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Fruit quality and antioxidant properties of 'KluaiKhai' banana (*Musa* AA group) at different stages of harvest maturity

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Abstract

This study was designed to determine the effects of harvest maturity and harvesting season on fruit quality and shelf life and evaluate the relationship between maturity and antioxidant properties in 'KluaiKhai' banana (*Musa* AA group) for export in Thailand. Three harvest season trials (cool, summer and rainy seasons) were conducted using fruit bunches of different ages (30-50 days from removal of the blossom when the last fruit hand emerged). Fruit samples were stored at 13°C for 2 weeks to simulate export shipment and holding. Fruits were ripened with ethylene for measuring total soluble solids (TSS) and shelf life of ripe fruits. Results showed that the degree of maturity of fruits from the same bunch age differed with harvest season; fruits matured the slowest in the cool season and fastest in the rainy season. Hand weight and finger diameter generally increased with increasing harvest maturity and were highest in the most mature fruits regardless of harvest season. Fruit length did not widely vary with maturity. Similarly, TSS and shelf life of ripe fruits were not significantly affected by harvest maturity and season. Fruits were 70-80% mature, the recommended maturity for export, at 45-50, 37-40, and 33-37 days from blossom removal during the cool, summer and rainy season, respectively. A fourth trial was conducted to determine fruit quality and antioxidant properties at table ripe and overripe stages. Firmness and TSS showed no significant variations due to maturity but starch content was comparably higher in fruits harvested at 35-40 days (75-90% mature) than those at 30-33 days (60-70% mature) from blossom removal. Ascorbic acid, phenolics, flavonoids and β-carotene contents at table ripe stage increased with increasing harvest maturity while lycopene content was low and was not clearly affected by maturity. Similar trend and amount of ascorbic acid were obtained in overripe fruits but phenolics, flavonoids and β-carotene contents decreased. Ferrous ion chelating ability and DPPH scavenging activity at the table ripe stage generally increased with harvest maturity while reducing power decreased with increasing maturity. These antioxidant activities decreased slightly in overripe fruits. Ferrous ion chelating ability was higher than DPPH scavenging activity in fruits at 35-45 days from blossom removal. The results indicate the nutritional advantage of harvesting fruit at the more mature stage. Considering both export requirement and nutritional potential, fruits could be harvested when 75-85% mature.

Introduction

Banana is the most consumed tropical fruit in the world. Its production and trade are expanding driven by global economic realities (e.g. trade liberalization, global food chains and consumers' increasing living standards and health awareness) and the global drive to increase fruit and vegetable consumption to reduce risks of chronic diseases. The banana fruit is a health food that contains beneficial phytochemicals such as antioxidants (De Mejia and Prisecaru, 2005; Kanazawa and Sakakibara, 2000; Proteggente *et al*., 2002; Shian *et al*., 2012) that can reduce risks of cancers (Rashidkhani *et al*., 2005; Sun *et al*., 2002), heart diseases (Yin *et al*., 2008), and brain disorders such as Alzheimer's and Parkinson's diseases (Heo *et al*., 2008). Interestingly, the same antioxidants are also essential for the health of fruit; thus, maintaining high levels of antioxidants in fruits throughout the supply chain is of utmost importance to maintain fruit quality and to deliver to consumers the health benefits of fruit consumption (Silva *et al*., 2010).

A quality management essential that has become more critical as markets become globalized and

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more competitive is harvesting bananas at the optimum stage of maturity. Harvest maturity is the most important factor that determines fruit quality and storage life (Kader, 1999). Immaturity results in inferior quality while over-maturity decreases storage life and constrains transport to distant markets. In Thailand, maturity problem contributes to the low quality out turn of export golden banana (*Musa* AA group), locally known as 'KluaiKhai' (Sangudom *et al.*, 2012). About 30-40% of total produce of farmers fail export standards and are marketed domestically at a price of 8-10 times lower than the price for export-grade fruits.

Although maturity is more a matter of age than size, the latter or fullness of fruit fingers is widely used as harvest maturity index of bananas. For 'KluaiKhai' banana, fruits for export are harvested when 70-80% mature, i.e. light three-quarters (very prominent angularity) to light full three-quarters (prominent angularity) (Department of Agriculture-Thailand, 2007). This ensures arrival of fruits at the green stage at importing countries where fruits are ripened with ethylene before retail distribution. The time period to reach desired maturity may vary with harvest season. In Thailand, KluaiKhai' banana is produced year-round and harvesting may coincide with summer (March-May), rainy (June-September) or cool (October-February) season.

Quality of fruit with lower maturity is believed to be lower than fruit with higher maturity. This was obtained in a recent study comparing 60% and 80% mature 'Gros Michel' bananas (*Musa* AAA group) ripened naturally (Li *et al*., 2011). When ripening was accelerated by ethylene, harvest maturity did not influence fruit quality. Ahmad *et al.* (2007) also found no effect of harvest maturity on color and firmness of bananas ripened with ethylene but more mature fruits had higher soluble solids, sweetness and flavor. Thaiphanit and Anprung (2010) further found that antioxidant activities in ripe bananas increased with increasing harvest maturity. In the present study, the effects of harvest maturity and harvesting season on fruit quality and shelf life were determined. The relationship between maturity and antioxidant properties was also examined.

Materials and Methods

Experimental orchards

Orchards used in this study were located at Chanthaburi and Sukhothai, the first and third leading 'KluaiKhai' banana-producing provinces that are exporting to Japan and China. In Chanthaburi, a farmer's orchard and the banana experimental farm of Chanthaburi Horticultural Research Center were used while in Sukhothai, the banana experimental farm of Sukhothai Horticultural Research Center was used. At least 10 hills of plants from each experimental site were used for each treatment. Recommended cultural practices were followed.

Harvest season and maturity effects

Three harvesting season experiments were conducted in randomized complete block design with three replicates. For the cool season experiment (October 2010-February 2011), fruit bunches were harvested at 30, 35, 40, 45 and 50 days from removal of the blossom when the last fruit hand had emerged. For the summer season experiment (March-May 2011), fruit bunches were harvested at 35, 37, 40 and 45 days after blossom removal while for the rainy season experiment (June-August 2011), 30, 33, 35, 37, 40, 45 and 50 days after blossom removal. Degree of fruit maturity at each age of fruit bunch was assessed based on the standard maturity index for 'KluaiKhai' according to fullness of fingers: full – no angularity, >90-100% mature; full three-quarters – angularity not prominent,>80-90% mature; light full three-quarters – prominent angularity, >70-80% mature; light three-quarters – very prominent angularity, >60-70% mature, and light – <60% mature.

Fruit hand weight and finger length and mid portion diameter were determined using 5 sample fruit bunches per treatment per replicate.Ten sample fruit hands per treatment were ripened by dipping in 200 ppm ethephon for 5 min and at the table ripe stage (after 3 days from treatment) total soluble solids (TSS) content was measured using a digital refractometer.

For the summer and rainy season harvests, shelf life of fruits was determined. Fifteen (15) sample fruit hands per treatment were cool-stored for 2 weeks at 13^{o}C to simulate export shipment and holding at arrival in importing country. After which, the fruits were taken out and treated with 200 ppm ethephon for 5 min to simulate ripening treatment, and stored at ambient (26^{o}C). At the table ripe stage, TSS was measured. Shelf life was taken as the number of days from table ripe stage to first appearance of senescent spots.

Harvest maturity effects on antioxidant properties

This experiment was conducted from August-October 2011 (partly rainy and cool season) following the same procedures in earlier experiments. Fruits were harvested at 30, 33, 35, 37, 40 and 45 days from blossom removal. Harvested fruit bunches were dehanded and fruit hands were ripened by dipping in

200 ppm ethephon solution for 5 min before ambient holding. At the table ripe stage, fruit pulp quality, antioxidant compounds and antioxidant activity were measured. Fruit quality was characterized in terms of firmness which was measured using fruit texture analyzer, TSS, and starch content which was analyzed following the AOAC Method 996.11 (AOAC, 2000).

Antioxidant compounds included ascorbic acid, phenolics, flavonoids, and carotenoids (β-carotene and lycopene) which were determined following the method of Klein and Perry (1982), Singleton and Rossi (1965), Chang *et al.* (2006) and Talcott and Howard (1999), respectively.

Antioxidant activity was measured based on reducing power, ferrous ion chelating ability and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, following the method of Lin *et al.* (2006) with slight modifications. In these three assays, methanolic extract was prepared by soaking 1 g pulp sample in 9 ml of methanol and leaving it at room temperature for 3 h before filtering through Whatman No.1 filter paper under suction.

For reducing power assay, 5 ml of the methanolic extract was mixed with 1.25 ml of 0.2M phosphate buffer (pH 6.6) and 1.25 ml of 1% (w/v) potassium ferricyanide, incubated in a hot water bath(50°C) for 20 min, and then transferred to an ice bath for cooling before adding 1.25 ml of 10% (w/v) of trichloroacetic acid. The clear solution was separated (2.5 ml) and mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride and left at room temperature for 10 min. The absorbance was read at 700 nm.The intensity in absorbance was taken as a measure of antioxidant activity.

For ferrous ion chelating ability, 1 ml of the methanolic extract was mixed with 3.7 ml methanol, 0.1 ml of 2 mM ferrous chloride and 0.2 ml of 5 mM ferrozine and left at room temperature for 10 min. The absorbance was read at 562 nm, with ferrous chloride and ferrozine as control. Ferrous ion chelating power was the quotient of the absorbance of the sample and that of the control, which was expressed in percentage.

For DPPH scavenging activity assay, 4 ml of the methanolic extract was mixed with 1 ml of 1 mM of DPPH and kept at room temperature for 30 min. The absorbance was read at 517 nm. As control, 1 mM of DPPH and methanol was used. DPPH scavenging activity was the quotient of the absorbance of the sample and that of the control expressed in percentage.

Antioxidant analyses were also performed at the early overripe stage (appearance of first symptom of senescent spotting). All analyses were done in triplicates.

Statistical analysis

Results were subjected to analysis of variance (ANOVA) and treatment mean comparison by the least significant difference test (LSD) using the SAS Statistical Software.

Results and Discussion

Harvest season and maturity effects

The degree of maturity of the fruits from the same age of the fruit bunch differed with harvest season (Table 1). Fruits matured the slowest in the cool season and fastest in the rainy season. This can be seen from fruits harvested after 35 days from blossom removal; they were 60, 65 and 75% mature in the cool, summer and rainy season harvest, respectively. This trend persisted in fruits at 40 and 45 days from blossom removal. Summer and rainy season fruits developed the maximum export-grade of 80% maturity at a bunch age of 40 and 37 days from blossom removal, respectively. Cool season fruits were 80% mature 10-13 days later.

Fruits in the cool season harvest were lighter (<1 kg hand weight) and smaller (<100 mm fruit length and <30 mm fruit diameter) than those in the summer and rainy season harvests (Table 1). Hand weight and finger diameter generally increased with increasing harvest maturity and were highest in the most mature fruits regardless of harvest season. In the cool season harvest, only fruits at 45 days from blossom removal had comparable fruit diameter as the most mature fruits (50 days from blossom removal). Hand weight did not significantly differ with maturity in the summer season harvest while in the rainy season harvest, fruits harvested at 30-40 days from blossom removal (60-85% mature) had statistically comparable hand weights. On the other hand, fruit length did not vary with maturity regardless of harvest season, except in the cool season harvest in which significant differences were obtained but the trend was inconsistent with increasing maturity.

Harvest maturity did not affect TSS of fruits at the table ripe stage in the three harvest seasons (Table 2). In general, cool season fruits had higher TSS (28.4-28.9%) than fruits from the summer season (21.9-25.8%) and rainy season (20.9-23.6%) harvests. Similarly, after 2 weeks of cold storage and subsequent ripening with ethylene at ambient, TSS at the ripe stage and shelf life did not significantly vary with harvest maturity (Table 3). TSS ranged from 23.5-27% in the summer season harvest and

Table 1.Degree of maturity and fruit characteristics of 'KluaiKhai' banana as affected by harvest season and maturity

Harvest season/ Maturity (days from blossom removal)	Degree of maturity[1]	Fruit hand weight (kg)	Fruit length (mm)	Fruit diameter (mm)
Cool season				
30	52	0.65b	81.1ab	25.4b
35	60	0.65b	78.0ab	24.7b
40	67	0.65b	72.4b	25.3b
45	73	0.68b	75.5ab	27.1ab
50	80	0.84a	85.8a	29.0a
CV (%)		18.4	5.8	6.9
Summer season				
35	65	1.41	110.2	34.1b
37	70	1.36	101.5	34.3b
40	80	1.39	104.0	34.6b
45	85	1.62	106.4	37.3a
CV (%)		9.1	8.1	3.9
Rainy season				
30	60	1.05b	96.1	28.4c
33	70	1.39ab	106.7	30.4c
35	75	1.16b	104.7	31.7bc
37	80	1.26b	100.5	31.3bc
40	85	1.42ab	105.5	32.9bc
45	90	1.58a	105.9	33.3b
50	>90	1.83a	106.9	36.7a
CV (%)		10.7	6.1	3.2

[1]Fruit maturity index': full – no angularity, >90-100% mature; full three-quarters – angularity not prominent, >80-90% mature; light full three-quarters – prominent angularity, >70-80% mature; light three-quarters – very prominent angularity, >60-70% mature, and light – <60% mature.

Mean separation within columns per harvest season by LSD, 5%. CV-coefficient of variation

Table 2.Total soluble solids (TSS, %) at the ripe stage of 'KluaiKhai' banana as affected by harvest season and maturity

Harvest maturity (days from blossom removal)	Cool season	Summer season	Rainy season
30	28.8		21.7
33			20.9
35	28.9	23.9	23.1
37		25.8	23.0
40	28.9	21.9	22.8
45	28.7	22.1	22.3
50	28.4		23.6
CV (%)	5.3	8.6	7.4

No significant differences between means within columns were obtained based on analysis of variance.
CV-coefficient of variation

21-23.5% in the rainy season harvest. Shelf life was about 4-5 days irrespective of harvest season and maturity.

Fruit maturation was expected to be slow during the cool season when low temperatures and short daylength prevailed. Although more favorable temperatures and photoperiods existed during the summer season, water became a limiting factor to growth and maturation of the fruits which was not adequately replenished by supplementary irrigation. The favorable growth factors were present during the rainy season and could account for the faster and earlier development of the fruits as compared to that in the cool and summer seasons. Overall, the results indicate that harvest season has profound effect on fruit maturation and could be used as a benchmark in deciding when to harvest fruits. Furthermore, it was surprising that even the least mature fruits (e.g. 52-60% mature fruits) had TSS contents comparable to that of more mature ones after ripening with ethylene right after harvest and after 2 weeks of cold storage. Earlier, it was revealed that after ripening with ethylene, more mature bananas developed higher TSS, sweetness and flavor despite similar rates of yellowing and softening as less mature fruits (Ahmad *et al*., 2007). However, Li *et al.* (2011) found that 60% mature bananas had comparable sugar metabolism and fruit quality as 80% mature fruits when ripening with ethylene was employed. The latter findings seem to be corroborated by the results of the present study.

Table 3.Total soluble solids (TSS) and shelf life of 'KluaiKhai' banana ripened with ethephon after 2 weeks of cold storage as affected by harvest season and maturity

Harvest season/Maturity (days from blossom removal)	TSS (%)	Shelf life (days)
Summer season		
35	24.4	4.50
37	23.5	4.75
40	27.0	4.75
45	24.4	4.25
CV (%)	10.4	11.4
Rainy season		
30	21.0	4.03
33	21.7	3.96
35	22.3	4.00
37	22.7	4.03
40	23.5	4.03
45	23.0	3.97
50	21.9	4.07
CV (%)	8.2	1.6

No significant differences between means within columns were obtained based on analysis of variance.
CV-coefficient of variation

Table 4.Firmness, total soluble solids and starch content at the ripe stage of 'KluaiKhai' banana at different harvest maturities

Harvest maturity (days from blossom removal)	Degree of maturity[1]	Firmness (kg)	TSS (%)	Starch (%)
30	60	0.64	22.4	12.1b
33	70	0.60	23.2	13.5b
35	75	0.57	23.3	15.6ab
37	80	0.61	24.0	15.9ab
40	85	0.58	23.6	15.7ab
45	90	0.53	23.0	16.0a
CV (%)		5.3	8.6	7.4

Mean separation within columns by LSD, 5%. CV-coefficient of variation
[1]See additional notes in Table 1

It has been recommended that 'KluaiKhai' bananas for export should be harvested when fruits are 70-80% mature (Department of Agriculture-Thailand, 2007). Although this recommendation is regardless of harvest season, the results of the present study could provide supplementary basis. During the cool, summer and rainy seasons, the fruit bunches can be harvested at 45-50, 37-40, and 33-37 days from blossom removal, respectively. This could help in minimizing the problem of immaturity and/or over-maturity which contribute to the low quality outturn of banana growers engaged in the export trade.

Harvest maturity effects on antioxidant properties

Fruits used in this trial were 60% mature (30 days from blossom removal) to 90% mature (45 days from blossom removal) (Table 4). After ripening with ethylene, the table ripe fruits from all harvest maturities had comparable firmness of 0.53-0.64 kg and TSS of 22.4-24%. This result is similar to that in the preceding experiments. However, starch content differed and was distinctly lower in fruits harvested at 30-33 days from blossom removal relative to that of the most mature fruit which had the highest starch content. Fruits harvested at 35-40 days from blossom removal had comparable starch content as the most

Table 5.Antioxidant compounds at the table-ripe and overripe stages of 'KluaiKhai' banana harvested at different maturities and ripened with ethylene

Harvest maturity (days from blossom removal)	Ascorbic acid (mg 100 gFW^{-1})	Phenolics (mg gallic acid 100 gFW^{-1})	Flavonoids (mg rutin 100 gFW^{-1})	β-carotene (µg 100 gFW^{-1})	Lycopene (µg 100g FW^{-1})
Table ripe stage					
30	12.7e	237.7d	77.3e	235.1d	40.7b
33	13.0e	263.1c	85.5d	242.2c	37.3c
35	14.1d	229.7d	79.6de	251.0b	44.2a
37	14.8c	262.8c	93.5c	252.2b	36.3c
40	15.7b	338.3b	100.6b	256.4b	24.7d
45	16.2a	379.9a	123.4a	262.0a	ND
Overripe stage					
30	12.1c	218.8d	75.0bc	228.1b	5.5b
33	12.5c	231.3d	67.7c	228.0b	2.5c
35	14.8b	194.0e	68.7c	239.0a	10.4a
37	15.0b	258.5c	75.6bc	228.1b	3.6b
40	16.2a	304.2b	80.9b	238.6a	ND
45	16.7a	381.5a	94.7a	244.2a	ND

Mean separation within columns per ripening stage by LSD, 5%.
FW-fresh weight; ND-not detected

Table 6.Antioxidant activity at the table-ripe and overripe stages of 'KluaiKhai' banana harvested at different maturities and ripened with ethylene

Harvest maturity (days from blossom removal)	Ferrous ion chelating ability (%)	DPPH scavenging activity (%)	Reducing power (absorbance)
Table ripe stage			
30	33.6c	34.7e	2.42a
33	36.9b	36.9d	1.66b
35	53.7a	37.1d	1.54c
37	52.9a	37.6c	1.18d
40	54.3a	38.2b	1.03e
45	55.0a	38.9a	0.86f
Overripe stage			
30	30.1e	33.9d	2.11a
33	35.2d	34.6c	1.87a
35	45.7bc	36.6b	1.15c
37	44.3c	36.6b	0.85d
40	46.3b	37.3a	0.78d
45	51.1a	37.7a	0.51e

Mean separation within columns per ripening stage by LSD, 5%.

mature fruits.

Analysis of antioxidant properties showed marked differences with harvest maturity (Table 5-6). At the table ripe stage, antioxidant compounds increased with increasing age of the fruit bunch, except for lycopene. Ascorbic acid content increased from 12.7 to 16.2 mg per 100 g fresh weight (FW), phenolics content from 237.7 to 379.9 mg per 100 g FW, flavonoids content from 77.3 to 123.4 mg per 100 g FW, and B-carotene content from 235.1 to 262 µg per 100 g FW in fruits harvested at 30 and 45 days from blossom removal, respectively (Table 5). Lycopene content was very low and was not detected in the most mature fruit. The same maturity effect was obtained in the overripe fruits (first perceptible senescent spots) but phenolic, flavonoid, β-carotene and even lycopene contents were generally lower than that at the table ripe stage. Ascorbic acid content did not differ much between table ripe and overripe fruits.

Ferrous ion chelating ability and DPPH scavenging activity at the table ripe stage generally increased with harvest maturity (Table 6). Ferrous ion chelating ability increased from 33.6% in fruits at 30 days from blossom removal to 55% in fruits at 45 days from blossom removal. Chelating ability of fruits at 35-40 days from blossom removal did not significantly vary with that of the most mature fruit. DPPH scavenging activity progressively increased with increasing stage of maturity but the increase in activity was not as dramatic as that of ferrous iron chelating ability, i.e. from 34.7% in fruits at 30 days from blossom removal to 38.9% in fruits at 45 days from blossom removal. Reducing power exhibited an opposite trend as it progressively decreased with increasing harvest maturity. At the overripe stage, all antioxidant activities slightly decreased and showed similar pattern of harvest maturity effect.

The results show remarkable effect of harvest maturity on the antioxidant potential which increased with increasing maturity. Similar results were obtained earlier by Thaiphanit and Anprung (2010) in 'KluaiHom Thong' bananas (*Musa* AAA group). 'KluaiKhai; fruits were particularly high in phenolics about a third of which were flavonoids. Ascorbic acid was also present in significant amount while β-carotene proved to be the major carotenoid in the fruit. These four compounds are primary antioxidants that can stop free radical chain reaction by donating electron to the free radical to make it stable (Lobo *et al.*, 2010). Flavonoids could also act as secondary antioxidants by preventing the formation of free radicals. The activities of these antioxidants appeared to be depicted in the DPPH scavenging activity and ferrous ion chelating ability but not in reducing power. DPPH scavenging activity is a measure of the primary antioxidant potential while ferrous ion chelating ability is a measure of secondary antioxidant potential (Lim *et al*., 2007). Earlier, bananas were found to have higher secondary antioxidant potential than primary antioxidant potential (Lim *et al.*, 2007; Shian *et al.*, 2012). This is partly supported by the results of the present study as fruits at 35-45 days from blossom removal had higher ferrous ion chelating ability than DPPH scavenging activity. However, in fruits harvested at 30-33 days from blossom removal, the two measures of antioxidant potential did not differ much.

The results indicate the nutritional advantage of harvesting fruit at the more mature stage. However, this has to be weighed against export considerations including shipment duration and keeping the fruit green and unripe at arrival and holding at destination importing countries. Considering both export requirement and nutritional potential, the optimum harvest maturity from the present trial, which was conducted partly during the rainy and cool season (August to October), appeared to be reached at 35-40 days from blossom removal when fruits were 75-

85% mature.

Conclusion

Desired maturity of fruits for export was attained at different bunch ages due to influence of growing season. It took the longest period of time in the cool season and shortest in the rainy season. This can be used to supplement the present harvest maturity index which is based on fullness of fingers. Fruit weight and width differed with maturity but TSS and shelf life of ripe fruits did not. Higher maturity had also higher antioxidant potential. If this is considered in determining the best time to harvest, fruits for export should be harvested when 75-85% mature.

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