1-picrylhydrazyl.

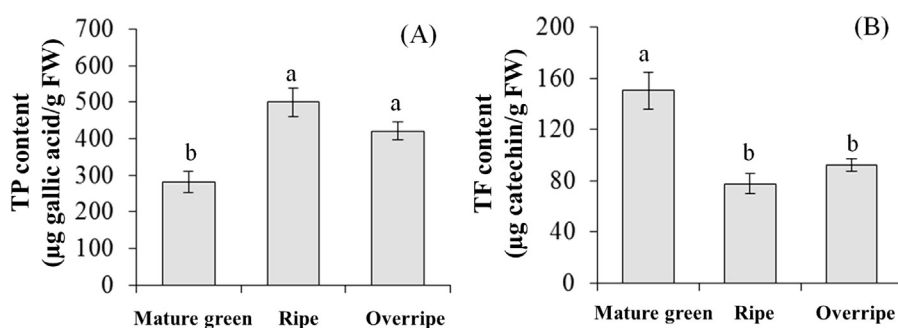


Fig. 4. Total phenols (TP) (A) and total flavonoids (TF) (B) content of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean ($n = 10$) \pm SD. Bars in the same subfigure with the same lowercase letter are not significantly different ($p \leq 0.05$). TP = total phenols; GA = gallic acid; FW = fresh weight; TF = total flavonoids.

the ripe and overripe fruit was found. The TF content of the mature green fruit was significantly higher than that of both the ripe and overripe fruit and no significant difference in the TF content between ripe and overripe fruit was found (Fig. 4B). The change in the TP content in this banana fruit was similar to the change in the total antioxidant capacity (Fig. 3A) as determined using the FRAP assay. This appeared to confirm that the antioxidant capacity in banana fruit can be attributed to the total phenolic compounds, of which gallic acid is the major component (Someya et al., 2002). Similarly, Bennett et al. (2010) showed that banana pulp was an excellent source of bioactive phenolics. Interestingly, the current study found a high content of TF and a low content of TP in the mature green fruit. This result was in contrast to Macheix et al. (1990) who reported high total phenols and tannin contents at the mature stage which then declined as ripening advanced. Bennett et al. (2010) reported a slight increase in the TP content in the pulp of 'Nanicão' banana fruit stored at 20 °C for 18 d whilst the TP content in fruits of other cultivars such as 'Figo', 'Terra', 'Mysore' and 'Pacovan' decreased during storage. These varying results showed that the changes in the TP and TF contents of banana fruit pulp during ripening are dependent on the cultivar.

During the ripening process, the greenness of the peel decreased, the yellowness increased markedly from the mature green stage to the ripe stage and then remained constant, and the redness increased continuously. With pulp color, the whiteness and lightness decreased as the ripening advanced while the pulp yellowness remained constant between the mature green stage and the ripe stage and then markedly increased at the overripe stage. The firmness of the fruit decreased rapidly when the fruit ripened and then remained constant. The lowest TSS and TA contents were detected in mature green fruit. The TSS content increased continuously while the TA content declined at the overripe stage. With the bioactive compounds, the highest total antioxidant capacity

and the lowest DPPH radical scavenging activity were detected in the ripe fruit. There was no significant difference in both antioxidant capacities between the mature green and overripe fruit. The change in the TP content was similar to the change in the total antioxidant capacity. There was no significant difference in the TF content between the ripe and the overripe fruit.

Conflict of interest

None.

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