Effects of bagging and storage temperature on anthocyanin content and phenylalanine ammonia-lyase (PAL) activity in mangosteen (*Garcinia mangostana* L.) fruit pericarp during maturation

Sujitra Ratanamarno[1], Jamnong Uthaibutra[2] and Kobkiat Saengnil[3]

Abstract

Ratanamarno, S.[1], Uthaibutra, J.[2] and Saengnil, K.[3]

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The purpose of this research is to investigate the effect of bagging and storage temperature on anthocyanin content and phenylalanine ammonia-lyase (PAL) activity of mangosteen fruit pericarp. Six maturity stages (stage 1 to stage 6, defined by the extension of red or purple colouration on the pericarp) of attached mangosteen fruits with bagged and unbagging were compared. It was found that sunlight had no significant effect on both anthocyanin content and PAL activity. The effect of storage temperature on anthocyanin content and PAL activity were also studied. Fruits at stage 1 (indicated by scattered of pink spot on pericarp) were harvested and allowed to develop red colour to stage 6 at different storage temperatures:

[1]Ph.D.(Biology), Asst. Prof., Department of Postharvest Technology, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290 Thailand. [2]Ph.D.(Postharvest Physiology), Asst. Prof., [3]Ph.D.(Applied Biochemistry), Asst. Prof., Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200 Thailand.
Corresponding e-mail: sujitra@mju.ac.th

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15º, 25º, 30º (room temperature) and 35ºC. It was found that temperature had no effect on anthocyanin content in any stage of fruit development. At all temperature levels, the anthocyanin content was increased accordingly and had the highest level at stage 6. Temperature affected on PAL activity at different stages. Levels of PAL activity decreased at the early stages and increased at the final stage of maturity except for fruits held at 25ºC, PAL activity remained at a low level through stage 6, while fruit at 35ºC had the highest level of PAL at stage 5.

Key words : mangosteen, anthocyanin, phenylalanine ammonia-lyase (PAL)

<div align="center">บทคัดย่อ</div>

สุจิตรา รตนะมโน[1] จำนงค์ อุทัยบุตร[2] และกอบเกียรติ แสงนิล[2]

ผลของการห่อผลและอุณหภูมิในการเก็บรักษาต่อปริมาณรงควัตถุและกิจกรรมของเอนไซม์
ฟีนิลอลานีนแอมโมเนียไลเอสในเปลือกผลมังคุดระหว่างการสุกแก่

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การทดลองนี้มีวัตถุประสงค์เพื่อศึกษาผลของการห่อผลมังคุดและอุณหภูมิในการเก็บรักษาที่มีต่อการเปลี่ยนแปลงปริมาณของแอนโทไซยานินและกิจกรรมของเอนไซม์ฟีนิลอลานีน แอมโมเนียไลเอส (PAL) ของเปลือกผลมังคุดในระหว่างการพัฒนาสีขณะที่ผลมีการสุกแก่ (ระยะที่ 1 ถึงระยะที่ 6 ดูจากการกระจายของสีม่วงแดงบนเปลือกผล) โดยทำการห่อผลและไม่ห่อผลบนต้นด้วยถุงกระดาษ พบว่าแสงไม่มีผลต่อการเปลี่ยนแปลงกิจกรรมของเอนไซม์ PAL และปริมาณแอนโทไซยานิน โดยกิจกรรมของเอนไซม์และปริมาณแอนโทไซยานินในผลที่ห่อและไม่ห่อเมื่อเปรียบเทียบกันในทุกระยะมีค่าไม่แตกต่างกันทางสถิติ ส่วนการศึกษาผลของอุณหภูมิขณะเก็บรักษานั้นทำโดยการเก็บผลมังคุดจากต้นในระยะที่ 1 นำมาเก็บรักษาไว้ที่อุณหภูมิแตกต่างกัน 4 ระดับ คือ 15, 25, 30 (อุณหภูมิห้อง) และ 35°C เมื่อผลมังคุดมีการพัฒนาของสีแดงเพิ่มขึ้นในระหว่างการสุกแก่ระยะต่าง ๆ ไปจนถึงระยะที่ 6 พบว่าอุณหภูมิในการเก็บรักษาไม่มีผลต่อปริมาณแอนโทไซยานินในทุกระยะ ทั้งนี้ในทุกระดับอุณหภูมิ ปริมาณแอนโท-ไซยานินมีแนวโน้มเพิ่มขึ้นในทิศทางเดียวกันและมีปริมาณสูงสุดในระยะที่ 6 แต่อุณหภูมิมีผลต่อการเปลี่ยนแปลงกิจกรรมของเอนไซม์ PAL ในแต่ละระยะของแต่ละอุณหภูมิที่เก็บรักษา โดยกิจกรรมของเอนไซม์มีแนวโน้มลดลงในระยะแรก ๆ และเพิ่มสูงขึ้นในระยะหลัง ยกเว้นผลที่เก็บที่อุณหภูมิ 25°C กิจกรรมของเอนไซม์ยังคงมีค่าต่ำในระยะที่ 6 ของการสุกแก่ของผลและผลที่เก็บรักษาที่อุณหภูมิ 35°C มีกิจกรรมของเอนไซม์สูงสุดที่ระยะที่ 5

[1]ภาควิชาเทคโนโลยีหลังการเก็บเกี่ยว คณะวิศวกรรมและอุตสาหกรรมเกษตร มหาวิทยาลัยแม่โจ้ อำเภอสันทราย จังหวัดเชียงใหม่ 50290 [2]ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ อำเภอเมือง จังหวัดเชียงใหม่ 50200

Fruit colour, an important marketing attribute of mangosteen (*Garcinia mangostana* L.), influences both consumer acceptance and sales. The attractive purplish-red of mangosteen is mainly anthocyanin. The major pigment is cyanidin-3-sophoroside, and the minor pigment is cyanidin-3-glucoside (Du and Francis, 1977). Mangosteen is ready to harvest when pericarp is light greenish yellow scattering with pinkish spots, minimum stage for harvesting (Tongdee and Suwanagul, 1989). Total anthocyanin content increased continuously during maturation and reaching a maximum value at fully ripe stage (Ratanamarno *et al*., 1999).

Environmental and cultural factors influence fruit colour development. Temperature and light are two environmental factors that are important for red pigment development in fruit (Saure, 1990). Anthocyanin synthesis in the apple fruit cv. Jonathan is light-dependent and may be further stimulated by several treatments including UV light (Chalmers and Faragher, 1977). Kliewer (1970)

found that the level of anthocyanin in Pinot noir berries was considerably less under low light than under high light. Further evidence indicating that light is important in the colouration of grapes is found by visually comparing berries from different positions on a cluster. The berries that are best exposed to solar radiation usually have darker colour than berries least exposed to light, especially in the low-pigmented table grape varieties. Bagging of mango cv. Keitt, the percentage of the skin with red colour, and its intensity decreased with increasing duration of bagging (Hofman *et al*., 1977). In the skin of mango fruit cv. Kent, the increase in red peel colour did not occur in the bagged group (Saengnil *et al*., 1997).

Anthocyanin synthesis in several plant tissue is associated with increased L-phenylalanine ammonia-lyase (PAL) activity. Aoki *et al.* (1970) observed PAL activity only in the red parts of the apple skin and concluded that PAL activity was closely related to the formation of anthocyanin. In apple skin, Faragher and Chalmers (1977) studied the effect of UV light and wounding on anthocyanin synthesis and PAL activity, and suggested that PAL controlled the rate of anthocyanin synthesis in whole fruit skin.

Although anthocyanin production is often associated with an increase in PAL activity, there are many examples of PAL activity without anthocyanin production. This is because PAL is active in the biosynthesis of a wide range of phenylpropanoid compounds, such as substituted cinnamic acids and their CoA-ester and conjugates of these such as chlorogenic acid, coumarin and lignin (Lister *et al*., 1996).

In this study, it was aimed to investigate anthocyanin synthesis and PAL activity in mangosteen fruit pericarp during maturation in response to light and storage temperature.

Materials and Methods

Fruit: Experimental fruits were harvested at random from the trees of 35 years old from the commercial orchard, "Wang Nam Kang" in Amphoe Maewang, Chiang Mai province, Thailand.

Experiment 1: To investigate the effect of light on anthocyanin content and PAL activity, fruits at the age of 14 days after full bloom were bagged using two layer paper bag. The fruit maturity stage was determined by visual examination according to Tongdee and Suwanagul (1989). Six different stages of fruits were defined by the extent of red or purple colouration on the pericarp: Stage 1 (pericarp light greenish yellow with scattered pinkish spots, minimum stage for harvesting), Stage 2 (pericarp light greenish yellow or yellowish pink with distinct irregular pink spots covering the entire fruit), Stage 3 (pericarp background pinkish, spots not as distinct as in stage 2, a stage commonly harvested commercially), Stage 4 (pericarp red to reddish brown, some with purple tinge), Stage 5 (pericarp darkened to reddish purple, best eating stage), Stage 6 (pericarp purple, dark purple to black with slight or no red colouration remaining). Each stage of fruits was taken for anthocyanin and PAL determination.

Experiment 2: The effect of storage temperature on anthocyanin content and PAL activity was examined. Fruit were harvested at stage 1 (pericarp light greenish yellow with scattered pinkish spots) and place in corrugated box of 30x 40x10 cm. Each side of the box has two holes of 2.3 cm in diameter. Fruits were stored at 15ºC (85 %RH), 25ºC (85 %RH), 30ºC (70-80 %RH) (room temperature) and 35ºC (65 %RH). When the colours of the fruits were developed to stage 2, 3, 4, 5 and 6 (indicated by colour as above), fruits from each stage were taken for anthocyanin and PAL determination.

Anthocyanin extraction and determination: Two grams of fruit pericarp was extracted in 100 ml of ethanol containing 1%HCL, using estimation of total anthocyanin method (Ranganna, 1977). Absorbance was measured at 535 nm using a Spectrophotometer 20 D+. Results were expressed as mg of total anthocyanin content per 100 g fresh weight.

Phenylalanine ammonia-lyase (PAL) extraction and assay: Two-hundred milligrams of acetone powder of fruit pericarp was extracted

with 20 ml of 0.1 M borate buffer (pH 8.8) at 4°C for 15 minutes and then was centrifuged at 13,500 rpm for 30 minutes at 4°C. The supernatant which was crude enzyme was used for enzyme assay.

The activity of PAL was determined by spectrophotometry by measuring the rate of trans-cinnamic acid formation as increased absorbance at 280 nm. The mixtures contained 2 ml of 0.1 M borate buffer (pH 8.8), 1 ml of 60 mM phenyl-alanine and 1 ml of the crude enzyme was incubated at 30°C for 1 hour, then the reaction stopped by adding 1 ml of 2 N perchloric acid. The reaction rate was measured by using a recording spectrophotometer. Results were expressed as nmole/mg protein•hr. (or unit/mg protein). Protein measurement was determined according to the modified procedure of Lowry *et al.* (1951).

Results and Discussion

Anthocyanin content increased continuously during maturation in both bagged and unbagged fruits (Figure 1). At stage 6 bagged fruits had higher anthocyanin content than those non-bagged with non-statistically significant difference. It was
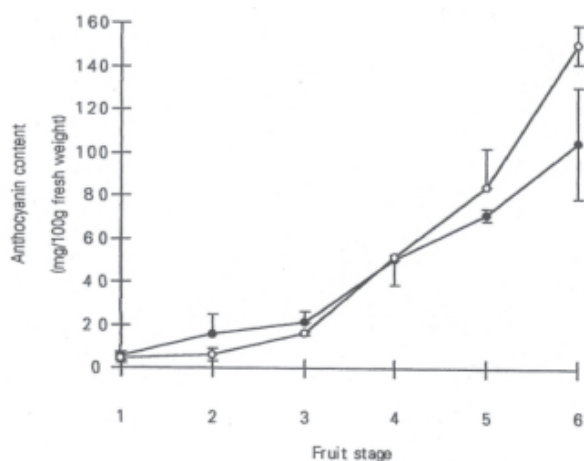
found that bagging mangosteen fruits had no effect on anthocyanin synthesis. It was contrary to many fruits which light had an effect on anthocyanin synthesis such as litchi cv. Ohea (Yindee, 1996), litchi cv. Tai So (Tyas *et al.*, 1998), mango cv. Kent (Saengnil *et al.*, 1997 and Rimpranam, 1998), grape cv. Emperor (Kliewer, 1977), apple cv. Starking Delicious and cv. Fuji (Arakawa *et al.*, 1986) and apple cv. Jonathan (Faragher and Brohier, 1984).

PAL activity in mangosteen fruit pericarp of both fruit with and without bagging was not significantly difference (Figure 2). PAL activity was decreased continuously from stage 1 to stage 6 in unbagged fruit, while in bagged fruit it was increased from stage 1 to stage 2, then decreased and remained relatively constant until stage 5 and increased again in stage 6.

According to Ju *et al.* (1995) who investigated the relationship between anthocyanin accumulation and PAL activity using apple fruits on the tree, the changes in anthocyanin accumulation can occur independently of changes in PAL activity; the increase of anthocyanin during maturation involved additional enzymes between
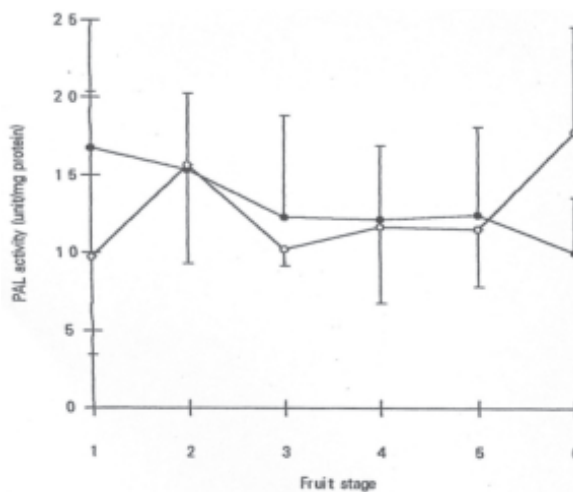

Figure 1. Changes in anthocyanin contents in mangosteen fruits pericarp during maturation bagged (o) and unbagging (●) (Vertical bars indicate standard deviations of nine replicates).

Figure 2. PAL activity in mangosteen fruits pericarp during maturation bagged (o) and unbagging (●) (Vertical bars indicate standard deviations of nine replicates).
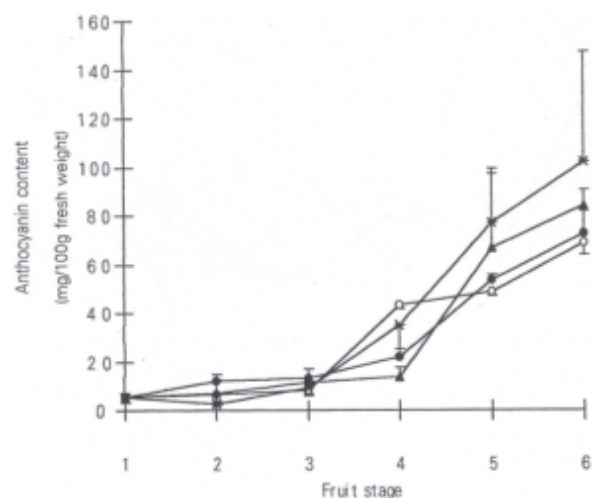
Figure 3. Changes in anthocyanin contents in mangosteen fruits pericarp during maturation at different storage temperatures 15ºC (●), 25ºC (o), 30ºC (RT) (▲) and 35ºC (✕) (Vertical bars indicate standard deviations of nine replicates).
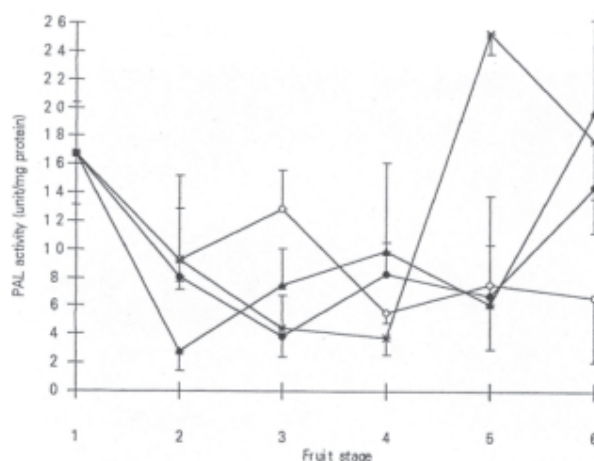
Figure 4. PAL activity in mangosteen fruits pericarp during maturation at different storage temperatures 15ºC (●), 25ºC (o), 30ºC (RT) (▲) and 35ºC (✕) (Vertical bars indicate standard deviations of nine replicates).

leucocyanidin and cyanidin glycocides. They also suggested that when the precursors of anthocyanin synthesis in bagged apples were deficient, PAL was critical for pigment formation. However, when such precursors were sufficient, as in the case of mature fruit, changes of PAL activity did not regulate anthocyanin accumulation.

PAL induction and anthocyanin formation responsed to different endogenous controls in previous studies. In apple, PAL can be induced by a wide range of factors including wounding, temperature, lights and chemicals and can be induced at any fruit developmental stages (Saure, 1990). Wang *et al*. (2000) reported that PAL is not the only regulating factor for anthocyanin accumulation in bagged mature and ripe Jonathan apples.

Anthocyanin contents in mangosteen fruit pericarp stored at different temperatures 15º, 25º, 30º (RT) and 35ºC were increased accordingly from stage 1 to stage 6 and had the highest level of anthocyanin at stage 6 (Figure 3). The fruits stored at 35ºC had the highest anthocyanin content

followed by at 30º, 15º and 25ºC, respectively, with non-statistically difference. Temperature had an effect on PAL activity at different stages of fruit development. Levels of PAL activity decreased at the early stage and increased at the final stage of fruit maturity except fruits held at 25ºC, PAL activity remained at low level through stage 6, while fruits at 35ºC had the highest level of PAL at stage 5 (Figure 4).

Faragher (1983) reported that a shift of temperature affects the course of fruit development: in detached 'Jonagold' apples, the optimum for anthocyanin accumulation was 12ºC in unripe and 16-24ºC in ripe fruit at constant illumination. Besides, anthocyanin accumulation in ripe apples at 23ºC exceeded that at 17ºC.

The role of temperature seems to be ambivalent for anthocyanin formation: low temperature may contribute to colour formation by directly reducing GA activity, but high temperatures are also required for proper fruit development and timely maturation, being the prerequisite for increased ethylene and ABA activity. Obviously,

proper temperature management with alternating temperatures could be helpful in anthocyanin formation, but is rarely feasible for economic reasons (Saure,1990).

Faragher (1983) also reported that levels of PAL activity were higher at low temperature than that of high temperatures, and increased inactivation of PAL by high temperature, in conjunction with ripening and light, was more likely than direct stimulation of PAL by low temperature.

This experiment was found that detached mangosteen fruits at early maturity stage (pericarp light greenish yellow with scattered pinkish spots) with bagging and unbagging were capable of accumulated anthocyanin during fruit colour development to fully ripe stage independently of changes in PAL activity. This research also showed that bagging of mangosteen fruits has potential to improve fruit quality and added benefits on visual appearance. Moreover, fruit bagging prevents fruits from insect infestation especially the fruit fly. This practice will enhance exporting quality of mangosteen fruit; however, further economic assessments are needed.

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