Emirates Journal of Food and Agriculture. 2020. 32(6): 443-452
doi: 10.9755/ejfa.2020.v32.i6.2114
http://www.ejfa.me/

RESEARCH ARTICLE

Determination of β-carotene content in *Musa* AA pulp (Kluai Khai) at different ripening stage and harvest period in Thailand

Kongaphisith Tongpoolsomjit[a,b], François Grandmottet[c], Ratri Boonruangrod[d], Akharapong Krueajan[a,b], Jarupa Viyoch[a,b]*

[a]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand, [b]Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand, [c]Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand, [d]Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand

ABSTRACT

Musa AA 'Kluai Khai' banana, is an important economic crop in Thailand. It was evaluated according to its morphology, total soluble solids (TSS), and pulp β-carotene content over different months according to its ripening state. The climate variability of Thailand in 2019 resulted in smaller bananas in May than in other months (March and July). TSS was found to be a directly proportional correlation to the ripening stage. The genotype and ploidy of *Musa* AA 'Kluai Khai' were confirmed using molecular markers and flow cytometry. The yellowish pigment of carotenoid was extracted from banana pulp using THF:MeOH (1:1, v/v). The utilization of HPLC displayed β-carotene content in *Musa* AA 'Kluai Khai' pulp as high as 5222.6 ± 83.8 µg / 100 g of fresh weight (gfw) in the 4th state of banana development in March, which was higher than those in July (4072.8 µg/ 100 gfw) and May (3121.8 µg/ 100 gfw) for the same ripening state. The relationship between β-carotene content and the ripening state was calculated to be a logarithmic correlation.

Keywords: Thai banana; β-Carotene; *Musa* AA; Kluai Khai; Provitamin A carotenoids

INTRODUCTION

Carotenoids are a group of red-orange pigments that composed of isoprene units to form tetraterpenoid produced in plants, algae, and bacteria (Rao and Rao, 2007; Zeb and Murkovic, 2013). They are fat-soluble natural organic compounds which play an important role in the photosynthetic system of plants (Amah et al., 2019). They are also required for various biological activities to sustain human and animal health as a precursor of vitamin A necessary for vision, reproduction, embryonic development, disease prevention and immunity (Amah et al., 2019; Lokesh et al., 2014; Stutz et al., 2015). As a dietary source of vitamin A, their antioxidant properties reduces proneness to cardiovascular diseases, cancers, and aged-related eye diseases (Fiedor and Burda, 2014). Many studies of the carotenoid content of plants have been reported for the last few decades (Heinonen et al., 1989; Saini et al., 2015; Setiawan et al., 2001; Yano et al., 2005).

Banana, as a sustainable source for alleviating vitamin A deficiency (Fu et al., 2019), is one of many fruits in which carotenoids have been explored in various cultivars (Aquino et al., 2018; Ekesa et al., 2013; Facundo et al., 2015; Fungo and Pillay, 2011; Gururao Kulkarni et al., 2011; Ngoh Newilah et al., 2009). It is also an economically important fruit consumed by people all over the world. In Thailand, various banana cultivars have been valued as crops for either the local or the export market, especially *Musa* AA group 'Kluai Khai', *Musa* AAA group 'Kluai Hom Thong', and *Musa* ABB group 'Kluai Nam Wa' (Youryon and Supapvanich, 2016). Pharmacologically, the nutritional properties of some Thai banana cultivars were recently reported, including the effects of *Musa* AA pulp in UVB-induced mouse skin damage by increasing γ-GCS

***Corresponding author:**
Jarupa Viyoch, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand, Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand. **Tel:** +665-594-3737.
E-mail: jarupav@nu.ac.th

Received: 01 March 2020; **Accepted:** 30 May 2020

expression and reducing the accumulation of oxidation in end products (Leerach et al., 2017; Viyoch et al., 2012) and the inhibition of melanogenesis through the ERK signaling pathway in B16F10 mouse melanoma cells by *Sucrier* banana peel extracts (Phacharapiyangkul et al., 2019). It is believed that these crucial properties result from the antioxidant ability of carotenoids in *Musa* AA 'Kluai Khai'.

Over the last decades, the relationship between the ripening stages of banana and the content of β-carotene were reported depending on various cultivars. The higher stage, the less β-carotene content was obtained (Aquino et al., 2018; Ngoh Newilah et al., 2009). Furthermore, either the highest (Borges et al., 2018; Ekesa et al., 2013) or the lowest (Ngoh Newilah et al., 2009) β-carotene content was observed in the middle of the ripening state. While, a few studies of β-carotene content in the *Musa* AA cultivar have been addressed with the correlation of ripening days and peel color (Charoensiri et al., 2009; Heng et al., 2017; Sangudom et al., 2014b) but not for total soluble solids (TSS). In this study, the β-carotene content in *Musa* AA pulp was examined using the correlation of different ripening stages defined by TSS at different harvest periods. The morphology and TSS depending on climate, temperature, and rainfall rate in different harvest periods were also evaluated.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used for the extraction were of analytical reagent grade; methanol (MeOH), tetrahydrofuran (THF), and butylated hydroxy toluene (BHT). The solvents for chromatography were HPLC grade; MeOH, ethyl acetate (EtOAc), and acetonitrile (ACN). The β-Carotene standard was purchased from Sigma-Aldrich.

Collection of banana

Bunches of mature fresh raw Thai banana and leaves [*Musa* AA group (var.), Kluai Khai in Thai] were purchased from the South Market in Phitsanulok Province, Thailand during March, May, and July 2019 with maturity of 45-50, 45-50, and 30-35 days since after anthesis until harvest in which an average temperature was 29.3, 30.0, and 32.1 °C, respectively (Thai Meteorological Department, 2019a; Thai Meteorological Department, 2019b; Thai Meteorological Department, 2019c). Three middle hands of the bunch were sampled, labelled according to triplicate (N=3), and left at room temperature to achieve uniform ripening. The ripening stages were evaluated based on peel color into 4 stages (1 = green, 2 = light green, 3 = yellow, and 4 = yellow with brown spots). Three fruits of each hand of the stage 1 were randomly selected for the preparation of dried banana pulp. The remaining fruits of each hand were left at room temperature for stages 2, 3, and 4, respectively.

Dried banana pulp preparation

Preparation of dried banana pulp was modified according to the previous report (Davey et al., 2007). Bananas at 4 stages of peel color were photographed, peeled and weighed. They were sliced once lengthwise and then sliced once again laterally. TSS of all pulp samples were determined using a portable refractometer (Trans Instrument, RBX0080). Collected pulp was frozen at −20 °C before lyophilisation using a freeze dryer (FTS systems Dura dry type FD 95C12, LabX). Lyophilized banana pulp was pooled and homogenized to a fine powder by blender and kept in sealed zip bags in the dark at −20 °C.

Genotype confirmation by molecular markers and ploidy analysis of plant material

The molecular marker and ploidy analysis using flow cytometry of *Musa* AA 'Kluai Khai' were performed according to the previous standard protocol (Boonruangrod et al., 2009). Genomic DNA was extracted from dried banana pulp to compare with five gene-pool specific primer pairs in PCRs experiments and visualized on agarose gel under UV light. For ploidy analysis, nuclear DNA content of *Musa* AA 'Kluai Khai' was extracted from young leaf tissue and then was analyzed using a Quantum P flow cytometer (Quantum Analysis GmbH, Germany) compared with a known diploid banana wild type M. balbisiana, HB224 'Kluai Tani' and a triploid ABB, HB106 'Kluai Namwa'.

Extraction of carotenoids

Carotenoids extraction protocol was modified from the previous study (Davey et al., 2006). 50 mg aliquot of powdered pulp was transferred to a 2 mL microtube containing 500 mL of 0.25% BHT in THF: MeOH (1:1, v/v) as an extraction solvent. The suspension was further homogenized by vortex mixer at setting 6.0 for 30 sec and then was centrifuged for 15 min at 14,000 rpm. The supernatant was collected and the extraction was then repeated twice more. The collected supernatants were combined and filtered through disposable 0.45 μm filters prior to HPLC analysis.

RP-HPLC analysis of β-carotene

Carotenoid extracts were analyzed by RP-HPLC using a Shimadzu LC-20AT system. The separations were carried out at room temperature on HyperClone (Phenomenex) (C_{18}, 250×4.6 mm, 5μm) column using isocratic run of 10% EtOAc:MeOH (1:1, v/v) in ACN at a flow rate of 1 mL/min. The signal of carotenoids was monitored at

450 nm at a resolution of 4 nm using a photodiode array detector. β-Carotene in all samples were quantified using a freshly prepared standard β-carotene as a standard curve. The HPLC system was controlled, data collected and integrated using LC solution software. The modified HPLC method was validated according to the previous report using the determination of analytical parameters: linearity range, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy (Pramote et al., 2018).

RESULTS AND DISCUSSION

Morphology and TSS of banana fruits in different harvest period

From our studies, hand and finger sizes of *Musa* AA 'Kluai Khai' in March, May and July, 2019 were measured (Fig. 1). In terms of hand size, their lengths were about 30 cm for bananas in March and July (Fig. 1a and 1c), while it was about 20 cm for the bananas in May (Fig. 1b). Corresponding to hand size, the finger length and diameter were about 7 cm and 3 cm for banana in May (Fig. 1e), respectively, while they were about 10.5 cm and 4 cm for banana in March and July (Fig. 1d and 1f), respectively. According to the previous report, the size of banana in this study was nearly the same with banana from Chanthaburi and Sukhothai provine in July, but larger and smaller than those in March and May, 2011, respectively (Sangudom et al., 2014a; Sangudom et al., 2014b). The smallest size of banana in May might be from the climate variability of Thailand in 2019. In May, the higher average temperature (32.1 °C) than normal caused the long drought weather including summer thunderstorm (Thai Meteorological Department, 2019c) which destroyed most of the banana threes in the area leaving only dwarf fruits. Although the average temperature in March and July (29.3 and 30.0 °C, respectively) were higher than normal, they were lower than May and there was no thunderstorm (Thai Meteorological Department, 2019a; Thai Meteorological Department, 2019b). This might be also explained by the occurrence of low rainfall amount in April (18.9 mm), the fruit development period of harvest in May, while, the higher rainfall amount in February (94.5 mm) and June (144.3 nm), the fruit development period of harvest in March and July, respectively, influenced the lager banana. (Meteorological Department, 2019d; Thai Meteorological Department, 2019e; Thai Meteorological Department, 2019f).

For TSS evaluation of banana pulp for each ripening stage and month (Fig. 2), a portable refractometer was used. It was found that TSS was directly proportional to ripening stage for all harvest periods (Table 1). Furthermore, banana in March had a little higher TSS (31.7 ± 0.8%) at the stage 4 than banana from May (26.9 ± 0.9%) and July (27.2 ± 11%) which was consistent to the previous study that addressed the highest TSS of cool season and the lower TSS of summer and rainy season (Sangudom et al., 2014b). The previous report also showed no effect of harvest maturity on TSS with or without ethephon ripening for all seasons, while, bagging materials affected to TSS in winter season (Sangudom et al., 2014a; Sangudom et al., 2014b).

Table 1: Total soluble solid of *Musa* AA 'Kluai Khai' of each stage in different month

Month	Total soluble solid of each ripening stage[a] (%)			
	1	2	3	4
March	4.3±0.8	18.0±1.5	27.2±0.3	31.7±0.8
May	2.4±0.4	10.5±3.7	24.0±1.6	26.9±0.9
July	3.2±0.2	18.0±0.6	25.6±1.4	27.2±1.1

[a]Stage 1, 2, 3, and 4 = green, light green, yellow, and yellow with brown spot, respectively. Data are shown as mean±standard deviation of three replicates.

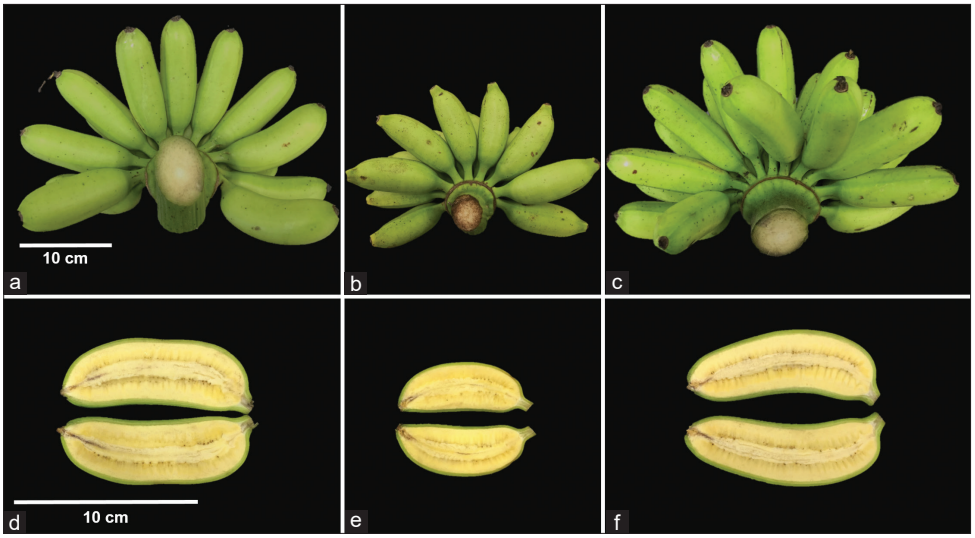

Fig 1. Size of *Musa* AA hand in a) March, b) May, and c) July and longitudinal section of the finger in d) March, e) May, and f) July.

Fig 2. *Musa* AA in March, May, and July at each stage.

Genotype confirmation by molecular markers and ploidy analysis

In order to confirm the genotype of the material used in the experiments, DNA from dried pulp of 'Kluai Khai' banana was extracted and the genotype was identified by M. acuminata gene-pool specific PCR compared with known genotypes. The results revealed that the material, 'Kluai Khai', used in this study had a genome composition of A1/A4a/A4b (Fig. 3), which was the same as an AA cultivar in Sucrier group ITC0653 Pisang mas, reported by previous report (Boonruangrod et al., 2009).

For ploidy analysis, a 'Kluai Khai' leaf was collected from the same merchant and placed with the banana and analyzed by comparing with two known diploid and triploid banana as controls, i.e., a diploid wild type *M. balbisiana* (HB224 'Kluai Tani') and a triploid ABB (HB106 'Kluai Namwa'), collected from the Banana Collection of the Department of Horticulture at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The diploid HB224 'Kluai Tani' was set at channel 200 (Fig. 4a) as a standard for diploid, then HB106 'Kluai Namwa' analysis was shown approximately on channel 300 (Fig. 4b). Analyses of leaf tissue mixtures of HB224 'Kluai Tani' and HB106 'Kluai Namwa' (Fig. 4c) and 'Kluai Khai' and HB106 'Kluai Namwa' (Fig. 4d) similarly showed two peaks at channels 200 and 300 confirming that the ploidy level of 'Kluai Khai' was diploid.

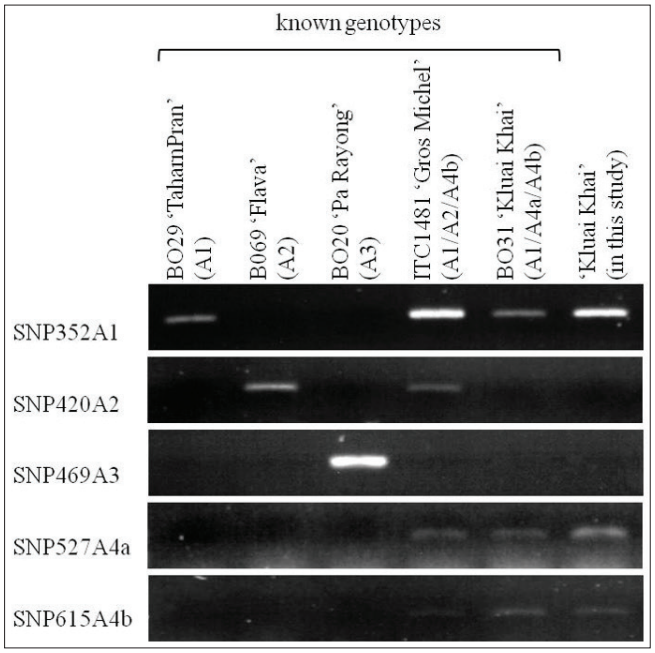

Fig 3. Agarose gel electrophoresis of five *M. acuminata* gene-pool specific PCRs: SNP352A1 (ssp. *zebrina* gene-pool), SNP420A2 (ssp. *malaccensis* gene-pool), SNP469A3 (ssp. *burmanica*/ ssp. *burmanicoides*/ ssp. *siamea* gene-pool) SNP527A4a (ssp. *banksii*/ ssp. *errans*/ ssp. *microcarpa* gene-pool) and SNP615A4b (ssp. *banksii*/ ssp. errans/ ssp. *microcarpa*/ ssp. *malaccensis* gene-pool), for five known genotypes and 'Kluai Khai' used in this study. Known genotypes: BO29 'Taharn Pran' (ssp. *zebrina* gene-pool), BO69 'Flava' (ssp. *malaccensis* gene-pool), BO20 'Pa Rayong' (ssp. *burmanica*/ ssp. *burmanicoides*/ ssp. *siamea* gene-pool) and ITC1481 'Gros Michel' containing both A4a and A4b alleles and confirmed genotype BO31 'Kluai Khai' (A1/A4a/A4b) (Boonruangrod, unpublished data).

Preparation of carotenoids extracts from *Musa* AA 'Kluai Khai'

The procedure of carotenoids extract preparation was composed of three major steps: lyophilization, homogenization, and extraction (Fig. 5). Lyophilization was utilized to remove moisture from the banana pulp under ultrahigh vacuum and low temperature. Drying via this process would prevent carotenoids compound from heat-assisted degradation. Homogenization using blender converted dried banana pulp to be fine power which easier to be suspended in the solvent used in extraction process.

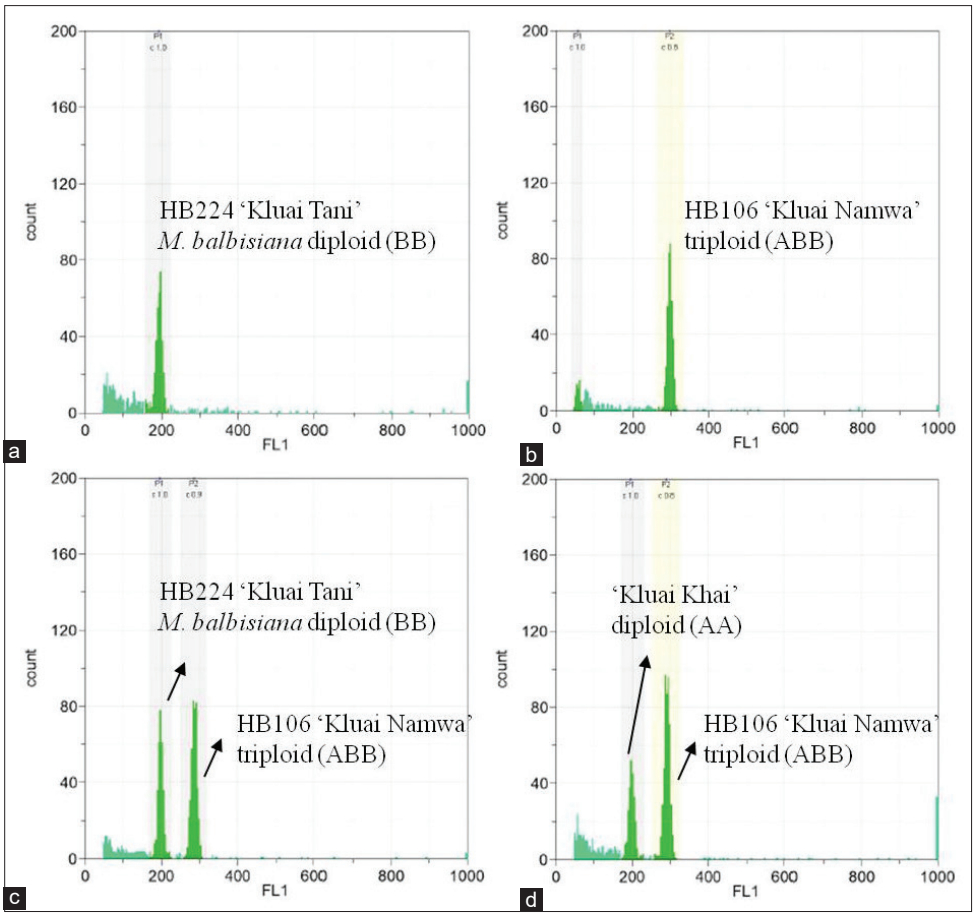

Fig 4. Histogram of relative nuclear DNA content from fresh leaves of 'Kluai Khai' compared with known genotypes HB224 'Kluai Tani'- *M. balbisiana* (BB) and HB106 'Kluai Namwa' - ABB group.

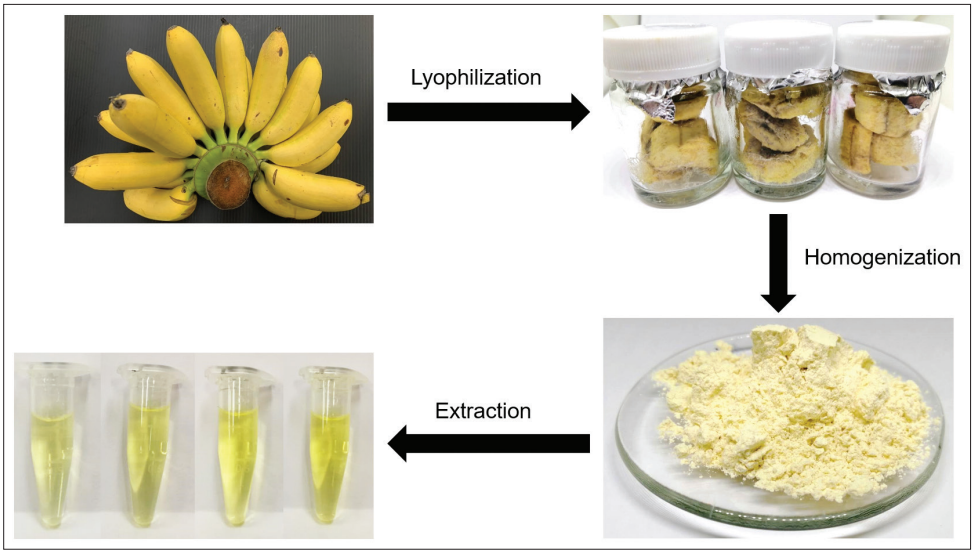

Fig 5. The carotenoid extracts from *Musa* AA 'Kluai Khai' of each stage in July.

β-Carotene is more nonpolar and non-volatile compound than others in carotenoid group, therefore, extraction conditions of β-carotene was reported using organic solvent rather than aqueous conditions including sole acetone (Amah et al., 2019; Aquino et al., 2018; Ngoh Newilah et al., 2009; Zeb and Murkovic, 2013), ethanol (Davey et al., 2006), and chloroform (Fungo and Pillay, 2011). Mixtures of various organic solvents such as hexane, methanol, ethanol, acetone, dichloromethane, and chloroform were also addressed as extraction buffers

for β-carotene (Englberger et al., 2006; Fu et al., 2019; Heng et al., 2017). In this study, as a popular extraction buffer for carotenoids from banana (Borges et al., 2018; Davey et al., 2009; Ekesa et al., 2013; Ekesa et al., 2015), mixture of methanol and tetrahydrofuran (1:1) was used to extract β-carotene to yield a yellowish solution. The process was modified from the previous reports for analysis of carotenoid-rich fruits to avoid the heating step and saponification which might cause the degradation and loss of β-carotene (Davey et al., 2006; Davey et al., 2009). Although saponification was recommended to simplify HPLC chromatogram in the analysis of plant carotenoids by several reports (Granado et al., 2001; Schierle et al., 2004), it did not affect carotenoids HPLC chromatogram for banana pulp (Davey et al., 2006). The color intensity of the extracts was shown as an absorbance at 450 nm using microplate reader spectrophotometer and clearly proportional to the ripening stage as observed by the naked eye due to the content of banana pulp pigment for each stage, for example the carotenoid extract from *Musa* AA 'Kluai Khai' in July (Fig. 6). The color intensity of the stage 2 was rapidly increased from the stage 1 but gradually increased when it turned to stage 3 and 4.

Quantitative analysis of β-carotene content

There were various techniques reported for the analysis of carotenoids. In order to distinguish between all-*trans*-β-carotene and *cis*-β-carotene, the Raman and Fourier transform infrared (FTIR) spectroscopy techniques were used (Stutz et al., 2015), while the total carotenoid content was measured using UV-vis spectrophotometer (Talcott and Howard, 1999). For quantitative analysis of each compound in the group, several separation and detection methods were considered. Gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE) were utilized to separate carotenoids (Stutz et al., 2015). For mass spectrometry (MS), electrospray ionization (ESI) was not appropriate for β-carotene analysis because of its hydrophobicity (Rivera et al., 2011). To improve its ionization property, atmospheric pressure chemical ionization (APCI) and collision induced dissociation (CID) were performed (Facundo et al., 2015; Nakornriab et al., 2008; Rivera et al., 2011). In this study, as a simple, convenient, and popular technique for analysis of β-carotene in banana, reverse phase high performance liquid chromatography (RP-HPLC) coupled with diode array detection (DAD) were used to quantify β-carotene in *Musa* AA 'Kluai Khai' pulp extract. The HPLC separation chromatogram displayed a major peak of *all-trans*-β-carotene, a minor peak of *all-trans*-α-carotene, and trace of *cis*-β-carotene at 19.6, 18.5, and 22.0 min, respectively (Fig. 7b), when compare to standard β-carotene (Fig. 7a). All forms of carotenoids observed in the HPLC chromatogram and the sequence of elution time corresponding to their hydrophobicity were characterized by the previous report (Davey et al., 2006; Davey et al., 2009; Schierle et al., 2004; Zeb and Murkovic, 2013). Additionally, the validation of HPLC method used in this study was also conducted using the retention time and peak area according to the previous report (Pramote et al., 2018). The following analytical parameters were evaluated; linearity range, limit of detection (LOD), limit of quantification (LOQ), intra-day precision, inter-day precision, and accuracy as 3-50 ppm, 0.32 ppb, 1.1 ppb, 2.5 %RSD, 3.1 %RSD, and 95.4% recovery, respectively.

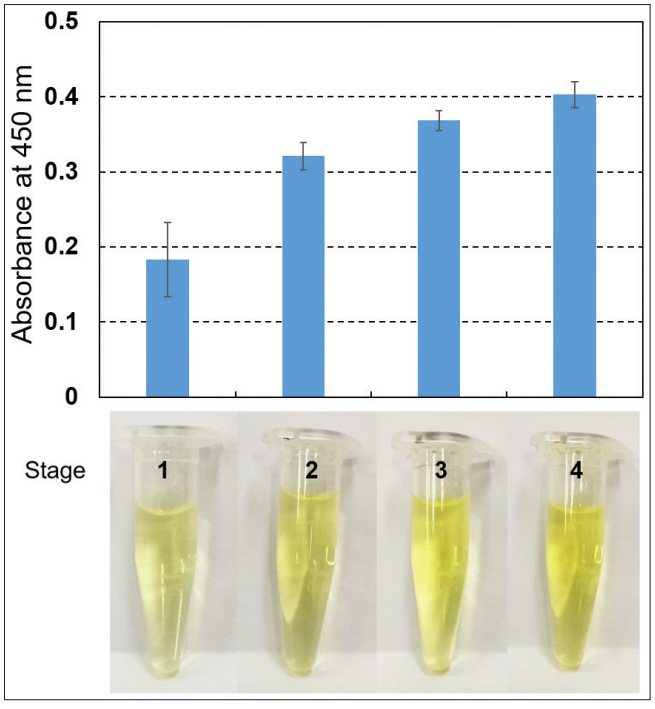

Fig 6. Logarithmic plot between β-carotene content (µg / 100 g of fresh weight) in *Musa* AA 'Kluai Khai' vs TSS (%Brix) of different month.

Content of β-carotene at different month and ripening stage defined by TSS

The content of β-carotene in various banana cultivars from all over the world have been evaluated over the last decade at different ripening stages defined by various parameters including days from blossom removal (Sangudom et al., 2014b), days from climacteric (Lokesh et al., 2014), and peel color (Amah et al., 2019; Aquino et al., 2018; Borges et al., 2018; Ekesa et al., 2013; Ekesa et al., 2015; Englberger et al., 2006; Fu et al., 2019; Ngoh Newilah et al., 2009) but not for TSS. The correlation between TSS and ripening stage was described by Soltani and Omid (Soltani et al., 2011). TSS is a quadratic function to gauge the ripening stage as defined by days, but also by peel color. In this study, β-carotene content in *Musa* AA 'Kluai Khai' pulp was firstly exhibited with the correlation of different ripening stages as defined by TSS at different months (Table 2). For our results, the β-carotene content increased along with the

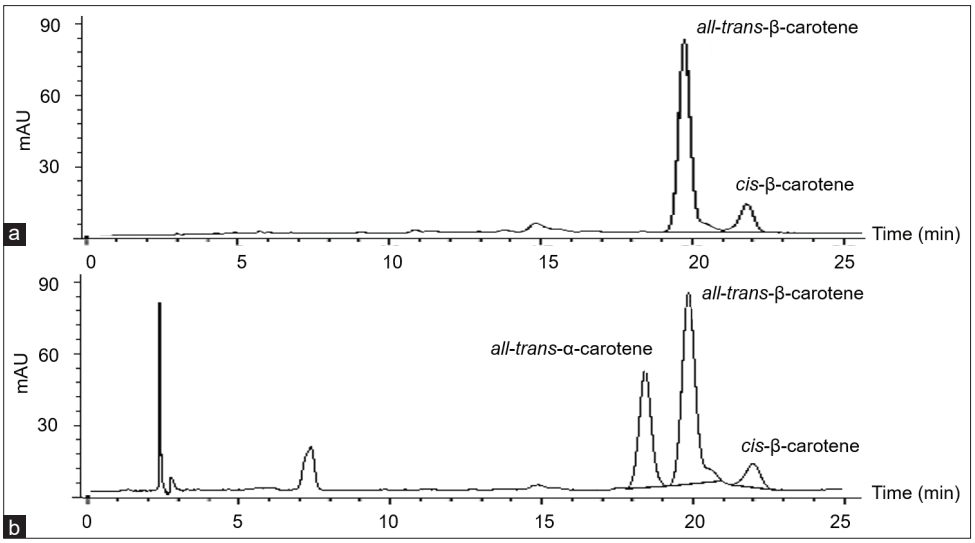

Fig 7. Quantitative analysis of ß-carotene content.

Table 2: β-Carotene content in *Musa* AA 'Kluai Khai' of each stage in different month

Month	β-Carotene content of each ripening stage[a] (µg/ 100 g of fresh weight)			
	1	2	3	4
March	645.9±38.0	4130.0±102.4	4567.8±118.9	5222.6±83.8
May	1179.5±84.8	2804.6±60.8	2853.2±20.3	3121.8±108.6
July	1686.4±38.8	3324.4±57.1	3879.4±74.8	4072.8±33.4

[a]Stage 1, 2, 3, and 4 defined by total soluble solid (Table 1). Data are shown as mean±standard deviation of three replicates.

ripening stages for all month which was consistent to other reports (Aquino et al., 2018; Ekesa et al., 2015; Lokesh et al., 2014; Ngoh Newilah et al., 2009). In *Musa* AA 'Kluai Khai' pulp, as shown in Table 2, the β-carotene content ranged from 645.9 - 5222.6 µg/ 100 g of fresh weight (gfw). The highest content at each month was observed at the fourth state, while the highest content at March (5222.6 µg/ 100 gfw) was higher than July (4072.8 µg/ 100 gfw) and May (3121.8 µg/ 100 gfw), respectively, and higher than those in 'Kluai Khai' from other reports (Charoensiri et al., 2009; Englberger et al., 2006; Heng et al., 2017; Sangudom et al., 2014b).

Additionally, the relationship between the ripening stages of banana and the content of β-carotene depended on various cultivars. There were both reciprocal proportion relation (Aquino et al., 2018; Ngoh Newilah et al., 2009) and ripening state-independent relation (Borges et al., 2018; Ekesa et al., 2013; Ngoh Newilah et al., 2009). For our study, on the other hand, it was a logarithmic relationship to TSS (%Brix) with R^2 = 0.9901, 0.9131, and 0.9857 for March, May, and July, respectively (Fig. 8). The lowest β-carotene content of banana pulp in May might be from the long drought weather and the higher average temperature (32.1 °C) than normal of Thailand in 2019

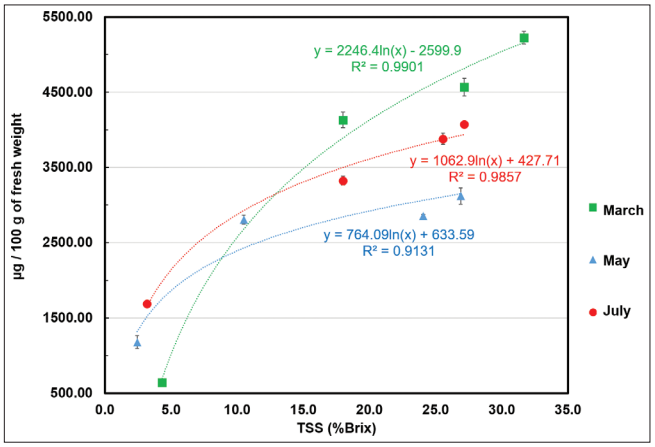

Fig 8. Content of ß-carotene at different month and ripening stage defined by TSS.

(Thai Meteorological Department, 2019c) which influenced the availability of isoprenoid precursors for β-carotene (Saini and Keum, 2018).

The carotenoid biosynthesis and storage are well established in the plastids of plant cells (Sun et al., 2018). Plastid-localized 2-C-methyl-D-erythretol 4-phosphate (MEP) derived from isoprenoid unit is a precursor for β-carotene biosynthesis or MEP pathway (Phillips et al., 2008). Drought and high temperature in summer are two of abiotic factors that limit the availability of isoprenoid unit and alter the downstream of MEP pathway (Cazzonelli and Pogson, 2010). Amyloplast and chromoplast are two organs in the group of plastids that play an important role of carotenoid biosynthesis in colored plants (Sun et al., 2018). In amyloplast, limited carotenoid production is observed because of the low transcription of crucial enzyme activity in carotenoid biosynthesis (Bai et al., 2016;

Vallabhaneni and Wurtzel, 2009) and the lack of lipoprotein sequestering substructure responsible for carotenoid synthesis and storage (Lopez et al., 2008). Nevertheless, chromoplasts developed from other plastids, including amyloplast and chloroplast is a main organelle in which carotenoid biosynthesis is highly productive due to the presence of various lipoproteins (Sun et al., 2018). For the duration of banana ripening states, the higher conversion of amyloplasts to chromoplasts between full green state to full ripe state was detected (Buah et al., 2016), therefore, the burst of β-carotene content is observed when the banana was turning to yellow. However, due to the unexpected long drought weather and thunderstorm in year 2019, the study was amended to report only for year 2019, not represent for a normal climate in Thailand. Thus, for further improvement of the study, the data will be collected at least 2 years for the effect of time, climate, temperature, and cultivation practice during fruit development.

CONCLUSIONS

A banana that is well-known in Thailand for its high β-carotene content, is the *Musa* AA 'Kluai Khai' which was studied over a period of several months. The banana in May was found to be smaller than the other months due to the climate variability in Thailand during 2019. However, the study of TSS in banana pulp for March, May, and July disclosed a directly proportional correlation to the ripening stage. The genotype and ploidy of *Musa* AA 'Kluai Khai' were confirmed using molecular markers and flow cytometry, respectively. An efficient extraction protocol yielded intense yellowish extracts of banana pulp pigment. The utilization of the HPLC revealed β-carotene content in *Musa* AA 'Kluai Khai' pulp as high as 5222.6 ± 83.8 μg / gfw in the 4th state of the banana in March. The relationship between β-carotene content and the ripening state were calculated to be a logarithmic correlation. The climate and ripening state may influence the availability of the precursor in β-carotene biosynthesis and the conversion of amyloplast to chromoplast, respectively, which directly affected the β-carotene content in banana. This finding might be useful as preliminary data of β-carotene in *Musa* AA 'Kluai Khai' pulp for further exploration in breeding of banana, nutraceutical as well as food preservation industry.

ACKNOWLEDGEMENTS

The authors acknowledge the Thailand Research Fund (TRF) for financial support (grant number IRN61W005) for this study. We also would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC) for partial financial support and Faculty of Pharmaceutical Sciences, Naresuan University for facilities support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Authors' contributions

Jarupa Viyoch: Research supervision; Funding acquisition; Conceptualization; Experiment design; Manuscript review. Kongaphisith Tongpoolsomjit: Raw material resource; Conceptualization; Experiment design; Experiment performer; Data analysis; Scientific writing. François Grandmottet: Manuscript review. Akharapong Krueajan: Raw material resource; Experiment performer. Ratri Boonruangrod: Experiment design, Experiment performer; Data analysis; Scientific writing.

REFERENCES

Amah, D., E. Alamu, M. Adesokan, A. van Biljon, B. Maziya-dixon, R. Swennen and M. Labuschagne. 2019. Variability of carotenoids in a *Musa* germplasm collection and implications for provitamin A biofortification. Food Chem. 12: 100024.

Aquino, C.F., L. C. C. Salomão, H. M. Pinheiro-Sant, S. M. R. Ribeiro, D. L. D. Siqueira and P. R. Cecon. 2018. Carotenoids in the pulp and peel of bananas from 15 cultivars in two ripening stages. Rev. Ceres. 65: 217-226.

Bai, C., T. Capell, J. Berman, V. Medina, G. Sandmann, P. Christou and C. Zhu. 2016. Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor product balance. Plant Biotechnol. J. 14: 195-205.

Pramote, B., N. Waranuch and O. Kritsunankul. 2018. Simultaneous determination of gallic acid and catechins in banana peel extract by reversed-phase high performance liquid chromatography. NUJST. 26: 189-200.

Boonruangrod, R., S. Fluch and K. Burg. 2009. Elucidation of origin of the present day hybrid banana cultivars using the 5'ETS rDNA sequence information. Mol. Breed. 24: 77-91.

Borges, C.V., I. O. Minatel, E. P. Amorim, M. A. F. Belin, H. A. Gomez-Gomez, C. R. Correa, G. P. P. Lima. 2019. Ripening and cooking processes influence the carotenoid content in bananas and plantains (*Musa* spp.). Food Res. Int. 124: 129-136.

Buah, S., B. Mlalazi, H. Khanna, J. L. Dale and C. L. Mortimer. 2016. The quest for golden bananas: Investigating carotenoid regulation in a Fe'i group *Musa* cultivar. J. Agric. Food Chem. 64: 3176-3185.

Cazzonelli, C.I. and B. J. Pogson. 2010. Source to sink: Regulation of carotenoid biosynthesis in plants. Trends Plant Sci. 15: 266-274.

Charoensiri, R., R. Kongkachuichai, S. Suknicom and P. Sungpuag. 2009. Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. Food Chem. 113: 202-207.

Davey, M. W., J. Keulemans and R. Swennen. 2006. Methods for the efficient quantification of fruit provitamin A contents. J. Chromatogr. 1136: 176-184.

Davey, M. W., J. Mellidou and W. Keulemans. 2009. Considerations to prevent the breakdown and loss of fruit carotenoids during extraction and analysis in Musa. J. Chromatogr. 1216: 5759-5762.

Davey, M. W., E. Stals, G. Ngoh-Newilah, K. Tomekpe, C. Lusty, R. Markham, R. Swennen and J. Keulemans. 2007. Sampling strategies and variability in fruit pulp micronutrient contents of

west and central african bananas and plantains (*Musa* species). J. Agric. Food Chem. 55: 2633-2644.

Ekesa, B., J. Kimiywe, I. Van den Bergh, G. Blomme, C. Dhuique-Mayer and M. Davey. 2013. Content and retention of provitamin A carotenoids following ripening and local processing of four popular *Musa* cultivars from eastern Democratic Republic of Congo. Sustain. Agric. Res. 2: 60-75.

Ekesa, B., D. Nabuuma, G. Blomme and I. Van den Bergh. 2015. Provitamin A carotenoid content of unripe and ripe banana cultivars for potential adoption in eastern Africa. J. Food Compos. Anal. 43: 1-6.

Englberger, L., R. B. H. Wills, B. Blades, L. Dufficy, J. W. Daniells and T. Coyne. 2006. Carotenoid content and flesh color of selected banana cultivars growing in Australia. Food Nutr. Bull. 27: 281-291.

Facundo, H. V. D, P. D. Gurak, A. Z. Mercadante, F. M. Lajolo and B. R. Cordenunsi. 2015. Storage at low temperature differentially affects the colour and carotenoid composition of two cultivars of banana. Food Chem. 170: 102-109.

Fiedor, J. and K. Burda. 2014. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 6: 466-488.

Fu, X., S. Cheng, C. Feng, M. Kang, B. Huang, Y. Jiang, X. Duan, D. Grierson and Z. Yang. 2019. Lycopene cyclases determine high α-/β-carotene ratio and increased carotenoids in bananas ripening at high temperatures. Food Chem. 283: 131-140.

Fungo, R. and M. Pillay. 2011. β-Carotene content of selected banana genotypes from Uganda. Afr. J. Biotechnol. 10: 5423-5430.

Granado, F., B. Olmedilla, E. Gil-Martinez and I. Blanco. 2001. A fast, reliable and low-cost saponification protocol for analysis of caretenoids in vegetables. J. Food Compos. Anal. 14: 479-489.

Gururao, K. S., V. N. Kudachikar and K. M. Prakash. 2011. Studies on physico-chemical changes during artificial ripening of banana (*Musa* sp) variety "Robusta". J. Food Sci. Technol. 48: 730-734.

Heinonen, M. I., V. Ollilainen, E. K. Linkola, P. T. Varo and P. E. Koivistoinen. 1989. Carotenoids in finnish foods: Vegetables, fruits, and berries. J. Agric. Food Chem. 37: 655-659.

Heng, Z., O. Sheng, S. Yan, H. Lu, I. Motorykin, H. Gao, C. Li, Q. S. Yang, C. Hu, R. Kuang, F. Bi, T. Dou, S. Xie, G. Deng and G. Yi. 2017. Carotenoid profiling in the peel and pulp of 36 selected *Musa* varieties. Food Sci. Technol. Res. 23: 603-611.

Leerach, N., S. Yakaew, P. Phimnuan, W. Soimee, W. Nakyai, W. Luangbudnark and J. Viyoch. 2017. Effect of Thai banana (*Musa* AA group) in reducing accumulation of oxidation end products in UVB-irradiated mouse skin. J. Photochem. Photobiol. B Biol. 168: 50-58.

Lokesh, V., P. Divya, B. Puthusseri, G. Manjunatha and B. Neelwarne. 2014. Profiles of carotenoids during post-climacteric ripening of some important cultivars of banana and development of a dry product from a high carotenoid yielding variety. LWT Food Sci. Technol. 55: 59-66.

Lopez, A. B., Y. Yang, T. W. Thannhauser and L. Li. 2008. Phytoene desaturase is present in a large protein complex in the plastid membrane. Physiol. Plant. 133: 190-198.

Nakornriab, M., T. Sriseadka and S. Wongpornchai. 2008. Quantification of carotenoid and flavonoid components in brans of some thai black rice cultivars using supercritical fluid extraction and high-performance liquid chromatography-mass spectrometry. J. Food Lipids 15: 488-503.

Ngoh, N. G., C. Dhuique-Mayer, J. Rojas Gonzalez, K. Tomekpe, E. X. Fokou and F. Etoa. 2009. Carotenoid contents during ripening of banana hybrids and cultivars grown in Cameroon. Fruits. 64: 197-206.

Phacharapiyangkul, N., K. Thirapanmethee, K. Sa-Ngiamsuntorn, U. Panich, C. H. Lee and M. T. Chomnawang. 2019. Effect of sucrier banana peel extracts on inhibition of melanogenesis through the ERK signaling pathway. Int. J. Med. Sci. 16: 602-606.

Phillips, M. A., P. León, A. Boronat and M. Rodríguez-Concepción. 2008. The plastidial MEP pathway: Unified nomenclature and resources. Trends Plant Sci. 13: 619-623.

Rao, A. V. and L. G. Rao. 2007. Carotenoids and human health. Pharmacol. Res. 55: 207-216.

Rivera, S., F. Vilaró and R. Canela. 2011. Determination of carotenoids by liquid chromatography/mass spectrometry: Effect of several dopants. Anal. Bioanal. Chem. 400: 1339-1346.

Saini, R. K. and Y. S. Keum. 2018. Significance of genetic, environmental, and pre- and postharvest factors affecting carotenoid contents in crops: A review. J. Agric. Food Chem. 66: 5310-5324.

Saini, R. K., S. H. Nile and S. W. Park. 2015. Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. Food Res. Int. 76: 735-750.

Sangudom, T., T. Wasusri, C. Wongs-Aree, V. Srilaong and S. Kanlayanarat. 2014a. Maruity index as related to growing season and supply chain managment of *Musa* (AA group) "Kluai Khai" in Thailand. Acta Hortic. 1026: 67-78.

Sangudom, T., C. V. Wongs-Aree, V. Srilaong, S. Kanlayanarat, T. Wasusri, S. Noichinda and W. Markumlai. 2014b. Fruit quality and antioxidant properties of "KluaiKhai" banana (*Musa* AA group) at different stages of harvest maturity. Int. Food Res. J. 21: 583-588.

Schierle, J., B. Pietsch, A. Ceresa, C. Fizet and E. H. Waysek. 2004. Method for the determination of β-carotene in supplements and raw materials by reversed-phase liquid chromatography: Single laboratory validation. J. AOAC Int. 87: 1070-1082.

Setiawan, B., A. Sulaeman, D. W. Giraud and J. A. Driskell. 2001. Carotenoid content of selected Indonesian fruits. J. Food Compos. Anal. 14: 169-176.

Soltani, M., R. Alimardani and M. Omid. 2011. Evaluating banana ripening status from measuring dielectric properties. J. Food Eng. 105: 625-631.

Stutz, H., N. Bresgen and P. M. Eckl. 2015. Analytical tools for the analysis of β-carotene and its degradation products. Free Radic. Res. 49: 650-680.

Sun, T., H. Yuan, H. Cao, M. Yazdani, Y. Tadmor and L. Li. 2018. Carotenoid metabolism in plants: the role of plastids. Mol. Plant 11: 58-74.

Talcott, S. T. and L. R. Howard. 1999. Phenolic autoxidation is responsible for color degradation in processed carrot puree. J. Agric. Food Chem. 47: 2109-2115.

Thai Meteorological Department. 2019a. Monthly Weather Summary in Thailand July Meteorological Development Division, Climatological Center, 2019. Available from: https://www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 Aug 30].

Thai Meteorological Department. 2019b. Monthly Weather Summary in Thailand March Meteorological Development Division, Climatological Center, 2019. Available from: https://www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 Apr 30].

Thai Meteorological Department. 2019c. Monthly Weather Summary in Thailand May Meteorological Development Division, Climatological Center, 2019. Available from: https://www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 Jun 25].

Thai Meteorological Department. 2019d. Monthly Weather Summary in Thailand April Meteorological Development Division, Climatological Center, 2019. Available from: https://

www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 May 30].

Thai Meteorological Department. 2019e. Monthly Weather Summary in Thailand February Meteorological Development Division, Climatological Center, 2019. Available from: https://www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 Mar 30].

Thai Meteorological Department. 2019f. Monthly Weather Summary in Thailand June Meteorological Development Division, Climatological Center, 2019. Available from: https://www.tmd.go.th/en/climate.php?FileID=4. [Last accessed on 2019 Jul 25].

Vallabhaneni, R. and E. T. Wurtzel. 2009. Timing and biosynthetic potential for carotenoid accumulation in genetically diverse germplasm of maize. Plant Physiol. 150: 562.

Viyoch, J., K. Mahingsa and K. Ingkaninan. 2012. Effects of Thai *Musa* species on prevention of UVB-induced skin damage in mice. Food Chem. Toxicol. 50: 4292-4301.

Yano, M., M. Kato, Y. Ikoma, A. Kawasaki, Y. Fukazawa, M. Sugiura, H. Matsumoto, Y. Oohara, A. Nagao and K. Ogawa. 2005. Quantitation of carotenoids in raw and processed fruits in Japan. Food Sci. Technol. Res. 11: 13-18.

Youryon, P. and S. Supapvanich. 2016. Quality and bioactive compounds of ripe “Kluai Nam Wa” and “Kluai Khai” bananas during storage. Int. Food Res. J. 23: 1027-1032.

Zeb, A. and M. Murkovic. 2013. Determination of thermal oxidation and oxidation products of β-carotene in corn oil triacylglycerols. Food Res. Int. 50: 534-544.