# ภาคผนวก

## บทความฉบับร่าง จำนวน 2 เรื่อง

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2) Youryon and Supapvanich Effect of storage temperatures on physical quality and bioactive compounds in ‘Leb Muer Nang’ banana (*Musa* AA) fruit. (submit)

# Physicochemical quality changes in ‘Leb Mue Nang’ banana fruit during ripening

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**Abstract**

The purpose of this study was to investigate the physicochemical changes of ‘Kluai Leb Mue Nang’ banana fruit (Musa AA group) during ripening. Visual appearance, peel and pulp color, firmness, total soluble solids (TSS) content, total acidity (TA) and bioactive compounds of the fruit at tree stage of ripening including mature green, ripe and overripe stages were monitored. A typical changes in peel colour during banana ripening was found which brightness (L*) and yellowness (b*) values increased and greenness (-a*) value disappeared from mature green stage to ripe stage whilst the L* value decreased, b* value remained constant and redness (a*) value increased in

overripe stage of the banana fruit. In pulp colour, L* value and whiteness index (WI) decreased during ripening while the b* value increased. The firmness decreased markedly from mature green stage to ripe stage and then remained constant. TSS increased during ripe stage whilst TA increased at ripe stage and then decreased. The highest total antioxidants capacity and total phenols (TP) content were found in the ripe banana fruit. DPPH scavenging activity remained constant and the highest total flavonoids (TF) content was found in the mature green fruit.

**Keywords**

'Kluai Leb Mue Nang' banana fruit, physicochemical changes, ripening stages, bioactive compounds

## INTRODUCTION

Banana (*Musaceae*) is an economically important climacteric fruit for local and export markets worldwide. Banana fruit is considered to be a good source of nutrients including bioactive phenols, antioxidants and potassium. In Thailand, banana is a commercial fruit following mango, mangosteen, durian and longan 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). Asc climacteric fruit, the ripening process of banana fruit is related to various aspects, including the ethylene burst and evolution of respiratory (Siriboon and Banlusilp, 2004), fruit softening, starch degradation, sugar accumulation, the changes in organic acids content, the production of volatile compounds and bioactive compounds benefiting to health, including total phenols, total flavonoids and antioxidant activitiy (Ummarat et al., 2011). Li et al. (2011) reported

that the loss of pulp firmness the increase in reducing sugar and disease incidence and the reduction of starch content were detected in ‘Baxi’ (*Musa* AAA group) banana fruit during ripening. Previous works reported that the peak of ethylene production, fruit softening, the increase in moisture content, total acidity and total soluble solids and the occurrence of fruit drop and senescence spot were detected during ripening of Thai banana fruit such as ‘Kluai Namwa’ (Siriboon and Banlusilp, 2004), ‘Kluai Hom Thong’ (Imsabai et al., 2006). Moreover, Ummarat et al. (2011) reported the increase of certain bioactive compounds including ascorbic acid, free phenolic compounds and free flavonoids in ‘Kluai Hom Thong’ banana fruit during ripening.

Regarding to previous studies of commercial Thai banana fruits, most of them investigated the physiological changes in ‘Kluai Hom Thong’ banana fruit (Nguyen et al., 2003; Kyu Kyu Win et al., 2007), ‘Kluai Khai’ banana fruit (Nguyen et al., 2003, Nguyen et al., 2004) and ‘Kluai Namwa’ banana fruit (Siriboon and Banlusilp, 2004; Imsabai et al., 2006) during storage and ripening. However, a study of physicochemical changes in ‘Kluai Leb Mue Nang’ banana fruit during ripening has not been found. The origin of ‘Kluai Leb Mue Nang’ is from the south of Thailand and it has recently spread over the country. As small finger and the shape like lady finger, firm texture, sweet taste, yellow flesh and desirable odour, the demand of the fruit in market has been recently increased and the price per hand is higher than ‘Kluai Namwa’ banana. Thus, we were interested in investigating physicochemical changes including physical quality attributes, certain chemical quality attributes and bioactive compounds in ‘Kluai Leb Mue Nang’ banana fruit during ripening.

## MATERIALS AND METHODS

### Raw materials

'Kluai Leb Mue Nang' (*Musa* AA group) banana fruit at full mature green stage (2 months after full bloom), ripe stage (leaved for 4 days at room temperature (27 ±2 ºC) after harvest) and overripe stage (leaved for 8 days at room temperature after harvest) were derived from a local banana orchard at Prateaw District, Chomphon Province. Ten hands of each stage were selected with being uniformity of skin colour and free from any defects including physical damages and diseases. The fruit hands were cleaned by dipping in circulated tap-water and dried at room temperature before physicochemical quality attributes involving peel and pulp colour, firmness, TSS and TA content and certain bioactive compounds were investigated.

**Color measurement**

Peel and pulp colour of the fruit were measured at the middle part by using a HunterLab MiniScan@ XE Plus (Hunter Associates Laboratory Inc., 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 the formular (1) described by (Bolin and Huxsoll 1991).

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

**Firmness measurement**

Ten fingers from each hand were sampled for firmness measurement. The fruit were peeled and the measurement was taken at the middle part of the fruit using a TA Plus Texture Analyzer (Lloyds, England) with a 6 mm cylindrical probe. The result was expressed as the maximum force (N) of measurement.

**Total soluble solids and total acidity content measurements**

Ten fruit per hand were selected for these measurements. The TSS content of the fruit pulp was measured using a hand-held refractometer (ATAGO MNL-1125, Japan). The data were expressed as °Brix. Total acidity (TA) of the fruit pulp was determined using the standard method of AOAC (1995). A 10 g of the banana pulp was homogenized with 20 mL of distilled water and filtered through cloth sheet. A 5 mL of the extract was titrated with 0.1 N NaOH using 1% (w/v) phenophthalene 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 DPPH scavenging activity measurements**

Ten fruit per hand of the banana fruit were selected. The fruit were peeled and then blended together. A 5 g of the banana pulp was homogenized with 50 mL 80% methanol and stirred at room temperature for 15 min before filtration by using cloth sheet. The filtrate was collected and centrifuged at 10,000 x g for 15 min. The supernatant was used to assay bioactive compounds. Total antioxidant capacity of the fruit pulp was assayed using ferric reducing antioxidant potential (FRAP) method which described by Benzie and Strain (1996). FRAP reagent was the mixture of acetate buffer pH 3.0, 10mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20mM ferric chloride hexahydrate in the ratio of 10:1:1. The supernatant was diluted with distilled water in the ratio of 1:1(v/v). The reaction was started when 0.3 mL of the diluted supernatant was added into 3 mL of FRAP solution and then incubated at room temperature for at least 30 min before measuring absorbance at 630 nm. Total antioxidant capacity was expressed as µmole Trolox equivalents per g fresh weight (µmole TE/g FW). DPPH scavenging activity was determined using the method of Brand-Williams et al. (1995) with slight modification. The reactive 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 at dark place for 5 min. The capability to scavenge the DPPH free radical was calculated by using the following equation.

DPPH free radical scavenging activity (%) = [(A0 –A10)/A0] x 100

A0 = the absorbance of the sample at 0 min;

A10 = the absorbance of the sample at 10 min.

**Total phenols and total flavonoids content measurements**

The same supernatant of total antioxidant assay was used to determine total phenols (TP) and total flavonoids (TF) content in the banana pulp. 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 Na2CO3 solution. The mixture was incubated at room temperature for at least 30 min before measuring absorbance at 750 nm. The data was expressed in term of µg gallic acid per g fresh weight (µg GA/g FW). 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 the mixture of 1.25ml of distilled water and 75µL of 0.5% NaNO2 and then leaved for 6 min at room temperature. A 150 µL of 10% AlCl3-6H2O was added into the mixture and then allowed to stand for 5 min. A 0.5mL of 1 M NaOH was then added. The absorbance at 510nm was recorded. The data were expressed as µg catechin per g fresh weight (µg catechin/ g FW).

**Statistical analysis**

The data are shown as the mean of ten replications and standard deviation. Statistic analysis was carried out using ANOVA and the means compared by the least significant different (LSD) test at the significant level of 0.05 ($P<0.05$) using the SPSS software program (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

### Visual appearance and fruit colour

Figure 1 shows the visual appearance of 'Kluai Leb Mue Nang' banana fruit at mature green, ripe and overripe stages. The visual appearance of the fruit was related to the peel colour which showed green colour of the mature green fingers. The ripe fingers were yellow with green stalk and the overripe fingers had a few senescent spots and the black tip and stalk. The senescent spots generally appeared on banana finger caused the typical physiological disorder at the latter phase of fruit ripening and the spots gradually increase in size and number as the fruit advance in ripening process (Ketsa, 2000).


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

**Table 1.** Peel and pulp colour of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit.

| Colour | Mature green | Ripe | Overripe |
|---|---|---|---|
| Peel colour | | | |
| *L** | 47.85±3.37 c | 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 b |
| *b** | 9.09±1.35 a | 58.20±3.25 a | 59.47±4.29 a |
| Pulp colour | | | |
| *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 |

Values followed by the same letter within a row are not significantly different at $p < 0.05$ level.

As shown in Table 1, Peel and pulp colour of 'Kluai Leb Mue Nang' banana fruit was present as lightness (L*), redness to greenness (+a* to –a*), yellowness (b*) and WI. The change in peel colour of 'Kluai Leb Mue Nang' banana fruit was similar to other banana fruits such as Musa Cavendishii (Abdullah and Pantasico, 1990), Musa Acuminata (Buguad et al., 2009). and Musa Sapientum (Mustaffa et al., 1998). The L* value of the banana peel increased markedly from mature green stage to ripe stage and then decreased at overripe stage which this was similar to the change in L* value of Musa cavendishii cv. 'Montel'fruit and Musa Sapientum cv. 'Embun' fruit during ripening which the continuous increase in L* value was concomitant with the reduction of greenness and the increase in yellowness (Abdullah et al., 1985; Abdullah and Pantasico, 1990; Mustaffa et al., 1998). We found that the a* value of the fruit peel were changed from -19.06 (green colour) of mature green fruit to 3.11 and 9.09 (red

colour) of ripe and overripe fruit, respectively. The b* value of the fruit peel increased markedly from mature green stage to ripe stage and then remained constant. In the pulp color, L* value and WI decreased as the fruit ripening progressed. In another hand, the b* value of both mature green and ripe stages were similar but it markedly increased at overripe stage. The increase in b* values during ripening had been reported in 'Grande Naine' banana fruit (Buguad et al., 2009).

**Firmness, total soluble solids and total acidity**

Fruit softening, TSS and TA content are key factors indicating fruit maturity and quality. The typical changes in banana fruit firmness and TSS content were shown in Fig. 2A and 2B, respectively. The firmness of the fruit significantly decreased from 40 N at mature green stage to less than 10 N at ripe and overripe stages ($p>0.05$). No significant difference in firmness of ripe and overripe fruit. In the similar vein, a rapid decrease in firmness of mature green 'Baxi' banana (Musa spp. AAA group) during ripening had been reported (Li et al., 2009) which the softening of banana fruit is associated with the degradation of cell wall compounds, the reduction of starch and the increase in sugar content (Srivastava and Dwivedi, 2000; Li et al., 2009). We also found that the TSS content of the 'Kluai Leb Mue Nang' banana fruit increased significantly from 3.6 ºBrix at mature green stage to 27.8 and 29.4 ºBrix at ripe and overripe stages, respectively ($p>0.05$) (Fig. 2B). Li et al. (2009) reported that the increase in total sugar content of 'Baxi' banana (Musa sp. AAA Group) fruit was positively related to the increase in sucrose phosphate synthase, sucrose synthase and invertases activities during ripening. In another hand, we found that the TA content of the ripe and overripe banana fruits was significantly higher than that of the mature green fruit ($p>0.05$). The highest TA content was detected in the ripe fruit (Fig. 2C). In

the similar vein, the increase in TA of ‘Kluai Nam Wa’ banana fruit during ripening had been reported which the increase coincided with the peak of ethylene production and after that, started to declined onwards (Siriboon and Banlusilp, 2004) Wills et al. (1982) reported that the TA content of Musa sp. AAA group, cv. William banana fruit at mature green stage was markedly lower than that of the ripe fruit which this was associated with the reduction of pH. However, a contrast result had been reported for Musa sp., AAA group, cv. Zhonggang fruit (Jiang et al., 2004) and Musa acuminate, AAA group, cv. Gross Michel fruit (Thaiphanit and Anprung, 2010) which TA content decreased continuously during ripening.

**Total antioxidant capacity and DPPH radical scavenging activity**

Total antioxidant capacity and DPPH radical scavenging activity of ‘Kluai Leb Mue Nang’ banana fruit during ripening were shown in Fig. 3. The total antioxidant capacity of the ripe fruit was significantly higher than that of mature green and overripe fruit ($P<0.05$) (Fig. 3A) whilst the contrast result was shown in DPPH radical scavenging activity (Fig. 3B). The amount of total antioxidant capacity and DPPH radical scavenging activity of mature green fruit were similar to those of overripe fruit. The changes in DPPH radical scavenging activity in tis this was similar to the result reported for “Gross Michel” banana fruit (Thaiphanit and Anprung, 2010). Macheix et al. (1999) reported that antioxidant capacity of banana pulp may due to flavonoids and total phenolic contents. Someya et al. (2002) had discovered that gallocatechin, a phenol, in banana fruit and the antioxidant capacity of the fruit may be attributed to their gallocatechin content.

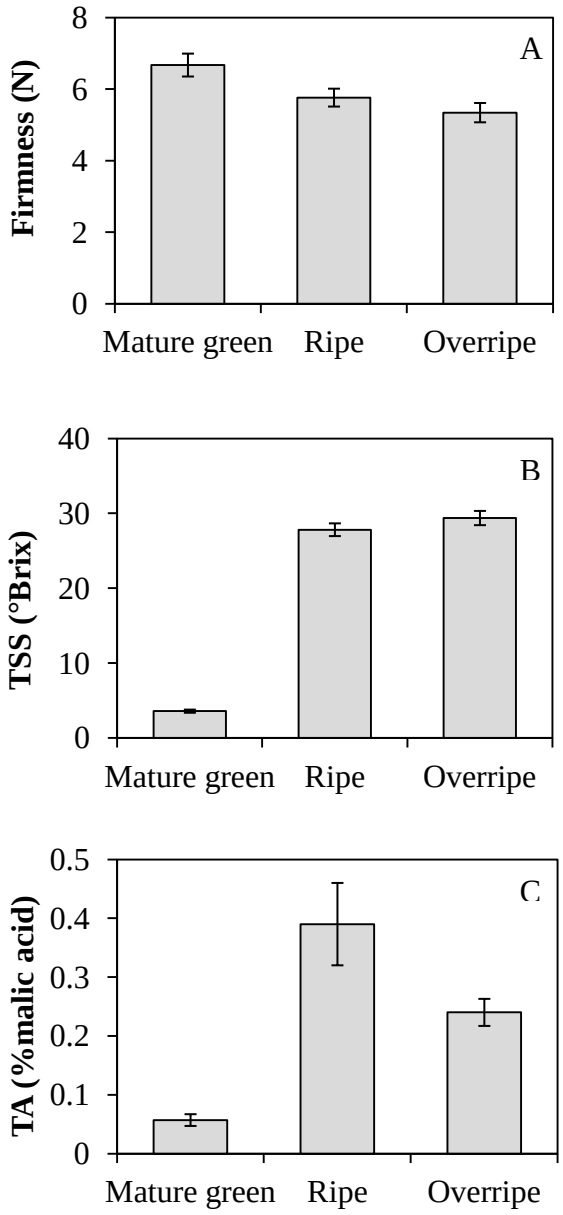


**Figure 2**. Firmness (A), total soluble solids (TSS) (B) and total acidity (TA) (C) of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean (n=10) ± SD. Samples followed by the same letter were not significantly different ($p>0.05$)

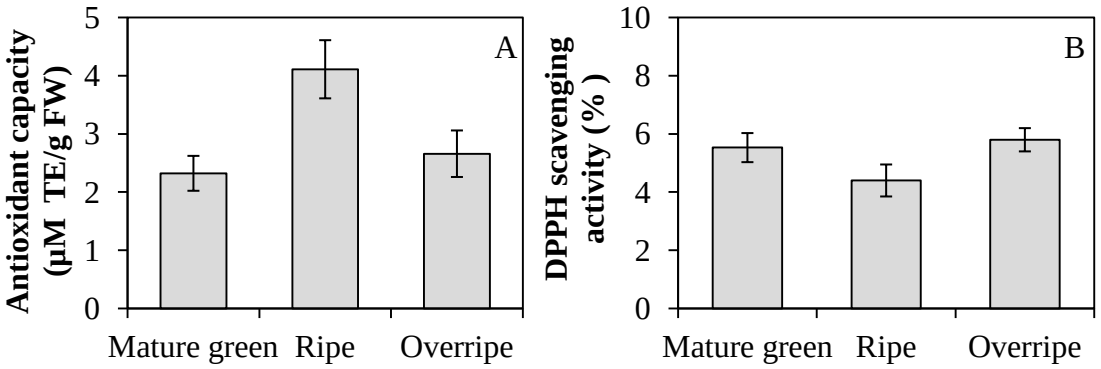


**Figure 3.** Total antioxidant capacity (A) and DPPH scavenging activity (B) of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean (n=10) ± SD. Samples followed by the same letter were not significantly different ($p>0.05$)

**Total phenols and total flavonoids content**

Regarding to the report of Macheix et al. (1999), the changes in TP and TF content of 'Kluai Leb Mue Nang' banana fruit at the three stages of ripening were investigated. TP content of the mature green fruit was significantly lower than that of both ripe and overripe fruit ($P<0.05$) (Fig 4A). No difference in TP content between the ripe and overripe fruit was found. As shown in Fig 4B, The TF content of the mature green fruit was significantly higher than that of both the ripe and overripe fruit ($P<0.05$). No significant difference in TF content between ripe and overripe fruit was found. The change of TP content in this banana fruit was similarly to the change of total antioxidant capacity (Fig. 3A) which determined using FRAP assay. These confirm that antioxidant capacity in banana fruit was attributed to total phenolic compounds which a phenolic compound, named gallocatechin, was identified as the major antioxidant compound in banana fruit (Someya et al., 2002). Similarly, Bennett et al. (2010) addressed that banana pulp was an excellent source of bioactive phenolics. Interestingly, we found a high content of TF content and a low content of total phenols

in the full mature green fruit. These contrast to the report of Macheix et al. (1999) which a high total phenols and tannin content at mature stage of banana fruit and these compounds then declined when ripening advanced. Bennett et al. (2010) reported a slight increase of TP content in the pulp of 'Nanicão' banana fruit stored at 20°C for 18 day whilst TP content of other cultivars such as 'Figo' banana, 'Terra' banana, 'Mysore' banana and 'Pacovan' banana fruits decreased during storage. These show that the changes in TP and TF content of banana fruit pulp during ripening are dependent on cultivars.

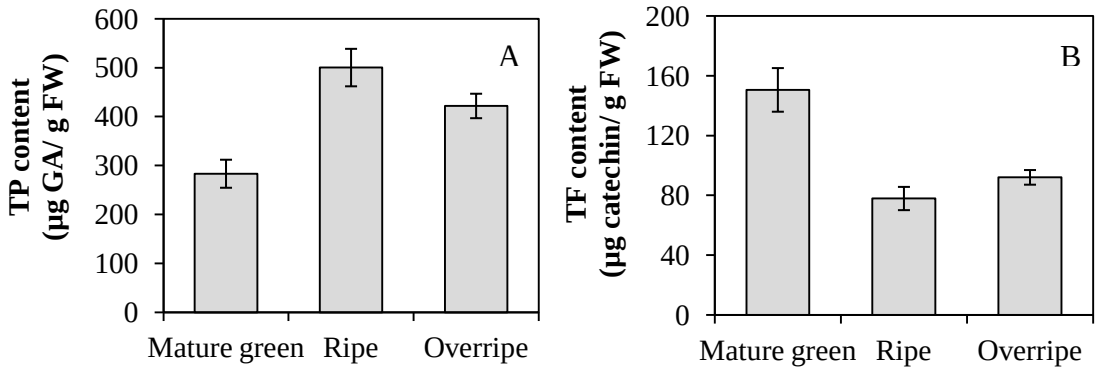


**Figure 4.** Total phenols (TP) (A) and total flavonoids (TF) (B) content of mature green, ripe and overripe stages of 'Kluai Leb Mue Nang' banana fruit. Bars represent mean (n=10) ± SD. Samples followed by the same letter were not significantly different ($p>0.05$)

## CONCLUSIONS

During the ripening progress, the greenness of the peel decreased, the yellowness increased markedly from mature green stage to ripe stage and then remained constant and the redness increased continuously. In the pulp color, the whiteness and lightness

decreased as the ripening advanced while the pulp yellowness remained constant between mature green stage and ripe stage and then markedly increased at overripe stage. The firmness of the fruit decreased rapidly when the fruit ripen and the remained constant. The lowest TSS and TA content were detected in mature green fruit. The TSS content increased continuously while TA content declined in overripe stage. For the bioactive compounds, the highest total antioxidant capacity and the lowest DPPH radical scavenging activity were detected in the ripe fruit. No significant difference of both antioxidant capacities between the mature green and overripe fruit. The change in TP content was similar to the change in total antioxidant capacity. There was no significant difference in TF content of ripe and the overripe fruit.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing the article.

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# Effect of storage temperatures on physical quality and bioactive compounds in 'Leb Muer Nang' banana (*Musa* AA) fruit

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**Abstract**

The purpose of this work was to investigate the quality changes in 'Leb Muer Nang' banana fruit (*Musa* AA group) during storage at various temperatures. The fruit at the onset of ripening stage were kept at 25°C and 13°C and 80%RH for 16 days. The shelf-life of the fruit held at 25°C was 8 days whilst that of the fruit held at 13°C could store for 16 days. The cold storage maintained the peel colour both lightness (L*) and yellowness (b*) and the whiteness of pulp over storage while the decrease in L* and the increase in b* of the fruit peel held at 25°C were found. The firmness was maintained by cold storage. The storage temperatures had no effect on the changes in bioactive compouds during storage as all of them decreased and no siginificant difference in these compounds of both fruit held at 25 °C and 13°C were detected. These suggest that storage at 13°C is a proper temperature extending the shelf-life of the ripe banana fruit.

**Keywords:** Ripe ‘Leb Muer Nang’ banana fruit, storage temperatures, shelf-life

Banana fruit have been claimed as an important commercial fruit in world market like apples, oranges, melon and grapes. As a climacteric tropical fruit, a peak of ethylene production and increased respiratory rate are typical ripening characteristics of banana fruit which induce peel de-greening, sugar accumulation, degradation of starch granules, pulp softening and the production of aroma compounds (Clendennen and May 1997, 463-469). The ripening-associated process is stimulated by storage environment such as high temperature and contaminated ethylene in the atmosphere. Approximately, 20-30% of banana fruit is lost during postharvest period due to poor handling and storage, especially in developing countries (Li and Jia 2008, 443-447). Generally, consumers prefer to purchase banana fruit at ripe stage rather than mature green stage which after the onset of ripening, the shelf-life and eating quality of the fruit change rapidly due to pulp softening and skin blackening. The shelf-life of banana fruit after the onset of ripening is approximate 4 to 5 days (Lima *et al*. 2014, 734-739), especially in tropical countries which the ambient temperature is normally higher than 28 ºC. Thus, cold storage is required to extend the shelf-life of banana fruit in market which the recommended temperature for banana storage is 13-15C (Payasi and Sanwal 2010, 679-710; Promyou et al. 2008, 132-138). Commercially, the banana fruit at mature green stage (pre-climateric phase) are typically held at the cold temperature to extend its postharvest life before sale (Lima *et al*. 2014, 734-739) and then leave them ripen at ambient temperature before sale. In market, the loss of banana fruit is related to rapid ripening and skin blackening. Thus, this work was to investigate the use of cold storage maintaining postharvest quality and extending shelf-life of ripe banana fruit.

‘Leb Muer Nang’ banana fruit (*Musa* AA) is a commercial banana fruit following ‘Gros Michel’ banana fruit (*Musa* AAA), ‘Sucrier’ banana fruit (*Musa* AA) and ‘Nam Wa’ banana fruit (*Musa* AAB). The demand of the fruit in market has been continuously increased. However, the research work involving postharvest quality of ‘Leb Muer Nang’ banana fruit is rare. Therefore, the effect of storage temperatures on physical quality and bioactive compounds of the banana fruit was investigated.

**Materials and methods**

Raw materials

Full mature ‘Leb Muer Nang’ (*Musa acuminate,* AA group) banana fruit were delivered from a banana orchard at Prateaw district, Chomphon province. The fruit bunches were then de-handed and the hands were selected for uniformity of colour, size and being free from physical damages. The hands were cleaned with tap water, air-dried and incubated at room temperature (25±2°C) for 48 hours (until the fruit peel turned to yellow). Twenty banana hands were then held at 25 ºC or 13 ºC and were sampled in every 4 days to determine visual appearance, peel and pulp colour, firmness and bioactive compounds including DPPH scavenging activity, ferric reducing antioxidant potential (FRAP), total phenolics (TP) content and total flavonoids (TF) content.

Visual appearance and colour measurement

Visual appearance of banana fruits was monitored by taking photo of the banana fruit. Superficial colour of both peel and pulp were measured by using a HunterLab MiniScan$^{@}$ XE Plus (Hunter

Associates Laboratory Inc., USA). The lightness ($L^*$), greenness or redness ($-a^*$ or $+a^*$) value and blueness or yellowness ($-b^*$ or $+b^*$) values were recorded. Peel colour was expressed as $L^*$, $a^*$ and $b^*$ values and pulp colour was expressed as $L^*$ value, whiteness index (WI) using the equation $100-[(100-L^*)^2 + a^{*2} + b^{*2}]^{0.5}$ (Bolin and Huxsoll 1991, 416-418) and yellowness ($b^*$).

Firmness measurement

Five fingers of each hand were randomly sampled for firmness measurement. The finger was peeled and the measurement was taken at the middle part of the finger using a TA Plus Texture Analyzer (Lloyds, England) with a 6 mm cylindrical probe. The result was expressed as the maximum force (N) of measurement.

Total antioxidant capacity measurements

A 5 g of the banana pulp was homogenized with 25 mL of 60% methanol and then filtered through cloth sheet. Supernatant was collected and then 1 mL of supernatant was diluted with 20 mL distilled water. The solution was used to assay biologically active compounds such as DPPH scavenging activity, FRAP, TP and TF content.

Ferric reducing antioxidant potential (FRAP) was determined using the method described by Benzie and Strain (1996, 70–76). FRAP reagent consisted of acetate buffer pH 3, 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20 mM ferric chloride hexahydrate in the ratio of 10:1:1 (v/v/v). The reaction was started by mixing 5 mL of FRAP reagent and 0.5 mL of the extract sample and leaved at room

temperature for 30 min. The absorbance at 630 nm was recorded. FRAP value was present in term of mmole trolox equivalents per g fresh weight (mmole TE/g FW).

DPPH scavenging activity was determined using the method of Brand-Williams et al. (1995, 25–30) with slight modification. The reaction was started when 3 mL of supernatant was mixed with 0.3 mL of 1 mM DPPH in methanol. The mixture was held at room temperature for 30 min and the absorbance measured at 517 nm. The capability to scavenge the DPPH free radical was calculated by using the following equation.

DPPH scavenging activity (%) = $[(A_0 - A_{30})/A_0] \times 100$

$A_0$ = the absorbance of the sample at 0 min; $A_{30}$ = the absorbance of the sample at 30 min.

Total phenols and total flavonoids content measurements

TP content was assayed using the method described by Slinkard & Singleton (1977, 49–55). The reaction was begun when 1 mL of the sample solution was added into the solution of 1 mL 50% (v/v) Folin–Ciocalteu reagent solution and 2 mL saturated $Na_2CO_3$ solution. The mixture was incubated at room temperature for 30 min. The absorbance at 750 nm was recorded. TP content was expressed in term of mg gallic acid per g fresh weight (mg GA/ g FW).

TF content was determined using a method described by Jia *et al*. (1999, 555–559). The reaction was started when 0.25 mL of the extract was mixed with 1.25 mL of distilled water, 75 µ L of 0.5% $NaNO_2$. The mixture was leaved for 6 min and then 150 µL of 10% $AlCl_3$-$6H_2O$ was added and allowed to stand for 5 min. After that, 0.5 mL of 1.0 M NaOH was added. The absorbance of the mixture was measured at 510 nm. The data were expressed as µg catechin equivalents per g fresh weight (µg catechin/ g FW).

Statistical analysis

All data were analyzed by using ANOVA and the deference between the means was performed with DMRT at $P < 0.05$ by using SAS (9.1) software. The results are presented as means (n=10) ± S.D.

**Results and discussion**

Visual appearance and superficial colour

We found that the shelf-life of the ripe banana fruit could extend by a proper cold storage. As shown in Fig 1, the appearance of the fruit held at 13°C for 16 days still looked good as the stem of fruit was still green and any black spots and flecks on the skin was not found. Whereas, the black spots and flecks were found on the skin of the fruit held at 25°C for 8 days and its stem end of the fruit was also shrivel and black. Senescence spots or black flecks are the typical physiological disorder happened on banana fruit skin at the last phase of ripening (Chen et al. 2008, 318-328). These physiological disorder is recognisely related to the dysfunction of cell membrane and cell wall degradation of the fruit skin due to senescence process. Choehom et al. (2004, 167-175) reported that browning spots of a *Musa* AA banan fruit, named 'Sucrier' or 'Klui Khai', during ripening relatively associated with the increased polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities which these reactions were obviously stimulated under high oxygen level and rapidly increased at 29-30 °C (tropical ambiene temperature). However, we found that storage at 13°C retarded browning spots and flecks on the fruit

skin and also maintained the fresh-liked appearance of ripe ‘Leb Meur Nang’ banana fruit for more than 16 days.

As the results shown in table 1, lightness (*L** value) of the banana peel held at both 25 °C and 13 °C decreased and the peel redness (*a** value) increased during storage. The yellowness (*b** value) of peel of the fruit held at 25°C decreased whilst that of the fruit held at 13°C remained constand during storage. This shows that the decrease in peel yellowness seemed depend upon the increase in the redness during storage which the obviouse correlation was shown in the fruit held at 25°C and storage at 13°C could maintain the banana peel yellowness during storage. Moreover, the decrease in peel *L** value positively related to the increase in peel *a** value during storage. Similarly, Chen et al. (2008, 318-328) reported that *L** value of banana fruit decreased as the fruit ripening increase. The result also shows that storage at 13°C maintained pulp colour over storage as no significant difference in *L**, WI and *b** values throughout storage. Wherease, the significant decrease in pulp *L** and WI values and the marked increase in pulp *b** value during storage were found the fruit held at 25°C ($P<0.05$). The increase in b* value might relate to the accumulation of carotenoids in the fruit pulp as the results reported for ‘Sucrier’ banana fruit during storage (Facundo et al. 2015, 102-109). Newilah et al. (2009, 197-206) also reported that the increase or decrease of carotenoids compound in banana pulp during ripening accorded to the banana genome.


**Figure 1** Visual appearance of ‘Leb Muer Nang’ banana (*Musa* AA group) fruit at initial day of storage and held at 25°C for 8 days and 13°C for 16 days.

**Table 1**. Superficial colour of peel and pulp of 'Leb Muer Nang' banana (*Musa* AA group) fruit during storage at 25C and 13 C.

| Temperature (°C) | Time (Days) | Peel | | | Pulp | | |
|---|---|---|---|---|---|---|---|
| | | ***L**** | ***a**** | ***b**** | ***L**** | **WI** | ***b**** |
| 25 | 0 | 70.20±2.5$^{a}$ | 4.07±0.3$^{b}$ | 59.01±1.7$^{a}$ | 81.93±0.5$^{a}$ | 71.20±1.4$^{a}$ | 22.26±2.1$^{b}$ |
| | 4 | 64.52±1.6$^{b}$ | 9.00±0.8$^{a}$ | 51.73±5.7$^{b}$ | 79.32±0.8$^{b}$ | 67.65±1.5$^{b}$ | 24.69±1.4$^{ab}$ |
| | 8 | 66.91±4.7$^{ab}$ | 10.22±1.6$^{a}$ | 53.80±3.6$^{b}$ | 79.32±1.5$^{b}$ | 65.78±2.2$^{b}$ | 27.03±1.6$^{a}$ |
| 13 | 0 | 71.56±3.0$^{a}$ | 4.27±0.7$^{b}$ | 58.50±4.9 | 81.17±0.8 | 70.90±1.4 | 21.87±1.2 |
| | 4 | 73.53±2.5$^{a}$ | 3.89±0.6$^{b}$ | 59.67±2.8 | 81.40±0.5 | 70.65±0.6 | 22.56±2.1 |
| | 8 | 67.45±3.1$^{ab}$ | 8.07±1.8$^{a}$ | 59.80±4.0 | 82.03±2.1 | 70.70±2.1 | 22.92±1.0 |
| | 12 | 65.73±1.9$^{b}$ | 7.75±0.9$^{a}$ | 56.73±4.8 | 80.40±0.3 | 70.86±0.3 | 21.50±0.5 |
| | 16 | 63.16±3.1$^{b}$ | 9.04±1.2$^{a}$ | 57.03±2.9 | 80.62±0.5 | 70.54±0.1 | 22.00±0.6 |

Data represent the mean of ten replication ±SD. Values of each storage temperature followed by the same letter within a column are not significantly different at $P < 0.05$ level.

**Firmness**

Figure 2 shows the changes in firmness of the ripe banana fruit during storage at both 25 and 13 °C. The result shows that the cold storage temperature maintained the firmness of the banana fruit over storage for 16 days which no significant difference in that of the banana fruit was detected. For the fruit held at 25 °C, the significant decrease in the fruit firmness was detected after day 4 of storage ($P<0.05$). Similarly, the softening of 'Goldfinger' banana fruit was delayed during stored at 10°C for 22 days

whilst a rapid decrease in firmness was found in the fruit held at 20 °C after day 4 of storage (Nunes *et al*., 2013).

Banana fruit softening is relatively related to the degradation of cell wall components involving the activity of pectic lyase, β-glucosidase and polygalacturonase (Medina-Suárez et al. 1997, 453-461.; Pathak and Sanwal 1998, 249-255) and the breakdown of accumulated starch (Shiga, et al. 2011, 1511-1516). Low storage temperature is the most important factor extending shelf-life and maintaining fresh-liked quality due to slow down metabolism process including the activity of enzymes in plants. Thus, the maintained firmness of the banana fruit held at 13°C might due to the slow down those cell wall hydrolases activity by cold temperature

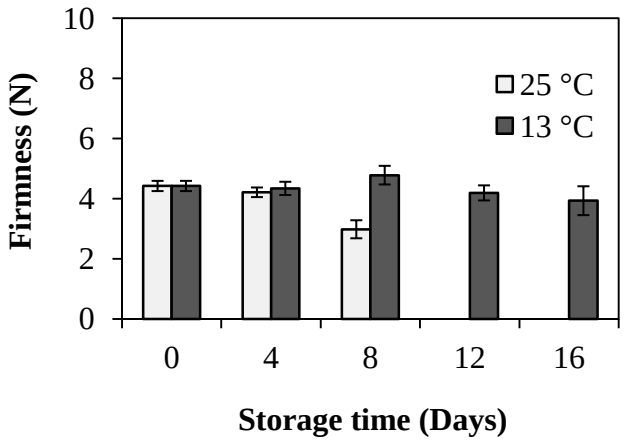


**Figure 2.** Firmness of 'Leb Muer Nang' banana (*Musa* AA group) fruit during storage at 25 °C and 13 °C. Data represent the mean of ten replications ± SD.

**Bioactive compounds**

Antioxidant activity in the banana fruit was identified by measuring DPPH scavenging activity and ferric reducing antioxidant potential (FRAP) (Fig. 3). Both DPPH scavenging activity and FRAP at the initial day were significantly higher than those at other days of storage ($P$<0.05). The significant

decrease in DPPH scavenging activity was found on day 4 ($P$<0.05) and then remained constant over storage at both 25 and 13°C (Fig. 3A). The similar result was also found in FRAP. As the results shown in Fig. 4, total phenolic compounds of the banana fruit held at the both temperatures decreased over storage and there is no significant difference in that between the fruit held at 25 and 13 °C. The TF content of the banana fruit held at 13 °C was lower than that of the banana fruit held at 25 °C over storage. Flavonoids is also widely recognised as a pigment compound in plant. Thus the higher TF content in the pulp of the banana fruit held at 25°C might relate to the increased yellowness ($b$* value), however, it might be not the main compound providing the yellow colour in the fruit pulp like carotenoids (Facundo et al. 2015, 102-109). We suggest that cold storage temperature had no influence to maintain bioactive compounds when compared to the ambient temperature (25°C).

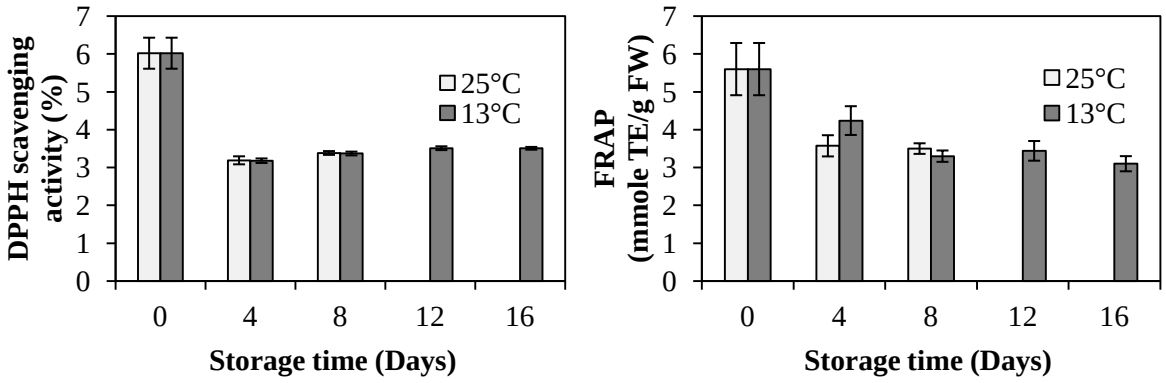


**Figure 3.** DPPH scavenging activity (A) and ferric reducing antioxidant potential (FRAP) (B) of 'Leb Muer Nang' banana (*Musa* AA group) fruit during storage at 25°C and 13 °C. Data represent the mean of ten replications ± SD.

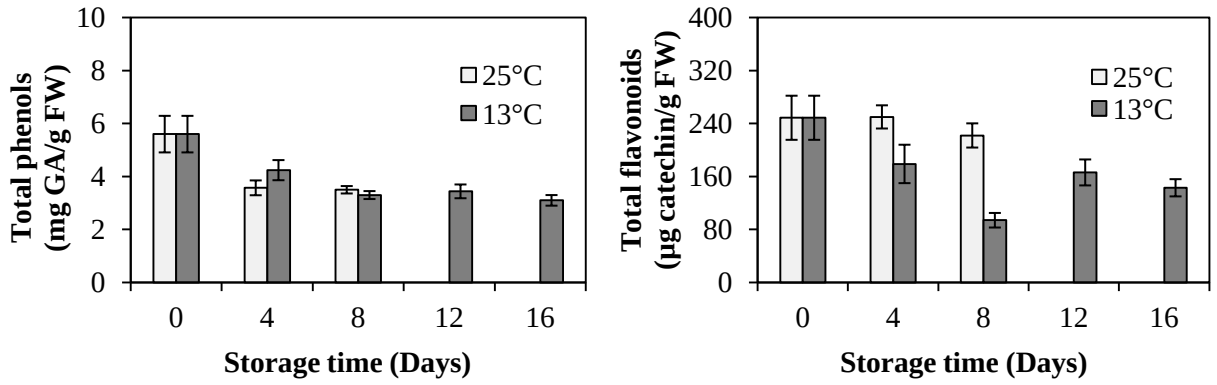


**Figure 4.** Total phenolic (TP) compounds (A) and total flavonoids (TF) content (B) of 'Leb Muer Nang' banana (*Musa* AA group) fruit during storage at 25 °C and 13 °C. Data represent the mean of ten replications ± SD.

## Conclusion

Storage at 13°C is a proper storage temperature maintaining shelf-life and physical quality involving visual appearance, peel and pulp colour and firmness of ripe 'Leb Muer Nang' banana fruit. The fruit could be stored at the cold temperature more than 16 days without black spots and flecks on skin whilst storage at 25 °C could not store the fruit longer than 8 days. The cold storage temperature had no influence to maintain the level of antioxidants, TP content, except TF content, during storage.

## Acknowledgement

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