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Changes in antioxidant properties and chemical composition during ripening in banana variety 'Hom Thong' (AAA group) and 'Khai' (AA group)

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Abstract

Massive changes in chemical composition and antioxidant properties usually occur in fruits during maturation and ripening. In this study, changes in ascorbic acid content, phenolics content, antioxidant activity (DPPH and FRAP), soluble solid content (TSS) and sugar content during ripening of two banana cultivars at 25 ± 2°C were investigated. Fully mature green banana fruit (cv. Hom Thong, AAA group and cv. Khai, AA group) were used for the experiment. The total ascorbic acid content of both cultivars was increased slightly with ripening and reduced with the starting of senescence. Total phenolic content in 'Khai' banana decreased in the first two days and then significantly increased until day 6. However, the total phenolic content was reduced at the fully ripened stage in both banana cultivars. Total antioxidant activities (DPPH and FRAP) in both cultivars, increased with ripening and decreased rapidly with senescence. 'Khai' banana had higher amounts of antioxidant components and total antioxidant activity than 'Hom Thong' banana. There was no correlation between antioxidant activities and ascorbic acid content or phenolics content. TSS and total sugar contents increased rapidly in both cultivars until fully ripen stage and then declined thereafter. TSS and sugar contents were highly correlated.

Introduction

Fruit consumption is very important for reducing the risk of many diseases including chronic diseases (Beecher, 1999), heart disease (Gordon, 1996), inflammation, cardiovascular diseases and cancers (Bae *et al.*, 2008). These diseases are related to elevated levels of oxidative stress in the body due to damage of lipids, proteins and nucleic acids molecules (Leong and Shui, 2002). Antioxidant compounds are substances that may protect from oxidative damages by reactive oxygen species thereby minimizing the incidence of the above diseases. Antioxidant compounds suppress the formation of reactive oxygen species and free radical reactions in the body.

Banana is a tropical plant which is able to protect from the oxidative stress caused by high intensity of sunlight and elevated temperature by raising its antioxidant ability (Kanazawa and Sakakibara, 2000). Banana fruits contain various antioxidant compounds in both pulp and peel tissues, such as vitamin C, vitamin E, β-carotene and flavonoids. Macheix *et al.* (1990) also reported that banana pulp contains high amount of total phenolics and tannin. Some enzymes in banana pulp are involved in elevating its antioxidant capacity (Someya *et al.*, 2002).

The antioxidant ability of many fruits including banana depends on the cultivar, maturity and ripening stage (Shewfelt, 1990; Mozafar, 1994; Lee and Kader, 2000). The ripening process of fleshy fruits affects the changes of chemical and nutritional contents (Sisler *et al.*, 2003). Change of antioxidant capacity and chemical compositions of banana has been reported during ripening (Thaipanit and Anprung, 2010). Suleiman *et al.* (2011) reported that levels of antioxidants compound varied with cultivar and maturity in Malaysian bananas. Highest antioxidant levels were exhibited in cv. 'Mas'. Wall (2006) found that Dwarf Brazilian banana has almost three times more vitamin C (12.7 mg/100 g fresh weight) than 'Williams' banana (4.5 mg/100 g) and Dwarf Brazilian banana had also higher β-carotene content than Williams banana. The present study aimed to compare the chemical composition and antioxidant activities and determine the correlation between antioxidant compounds and free radical scavenging ability in pulp extracts of 'Hom Thong' and 'Khai' banana during ripening at 25 ± 2°C.

Materials and Methods

Fruit sampling

Mature, green 'Hom Thong' (*Musa* sp., AAA group) and 'Khai' (*Musa* sp., AA group) bananas

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were obtained from a market in Bangkok. Fruit samples were selected for uniformity in green color and size and freedom from defects. Selected fruit hands were cut in clusters with 3-4 fruit fingers each, washed 0.5% $MgSO_4$ solution to remove the latex from the cut surfaces (Nguyen *et al*., 2004), rinsed in tap water, and then dipped in 100 ppm sodium hypochlorite solution as a disinfection treatment. The samples were then air dried before storage.

Determination of total ascorbic acid content

Total ascorbic acid content was measured following the DNPH method (Kapur *et al.*, 2012). 5 grams of fresh sample was extracted with 20 mL of 5% Meta phosphoric acid using a homogenizer in an ice bath. The extract was filtered using whatman # 01 filter paper and a clear sample was taken. 0.2 mL of 0.02% indophenol solution was added with 0.4 mL of sample extract and incubated 2-3 minutes until it became a stable reddish-pink color. After that, 0.4 mL of 2% thiourea and 0.2 mL of 2% DNP solution were added and then incubated 3 hours at 37ºC in a hot water bath. Then, 1 mL of 85% sulfuric acid was added and then incubated at room temperature for 30 minutes. The absorbance was determined at 540 nm using a UV visible spectrophotometer (Shimadzu, UV-1601, and Japan). A standard curve was prepared using standard ascorbic acid with concentrations of 20,40,60,80 and 100 mg L^{-1}.

Determination of total phenolics content

Total phenolics content was measured using the Folin- Ciocalteau method (Singleton, 1999). Three grams of fresh pulp sample was extracted with 25 mL of 80% ethanol using a homogenizer in an ice bath. The homogenate was centrifuged at 11,000 rpm and 4ºC for 25 minutes and a clear sample was taken. 2.5 mL of distilled water and 0.15 mL of 0.25 N fresh Folin-Ciocalteau solution were added with 0.1 mL of sample extract. The sample was incubated for 3 minutes and 300 µL of 1N sodium carbonate solution was added. Thereafter, samples were incubated in dark conditions at 23ºC for 2 hours. The absorbance of the resulting blue colored solution was determined at 760 nm using a UV visible spectrophotometer (Shimadzu, UV-1601 and Japan). A standard curve was prepared using the same procedure with a series of garlic acid.

Determination of total antioxidant activity

Total antioxidant activity of banana pulp was measured by the FRAP method (Benzie and Strain, 1996) and DPPH method (Krings and Berger, 2001). In the FRAP method, 3 grams of fresh sample was extracted with 25 mL of 80% methanol using a homogenizer in an ice bath. The homogenate was centrifuged at 15,000 rpm and 4ºC for 20 minutes. 2,850 µL of FRAP working solution was added with 150 µL of supernatant and incubated for 30 minutes in dark conditions. FRAP working solution contained 300 mM of acetate buffer (3.6 pH), 10 mM of TPTZ, and 20 mM of $Fecl_3.6H_2O$ with ratio of 10:1:1. The fresh FRAP working solution was warmed at 37°C for 4 minutes before using. The absorbance of the resulting blue colored solution with Fe^{2+} was determined at 593 nm using a UV visible spectrophotometer (Shimadzu, UV-1601 and Japan).

In the DPPH method, 3 grams of fresh samples were extracted with 25 mL of 80% ethanol using a homogenizer in an ice bath. The homogenate was centrifuged at 15,000 rpm and 4ºC for 20 minutes. 2,850 µL of DPPH fresh working solution was added with 150 µL of supernatant and incubated 30 minutes in dark conditions. The absorbance of the solution was determined at 515 nm using a UV visible spectrophotometer (Shimadzu, UV-1601 and Japan).

Determination of total sugar content

Total sugar content in the samples was measured following the sulfuric method (Dubois *et al.*, 1956). 1 gram of fresh pulp sample was extracted with 10 mL of 80% ethanol using a homogenizer in an ice bath. The homogenate was filtered using whatman #04 filter paper. 1 mL of 5% fresh phenol solution was added with 1 mL of sample extract and 5 mL of 98% sulfuric acid was added thereafter. The absorbance of the resulting brownish-yellow colored solution was determined at 490 nm using a UV visible spectrophotometer (Shimadzu, UV-1601 and Japan). A standard curve was prepared using the same procedure with a series of D - glucose, at 10, 20, 40, 60 and 80 µg mL^{-1}.

Determination of total soluble solids

Total soluble solids content was measured on a fresh juice sample from whole banana fruit using a digital Refractometer (Brix 0 - 32%; STAGO, Japan).

Data analysis

The experimental data were subjected to analysis of variance and mean comparison using the SAS computer software. Pearson's correlation test was used to determine the correlation between the antioxidant activities of two independent tests (FRAP and DPPH) and phenolic or ascorbic acid content. Same procedure was used to determine the correlation between TSS and total sugar contents.

The p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

Ascorbic acid content

Ascorbic acid content of 'Khai' banana was about two times higher than that of 'Hom Thong' banana (Figure 1). During storage and ripening at 25°C, ascorbic acid content generally increased until full ripe stage at 8 days of storage. Thereafter when the fruit became over-ripe and senescent, ascorbic acid content decreased.

The results support previous findings of several workers. Wall (2006) reported that AA bananas generally contain higher ascorbic acid or Vitamin C than AAA bananas. Wenkam (1990) earlier found lower vitamin C content of Cavendish bananas (AAA) due to their higher moisture content than that of AAB and AA cultivars. In 'Mas' bananas (AA group), Osman *et al.* (1998) obtained increasing ascorbic acid content with ripening, with highest level at the fully ripened stage, as similarly obtained in the present study. The decrease in ascorbic acid content when the fruit became over-ripe has been noted also in 'Williams' banana (Wills, 1983). The increase in ascorbic acid content with ripening has been attributed to the increase in lipid peroxidation considering that fruit ripening which is an oxidative phenomenon that requires turnover of active oxygen species (Jimenez *et al*., 2002). Under this condition, antioxidant compounds including ascorbic acid usually increased.

Phenolics content

'Khai' banana had higher phenolic content than 'Hom Thong' banana during whole storage period (Figure 2). With ripening, phenolic content increased in 'Khai' banana and was higher after 6-8 days when fruit ripened compared to that of green fruits. In Hom thong banana, no such increase in phenolic content was noted during ripening; instead, phenolic contents decreased after 2 days at the onset of ripening and remained almost at the same level the following 6 days when ripening advanced before decreasing again on the 10th day when the fruit became over-ripe. Banana cultivar variations in phenolic content have been observed in earlier studies (Award *et al*., 2001; Kondo *et al.*, 2005; Priya Darsini *et al.*, 2012). In general, phenolic content, particularly tannins which are responsible for astringency taste of unripe fruits, decreased with ripening mainly due to polymerization rendering them insoluble and undetectable to taste. In the present study, the decrease in phenolic content

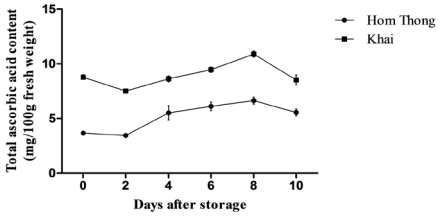

Figure 1. Change of total ascorbic acid content (mg/100 g fresh weight) of banana (●) cv. Hom Thong (■) cv. Khai during ripening at 25°C for 10 days

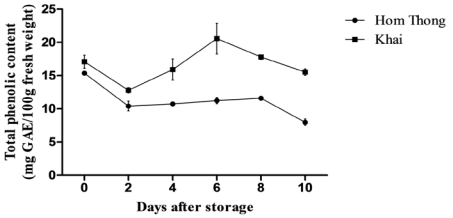

Figure 2. Change of total phenolic content (mg GAE/100g fresh weight) of banana (●) cv. Hom Thong (■) cv. Khai during ripening at 25°C for 10 days

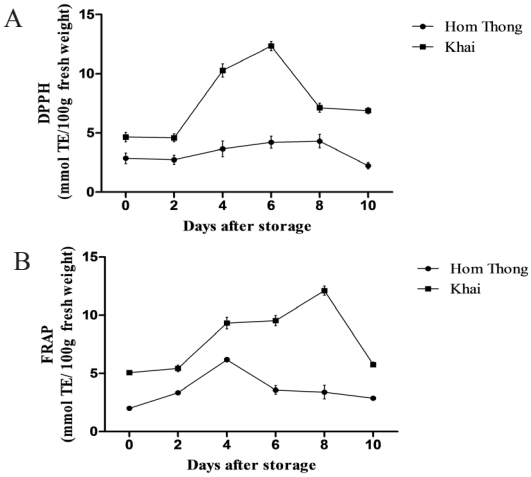

Figure 3. Change of total antioxidant activity (mmol TE/100 g fresh weight) of banana (●) cv. Hom Thong (■) cv. Khai in two different methods (A) DPPH and (B) FRAP method during ripening at 25°C for 10 days

with ripening was noted only in 'Hom Thong' banana whereas in 'Khai' banana, phenolic content increased with advancing ripening, particularly at 6 days of holding, before slightly decreasing at 8 days when the fruit fully ripened and markedly decreasing at 10 days when the fruit became over-ripe. Newilah *et al.* (2010) reported similar results in hybrid banana in which phenolic content increased during ripening before decreasing at the full ripe stage.

Antioxidant activity

DPPH scavenging and FRAP activities were higher in Khai banana than in Hom Thong banana which was maintained throughout the ripening period (Figure 3). Khai banana showed dramatic increases in DPPH and FRAP activities which were highest after 6 and 8 days when fruit ripened,

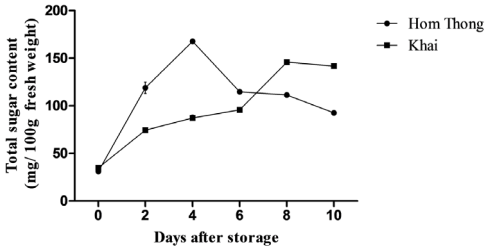

Figure 4. Change of total sugar content (mg/100 g fresh weight) of banana (●) cv. Hom Thong (■) cv. Khai during ripening at 25°C for 10 days

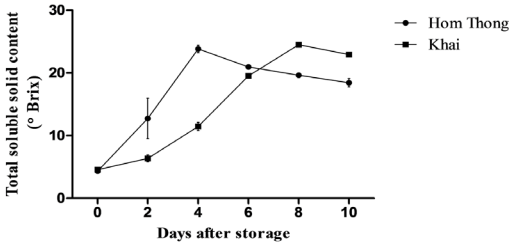

Figure 5. Change of total soluble solid content (°Brix) of banana (●) cv. Hom Thong (■) cv. Khai during ripening at 25°C for 10 days

respectively. In Hom Thong banana, DPPH activity slightly increased only with ripening while FRAP activity more than doubled after 4 days of storage and then decreased with advancing ripening. In general, DPPH and FRAP activities decreased in over-ripe or senescent fruits in both cultivars. Antioxidant activity expressed as radical scavenging activity usually differed with ripening stage due to differences in concentrations of antioxidant compounds (Raffo *et al*., 2002). Kondo *et al.* (2005) revealed that DPPH radical scavenging activity was associated with total phenolic content in the plant tissues. Pinelo *et al.* (2004) further showed that carotenoids, vitamin C, vitamin E, phenolic compounds and their interactions contribute to the overall antioxidant activity. In the present study, Khai banana had higher ascorbic acid and phenolic contents which could account for its higher antioxidant activity than that of Hom Thong banana. Ascorbic acid and phenolic contents increased with ripening in Khai banana while only ascorbic acid content increased during ripening of Hom Thong banana. These changes in antioxidant compounds relate well with the changes in antioxidant activities. Direct association of antioxidant activity and antioxidant compound was also obtained in other fruits (Baskar *et al*., 2011; Patthamakanokporn *et al.*, 2008; Sulaiman *et al.*, 2011).

Sugar content and TSS

Khai banana showed an increasing sugar content with advancing ripening and was highest after 8 days of storage when the fruits ripened fully (Figure 4). Sugar content leveled off two days later when the fruit became over-ripe. In contrast, Hom Thong banana had increasing sugar content during the first 4 days of storage; thereafter, sugar content decreased. TSS content showed the same trend and was highest after 8 days in Khai banana and after 4 days in Hom Thong banana (Figure 5). Peak sugar content was slightly higher in Hom Thong banana (165 mg/100 g fresh weight) than in Khai banana (145 mg/100 g fresh weight) while peak TSS content (about 23°B) was almost similar in both cultivars. Increasing sugar content and TSS is a typical characteristic in ripening bananas due to increased starch to sugar conversion (Pinto *et al*., 2004). Cordenunsi and Lajolo (1995) also found dramatic decreases in starch content concomitant with increases in sugar content. However, TSS may not totally reflect the sugar content of fruits. Khai and Hom Thong bananas showed differences in sugar content which were not reflected in their TSS levels. Similar results were reported in previous studies on bananas (Emaga *et al.*, 2007; Regina and Gloria 2005), strawberries (Tian *et al*., 2000), oranges (Porat *et al*., 1999), apricot and plums (Dong *et al*., 2002), custard apple and mango (Hofman *et al.*, 2001) and apples (Rupasinghe *et al.*, 2000).

Table 1. Correlations of antioxidant components to total antioxidant activity (FRAP and DPPH) and TSS to total sugar content of 'Hom Thong' and 'Khai' banana during ripening at 25°C

Parameters	Cultivar	Correlation coefficient (R^2)	*P* value
AsA and FRAP	Hom Thong	0.1158	0.5093
AsA and DPPH	Hom Thong	0.4414	0.1501
AsA and FRAP	Khai	0.6904	0.0405*
AsA and DPPH	Khai	0.1070	0.5268
TPC and FRAP	Hom Thong	0.1022	0.2022
TPC and DPPH	Hom Thong	0.0508	0.8060
TPC and FRAP	Khai	0.3157	0.2459
TPC and DPPH	Khai	0.4579	0.1399
TSS and total sugar	Hom Thong	0.7359	0.0289*
TSS and total sugar	Khai	0.8732	0.0063*

AsA= Total ascorbic acid content, TPC = Total phenolics content, TSS = Total soluble solids content. Correlation coefficient (R^2) was determined following Pearson's correlation test and the p-value less than 0.05 ($p < 0.05$) was considered as statistically significant*.

Correlation analysis

Table 1 shows the correlation coefficients (R^2) for ascorbic acid content or phenolic content and total antioxidant activity (FRAP and DPPH). There was no significant correlation between the antioxidant compounds and antioxidant activity except for ascorbic acid content and FRAP in Khai banana. On the other hand, sugar content was strongly correlated with TSS in both cultivars, with R^2 values of 0.7359 and 0.8732 in 'Hom Thong' and 'Khai' bananas, respectively (Table 1).

Previous works have shown positive correlations between antioxidant compounds and antioxidant activity in fruits (Pinelo *et al.*, 2004; Priya Darsini *et al*., 2012; Raffo *et al*., 2002). However, Sulaiman

et al. (2011) obtained only minor to moderate correlation between phenolic contents and antioxidant activities in nine Malaysian banana cultivars. Samee *et al.* (2006) working on 28 types of Thai fruits also noted only fair correlation between ascorbic acid content and antioxidant activity. They further found that multiple regressions that combined more antioxidant compounds (total acid, phenolic and ascorbic acid contents) produced higher correlations with antioxidant activity; when compared with single antioxidant compound, moderate or low correlation with antioxidant activity was found. This finding was also obtained in the present study.

On the other hand, sugar content and TSS are usually strongly correlated as 60-80% of sugars account for the TSS in ripe fruits (Winsor *et al*., 1962). Siriboon and Banlusilp (2004) reported that the increased breakdown of starch to soluble sugars contributed to the increase in TSS in banana fruit.

Conclusion

Total antioxidant activity, ascorbic acid content, phenolic content, TSS and sugar content increased with ripening and then declined when the fruits were at the over-ripe stage. These changes were more evident in Khai bananas which had higher antioxidant compounds and antioxidant activity compared to 'Hom Thong' banana.

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