International Journal of Agricultural Technology 2023 Vol. 19(4):1657-1668
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Changes in the activity of enzymes associated with enzymatic browning and chemical composition during *Musa sapientum* Linn. 'Kluai Khai' banana fruit ripening

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Makboriboon, N., Teerarak, M. and Saetiew, K. (2023). Changes in the activity of enzymes associated with enzymatic browning and chemical composition during *Musa sapientum* Linn. 'Kluai Khai' banana fruit ripening. International Journal of Agricultural Technology 19(4):1657-1668.

Abstract The results showed that the initial firmness of banana fruit harvested at the raw stage was 7 N and decreased to less than 2 N at overripe and very ripe stages. The total soluble solids increased throughout the ripening period, whereas the titratable acidity maintained a high level during the ripening process. The banana peel stayed green during the raw stage, and the yellow color formed between the unripe stage to the overripe stage. The lightness of the banana peel increased from the raw stage to the ripe stage, decreased at the overripe stage, and then dropped sharply at the very ripe stage. This color change was linked to the browning incidence of the fruit peel and an increasing browning area and soluble browning pigment content. The PPO and POD showed a low activity at the raw stage and then increased with advancing ripening and maintained a significantly higher level than the raw fruit. The findings of this study exhibited that the ripening stages of the 'Kluai Khai' banana influence the firmness, peel color, and browning incidence of the fruit peel and the polyphenol oxidase and peroxidase activities.

Keywords: Banana, Browning pigment, Polyphenol oxidase, Peroxidase

Introduction

Banana fruit is an important horticultural crop that is high in nutrients and a high performer economically in domestic and international markets. Bananas are usually harvested at the pre-climacteric stage when the fruit is bright green and angular and the pulp is still hard. Bananas undergo various physical and chemical changes, including fresh weight loss, fruit softening, chlorophyll degradation, starch hydrolysis, and the synthesis of several volatile compounds, when ripening is initiated (Sheehy and Sharma, 2011). These changes contribute to bananas with a good appearance and desirable quality (soft texture, sweetness, and good aroma). As a climacteric fruit, banana fruit ripening is controlled by the plant hormone ethylene (Bleecker and Kende, 2000). Commercial banana cultivars grown in Thailand include the *Musa* AAA group 'Kluai Hom

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Thong', *Musa* ABB group 'Kluai Nam Wa', and *Musa* AA group 'Kluai Khai', (Youryon and Supapvanich, 2017). However, the *Musa sapientum* Linn. 'Kluai Khai' banana has an undesirable visual appearance due to browning discoloration during the late phase of senescence, while the 'Kluai Hom Thong' banana and 'Kluai Nam Wa' banana show few symptoms of browning peel (Ketsa, 2000). The appearance of the undesirable brown color in banana peel, known as senescent spotting, severely reduces its marketing value and the attractiveness of the fruit. It is widely accepted that the browning of the fruit tissue is primarily attributed to the interaction of phenolic compounds with polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POD; EC 1.11.1.7) (Yang *et al*., 2004; Fortea *et al*., 2009). The brown-colored substances occur in cells from ripe fruits where the loss of membrane integrity allows for contact between oxidative enzymes such as POD and PPO and their substrates such as phenolics (Su *et al*. 2005). PPO and POD are responsible for enzymatic browning and cause undesirable quality changes in various plant sources, such as 'Nam Dok Mai No. 4' mango (Chimvaree *et al*., 2019), avocado (*Persea americana* Mill.) (Vanini *et al*., 2010), loquat (*Eriobotrya japonica* Lindl.) (Zhang and Shao, 2015), lettuce (Chen *et al*., 2017; Rico *et al*., 2008), 'Williams' banana (*Musa* AAA group) (Lóay and Dawood, 2017), and 'Brazil' banana (*Musa* AAA group) (Huang *et al*., 2013). Based on the literature, a correlation between the development of the brown color on the surface and the PPO and POD activities of bananas was observed in the 'Giant Dwarf' (*Musa* AAA, subgroup Cavendish) and FHIA-23 (tetraploid hybrid, AAAA) banana cultivars. The browning level was higher for the 'Giant Dwarf' than for the FHIA-23 tissues. This finding correlated with higher PPO and POD activities in the 'Giant Dwarf' cultivar than those of the FHIA-23 banana cultivars (Escalante-Minakata *et al*., 2018). Therefore, this study aimed to investigate the impact of the different ripening stages on the changes in the activity of enzymes associated with the enzymatic browning and chemical composition during 'Kluai Khai' banana peel fruit ripening. The study aimed to provide a better understanding of the relation between brown color in banana peel and browning-related enzyme activities and to gain information for further developing technologies for the commercial postharvest handling of 'Kluai Khai' banana fruit during storage.

Materials and methods

Fruit sampling

Banana cv. Kluai Khai was purchased from a local banana orchard in the Chanthaburi province, Thailand. The bananas were harvested at 70–80% maturity when the fruits were bright green and angular. The fruits were selected to be free from defects and to have uniformity in green color and

size. They were cleaned in tap water and air-dried at room temperature. The bananas were allowed to ripen naturally at ambient temperature and were taken randomly from each hand according to their color appearance and the development of brown spots. As shown in Fig. 1, the banana maturity stages were classified into 5 stages namely, the raw stage (harvest stage), unripe stage, ripe stage, overripe stage, and very ripe stage (stage 5), which fell on the 3rd, 5th, 7th, and 9th day after harvest, respectively. Five fingers from each stage were randomly selected to assess the peel color, firmness, total soluble solids concentration (TSS), total acidity content (TA) content, senescent spotting appearance, and enzymes related to enzymatic browning.

Color measurement

The color values (*CIE L*a*b**) of the banana peel were measured in the middle section of five individual banana fruits from each stage using a colorimeter (Minolta, CM 600d, US). Each fruit was measured at three points from bottom to top on the fruit skin. The color values of lightness (*L**), redness (*a**), and yellowness (*b**) were recorded, and the color difference (*ΔE*) was calculated according to the following equation (Larraín *et al*., 2008): $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Firmness measurement

Bananas with five fingers from each hand were randomly stage selected for the firmness measurement. The banana fruit was peeled, and the measurement was taken by pressing down on the pulp 3 times per fruit at the head, middle, and tip positions using a fruit hardness tester. The firmness value was expressed as Newtons (N).

Total soluble solids (TSS) and Titratable acidity (TA)

The banana fruit pulp was crushed with a mortar and distilled water was added at a ratio of 1:3. The mixture was centrifuged at 8000 rpm for 5 min. The obtained extract was collected for TSS and TA determination. The TSS concentration of the fruit was measured using a digital refractometer (ATAGO, Japan) and was adjusted to 0 with distilled water before use. The data were expressed as °Brix. For TA determination, a 1 mL aliquot of the extract was brought up to 50 mL with distilled water and was measured with a pocket Brix-acidity meter. Total acidity was defined as the percentage of TA (% malic acid).

Senescent spotting appearance, brown area, and browning index

Senescent spotting appearance was divided according to the intensity of the peel surface browning as follows**:** 1= yellow skin and no senescent spotting; 2 = more yellow skin and very small senescent spotting; 3 = the brown freckles spread all over the skin and larger scattering points are also distributed separately; 4 = the brown freckles increase in size and become dark brown and senescent spot may coalesce and become pitted.

The brown area was divided according to the amount of senescent spotting on the surface as follows: 1 = very little 1-20% of the area; 2 = little 21-40% of the area; 3 = moderate 41-60% of the area; 4 = 61-80% of the area; 5 = most 81-100% of the area.

The browning index was determined using a modification of the method described by Supapvanich *et al*. (2011). A banana peel sample (10 g) was extracted with 30 mL of 2% acetic acid for 10 min and blended for 30 seconds. The obtained extracts were filtered with filter paper and measured at 420 nm with a spectrophotometer. The browning index was expressed as arbitrary absorbance units.

Enzymic activity assays

Sixty grams of peel tissues from five fingers in each stage were homogenized in a blender with 150 mL of 0.05 M sodium phosphate buffer, pH 7.0 containing 3% polyvinylpolypyrrolidone and centrifuged at 10000 ×g for 20 min. The supernatant was collected for the enzyme assay.

PPO activity was measured according to Jung and Watkins (2011). The reaction mixture contained in 3 mL: 2.9 mL substrate solution (10 mM catechol solution in 0.05 M sodium phosphate buffer, pH 7.0) and 0.1 mL of the enzyme extract. POD activity was measured according to Jung and Watkins (2011). The assay mixture consisted of 0.1 mL of 4.0% guaiacol, 0.1 mL of 0.46% H_2O_2, 2.75 mL of 0.05 M sodium phosphate buffer, pH 7.0, and 0.05 mL of crude enzyme. The increase in absorbance at 398/470 nm for PPO/POD, respectively, was recorded at intervals of 30 s for 4 min by a spectrophotometer. The enzyme activity was expressed as Units/mL.

Statistical analysis

The data were statistically analyzed using a completely randomized design with four replicates using an analysis of variance. The means were compared at P<5% using Tukey's studentized range test.

Results

Color and ΔE

Samples harvested at the mature-green fruit stage were allowed to naturally ripe at ambient temperature. Table 1 shows the results of the color (*CIE L*a*b**) values of the banana peels and the *ΔE* during storage. For the color values, the lightness (*L** value) increased from the raw stage to the ripe stage, decreased at the overripe stage, and then dropped sharply at the very ripe stage. The redness (*a** value) sharply increased during the ripening process. The yellowness (*b** value) slightly increased and then decreased at the end of the ripening. The *ΔE* value increased as the storage advanced. The color of the banana peel stayed green at the raw stage, the yellow color formed between the unripe stage to the overripe stage, and brown spots began to appear on the yellow areas at the unripe stage.

Table 1. Changes in color value (*CIE L* a* b**) and color difference (ΔE) in 'Kluai Khai' banana fruit during ripening

Ripening stages	***L**** (Lightness)	***a**** (Redness)	***b**** (Yellowness)	**Δ*E*** (Color difference)
Raw	62.31±3.91 c[1/]	−11.70±0.66 d	43.50±0.40 b	0.00±0.00 c
Unripe	81.34±2.66 a	3.84±1.60 c	52.00±2.86 a	26.10±3.35 b
Ripe	81.70±3.50 a	11.70±1.79 b	52.52±2.86 a	31.80±3.73 b
Overripe	70.43±1.40 b	15.73±2.33 a	52.40±4.86 a	30.26±3.03 b
Very ripe	30.30±6.69 d	8.60±1.36 b	14.42±4.10 c	48.04±5.70 a

[1/] Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ by Tukey's studentized range test.

Firmness, TSS and TA

Changes in the fruit firmness, TSS and TA contents during banana ripening are shown in Figure 2. The results showed that the initial firmness of banana fruit harvested at the raw stage was 7 N and decreased to less than 2 N at overripe and very ripe stages (Figure 1A). There was a significant difference in firmness at all stages of the ripening process. The TSS in the 'Kluai Khai' banana fruit increased throughout the ripening period. The TSS concentration in the 'Kluai Khai' banana fruit increased significantly from 3.76 °Brix at the raw stage to 26.88 °Brix at the very ripe stages. The highest TSS concentration was detected in the overripe and very ripe banana fruits (Figure 2B). As shown in Figure 2C, TA in the banana pulp was at a high level at the harvest stage and then slightly decreased during ripening. In general, the TA content maintained a high level during the ripening process.

Raw stage **Unripe stage** **Ripe stage**

Overripe stage **Very ripe stage**

Figure 1. The appearance of raw, unripe, ripe, overripe, and very ripe stages of ‘Kluai Khai’ banana fruit during ripening

Senescent spotting appearance, browning area, and browning index

The senescent spotting appearance score tended to increase, which was dependent on storage as shown in Figure 3. The samples from the raw and unripe fruits had a score of 1 point, and the scores were 2, 3, and 4 points at the ripe, overripe, and very ripe stages, respectively. The browning areas of the samples from the raw and unripe fruits were also at a score of 1 point and were 2.5, 3.5, and 4.5 points at the ripe, overripe, and very ripe stages, respectively. The senescent spotting appearance in this banana fruit was similar to the change in the browning area. Result revealed the effect of the different ripening stages on the browning index of the banana peels (Figure 4.). There was a progressive increase in the browning of the banana peels with the increasing storage period. In the raw fruit, the browning index was 0.60, and it slightly increased to 0.190, 0.300, and 0.380 in the unripe, ripe, and overripe banana pulp, respectively. Then, there was a sharp increase in the very ripe fruit, with a value of 1.650. There were significant differences in browning with the different ripening periods.

PPO and POD activities

The lowest PPO activity was detected at the raw stage with a value of 3.38±1.15 U/mL. The PPO activity of the unripe fruit was significantly increased from the raw fruit, and then maintained a relatively stable level when it turned from ripe to very ripe and maintained a significantly higher level than the raw fruit (Figure 5A). Like PPO activity, the lowest POD

activity was also detected at the raw stage with a value of 7.58±1.15 U/mL. POD activity increased with advanced ripening, particularly at the unripe and ripe stages, before decreasing at the overripe stage and markedly increasing when the fruit became very ripe (Figure 5B).

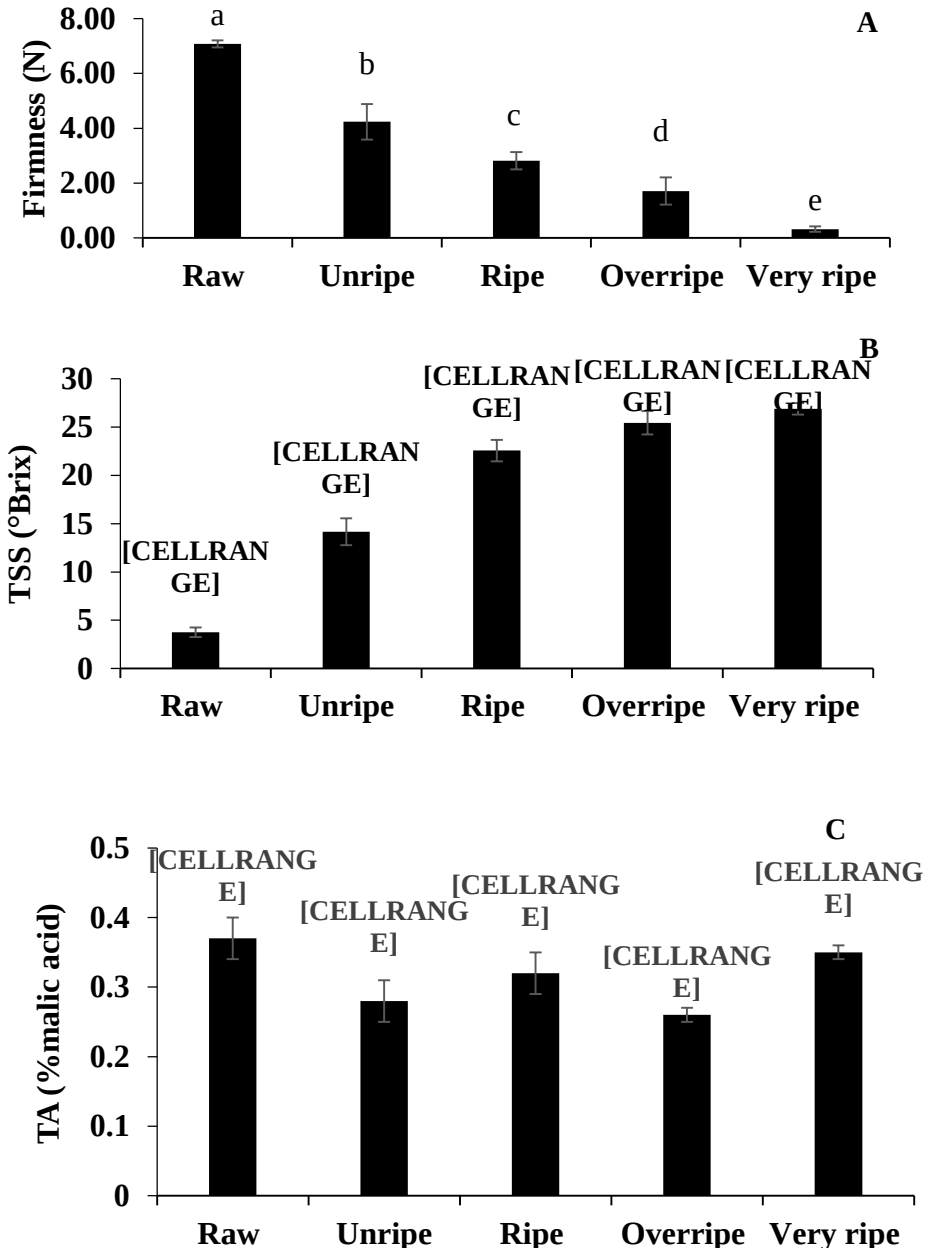

Figure 2. Firmness (A), total soluble solids (TSS) (B), and titratable acidity (C) in 'Kluai Khai' banana fruit during ripening

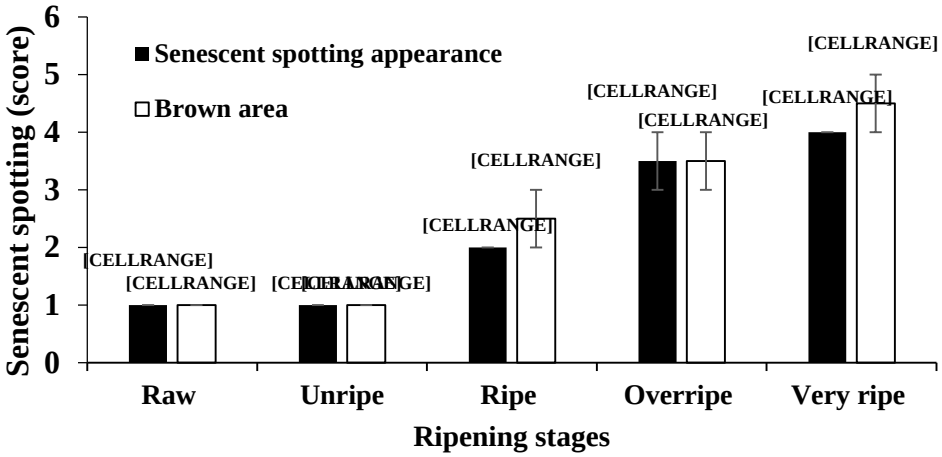

Figure 3. Senescent spotting appearance and brown area scores in 'Kluai Khai' banana fruit during ripening

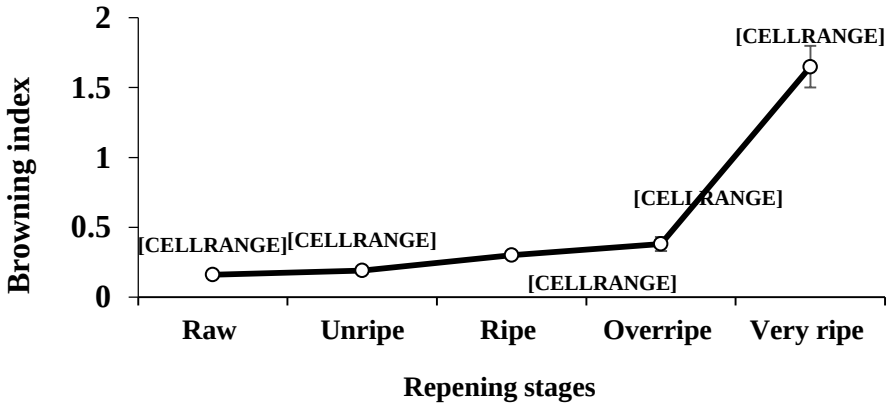

Figure 4. Changes in the browning index in 'Kluai Khai' banana fruit during ripening

Discussion

The color of the banana peel normally confirms changes in their stages from unripe to ripe (Mendoza and Aguilera, 2004). During the ripening process, 'Kluai Khai' banana peel color changes from bright green to yellow. Similar, these changes during ripening were also previously reported for, *Musa acuminata* (Bugaud *et al*., 2009), 'Kluai Leb Mue Nang' (*Musa* AA group) banana (Youryon and Supapvanich, 2017) and 'Grand Nain' (*Musa* AAA group) banana. Chlorophyll breakdown by the increase activity of chlorophyllase as well as β-carotene and xanthophyll accumulation during the ripening process is associated with changes in peel color (Seymour *et al*., 2008; Tongpoolsomjit *et al*., 2020). In this study, an increase in the lightness was accompanied by a decline in greenness and an

increase in yellowness when ripening was initiated, and a decreased lightness was concomitant with the browning spots, which increased in area and number at the overripe stage. The reduction in fruit lightness at the overripe stage was due to the browning appearance and continuous increase in area and number of browning spots during ripening. These results confirm those of Mendoza and Aguilera (2004) in bananas (*Musa cavendish*) where the lightness decreased at the later part of the ripening period as measured using a computer vision system and colorimeter.

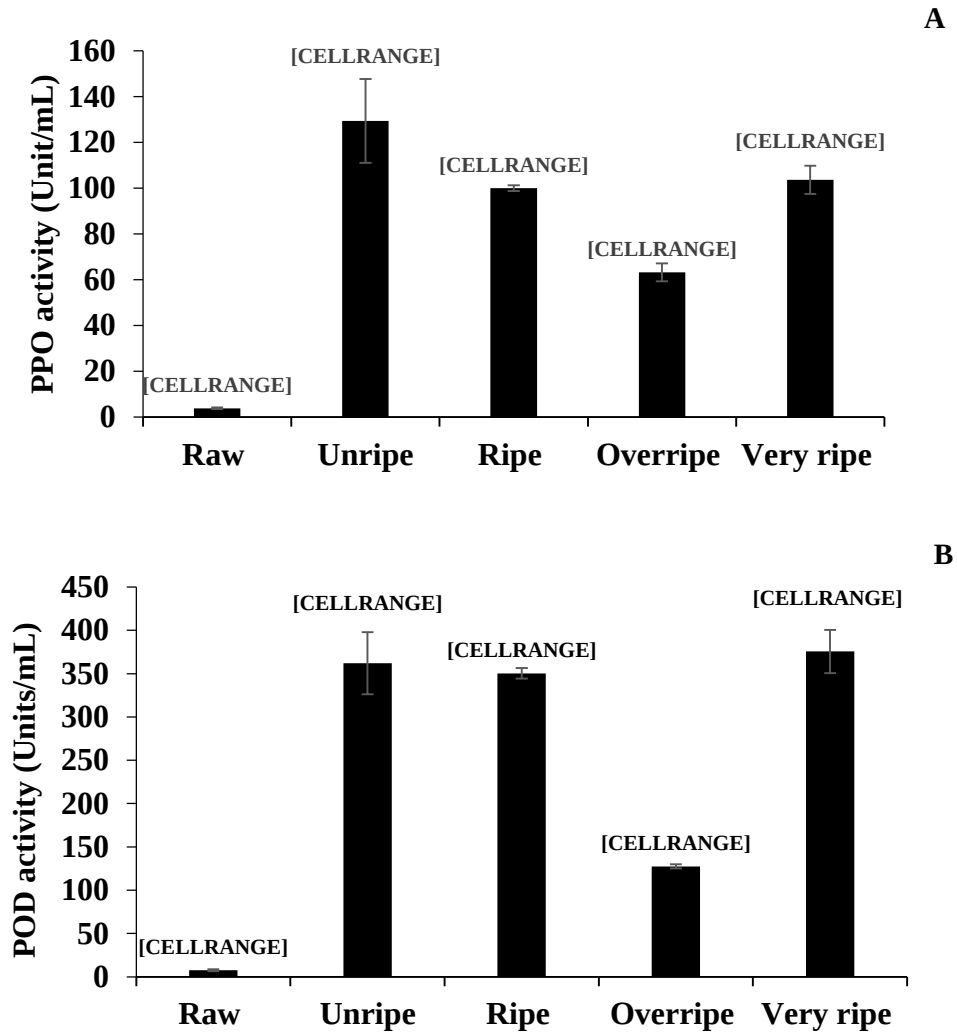

Figure 5. Changes in polyphenol oxidase (PPO) (A) and peroxidase (POD) (B) in 'Kluai Khai' banana fruit during ripening

'Kluai Khai' banana showed a decreasing firmness with advancing ripening. Fruit softening occurs mainly due to the degradation of insoluble protopectins into their soluble form and an increased starch to sugar conversion (Mattoo *et al*., 1975; Seymour *et al*., 1993). The TSS

concentration and TA content are most commonly associated with the taste of fruits. ‘Kluai Khai’ banana showed an increasing TSS concentration as ripening increased and was highest at overripe and very ripe fruits. TSS in banana fruit is most commonly associated with the breakdown of starch to soluble sugars (Siriboon and Banlusilp, 2004). Malic and citric acid are the predominant organic acids in ripe fruit and commonly measure in terms of titratable acidity (Seymour *et al.*, 1993). Differences in TA in ripe bananas vary among cultivars (Bugaud *et al.*, 2011). It has been reported that tomatoes, climacteric fruits, appear to utilize malate during respiration rise. However, the accumulation and utilization of organic acids might have been not directly related to the respiratory rate and climacteric characteristics of the fruit (Goodenough *et al*., 1985). For example, malate in bananas and mangos (*Mangifera indica*) appears to accumulate continuously throughout ripening, even at the peak of respiration (Selvaraj and Kumar, 1989). The TA of the banana pulp maintained a high-level content during ripening indicating that the ‘Kluai Khai’ banana may accumulate organic acids during ripening.

The important factors affecting the level of brown coloration on the fruit during ripening are the activity of enzymes and loss of cell membrane integrity (Lóay and Dawood, 2017). Browning is due to the hydroxylation of monophenols to o-diphenol and the oxidation of o-diphenol with oxygen to *o*-quinone by PPO (Pourcel *et al*., 2007). In the presence of hydrogen peroxide, POD can also catalyze the oxidation phenolic compounds leading to the formation of browning in fruit and vegetable tissues (Lengauer and Rarey, 1996). During ripening, the PPO and POD activities in the peels of the bananas when they turned from unripe to very ripe were considerably higher than that of the raw banana. Accordingly, the banana peel at the raw stage was free from brown spots and showed a continuous increase in brown spots from the unripe stage to the very ripe stage where brown freckles increased in size and became dark brown due to the fusion of spots and the concomitant enlargement of their size. These changes were similar to the trend observed for the browning index. Thus, these results suggest that increased PPO and POD activity leads to an increase in brown coloration on the peel of the banana fruit.

This study revealed that the increase in the lightness of the ‘Klua Khai banana peel was concomitant with a reduction of greenness and an increase in yellowness when ripening was initiated. Moreover, a decrease in lightness was concomitant with browning spots at the overripe stage where brown freckles increased in size and became dark brown due to the fusion of spots and the concomitant enlargement of their size. The TA content maintained a high level while the TSS content increased continuously during ripening. The lowest PPO and POD activities were detected in the mature green fruit. The highest browning index was detected in very ripe

fruit and was concomitant with an enlarged browning size and the fruit becoming dark brown.

Acknowledgements

This research was financially supported by the National Science Research and Innovation Fund (Grant No. RE-KRIS/FF65/01), Thailand. The authors thank King Mongkut's Institute of Technology Ladkrabang, Thailand for facility support.

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(Received: 15 October 2022, accepted: 18 June 2023)