

ความสัมพันธ์ของเอนไซม์โพลีฟีนอลออกซิเดส สารประกอบฟีนอล และการรั่วไหลของสารอิเล็กโทรไลต์  
ระหว่างเกิดอาการสะท้านหนาวของผลลำไย

The Relationship Between Polyphenol Oxidase, Phenolic Compounds and Electrolyte Leakage  
During Chilling Injury of Longan Fruit

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Abstract

Fresh longan fruit (cv. Daw) with 0.5 cm pedicels were packed in cardboard boxes and stored at  $5\pm 1^{\circ}\text{C}$ ,  $90\pm 1\%$  RH. Measurements were made of color of the outer and inner sides of pericarps, polyphenol oxidase (PPO) activity, concentration of phenolic compounds, electrolyte leakage and soluble protein content. Chilling injury symptoms, water soaking and/or browning areas on the pericarp, appeared on the inner side of pericarp after 6 days storage and on the outer side of whole fruit within 14 days. Both sides of the peel became brown in color. During chilling injury there was an increase in the PPO activity and electrolyte leakage and decreases in total phenolic compounds and protein content.

**Key words:** Longan pericarp, chilling injury, polyphenol oxidase, phenolic compounds

บทคัดย่อ

ผลลำไยสดพันธุ์ดอ ตัดให้เหลือขั้วติดผลยาวประมาณ 0.5 เซนติเมตร บรรจุในกล่องกระดาษ เก็บรักษาที่อุณหภูมิ  $5\pm 1$  องศาเซลเซียส ความชื้นสัมพัทธ์  $90\pm 1\%$ . นำเปลือกของผลลำไยมาวัดค่าสีเปลือกด้านนอกและด้านใน วัดกิจกรรมของเอนไซม์โพลีฟีนอลออกซิเดส ปริมาณสารประกอบฟีนอล การรั่วไหลของสารอิเล็กโทรไลต์ และปริมาณโปรตีน พบว่าผลลำไยของผลลำไยเริ่มแสดงอาการสะท้านหนาวที่เปลือกด้านใน ซึ่งมีลักษณะเป็นจุดสีน้ำตาลจ้ำน้ำ เมื่อเก็บรักษานาน 6 วัน และเกิดอาการสะท้านหนาวทั่วทั้งผลเมื่อเก็บรักษานาน 14 วัน เปลือกทั้งด้านนอกและด้านในมีสีน้ำตาลคล้ำมากขึ้น เมื่อระดับความรุนแรงของอาการสะท้านหนาวเพิ่มขึ้น กิจกรรมของเอนไซม์โพลีฟีนอลออกซิเดสและการรั่วไหลของสารอิเล็กโทรไลต์เพิ่มขึ้น แต่ปริมาณสารประกอบฟีนอลและโปรตีนลดลงระหว่างเกิดอาการสะท้านหนาว

**คำสำคัญ** เปลือกลำไย อาการสะท้านหนาว โพลีฟีนอลออกซิเดส สารประกอบฟีนอล

Introduction

Longan fruit has a very short postharvest life of 3-4 days under ambient conditions (Jiang, 1997; Tongdee, 1997). The major factors reduce the storage life at room temperature and marketability of longan fruit are microbial decay and pericarp browning (Jiang *et al.*, 2002). The dark brown skin results from desiccation and/or heat stress, senescence, and chilling injury (Jiang *et al.*, 2002). Chilling injury occurred on fruit stored at low temperature, indicated by water-soaking or drying and darkening of the pericarp (Paull and Chen, 1987). However, these symptoms have no effect on flavor or aril quality. Browning in fruit is initiated by the enzymatic oxidation of phenolic compounds by PPO. The most important factors that determine the rate of enzymatic browning are the concentrations of both active PPO, phenolic compounds present, the temperature and the oxygen availability of the tissue (Martinez and Whitaker, 1995). Discoloration lowers market price of the fruit and even become unmarketable (Jiang *et al.*, 2002). The objective of our research was to assess the relationship between polyphenol oxidase, phenolic compounds and electrolyte Leakage during chilling injury of longan fruit.

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## Materials and Methods

Commercial fresh longan fruit (cv. Daw) were obtained from a commercial orchard in Lumphun province. The pedicels were cut to 0.5 cm length and each replication fruits were packed in cardboard boxes containing 50 fruits/each then stored at  $5\pm 1^{\circ}\text{C}$ ,  $90\pm 1\%$  RH. The chilling injury was graded as follows: 1 = no water-soaking and browning (excellent quality); 2 = 1-25% water-soaking and/or browning area; 3 = 26-50% water-soaking and/or browning area; 4 = 51-75% water-soaking and/or browning area (poor quality); 5 = 76-100% water-soaking and/or browning area. The color of outer and inner sides of the pericarp were measured with a Chromameter (CR-300 Minolta, Japan). The electrolyte leakage was measured as described by King and Ludford (1983). The concentration of free and bound phenolic compounds (bound-ethyl acetate) was determined according to Amiot *et al.* (1992) and Sun *et al.* (2002). The total phenolic content was measured spectrophotometrically using the Folin-Ciocalteu reagent, calculated as gallic acid equivalent (GAE) (Singleton *et al.*, 1999). Polyphenol oxidase (PPO) was partially purified with solid ammonium sulfate (80% saturation) and its activity on catechol assayed with a spectrophotometric at 400 nm (Jiang, 1999), with one unit of enzyme activity defined as that amount causing 0.001 change in absorbance per minute. Soluble protein content was determined by dye-binding using bovine serum as a protein standard (Bradford, 1976).

## Results and Discussion

Longan fruit pericarp showed brown discoloration after storage for 6 days of at  $5^{\circ}\text{C}$ . This chilling injury symptom initially appeared to be a mild water soaking and/or browning on the inner side of pericarp. As chilling injury steadily increased, both sides of the pericarp became brown, until the entire fruit was affected within 14 days (Fig. 1). The  $L^*$  value and hue angle slightly decreased as the chilling injury increased (Fig. 2A and 2B). The electrolyte leakage of pericarp increased from 10.8% initially to 47.8% with water soaking and/or browning covering 100% of pericarp (Fig. 2C). Loss of electrolyte from chilled tissues was also noted previously (Smith and McWilliams, 1978; Mercado-Silva *et al.*, 1998). As chilling injury became more severe, the total amount of phenolics declined, although the PPO activity increased (Fig. 3C). The amount of free phenolics exceeded that of bound-E phenols (Fig. 3A and 3B).

## Conclusion

There is a strongly relationship between electrolyte leakage, PPO activity and total phenolic compounds during chilling injury of longan fruit.

## Acknowledgement

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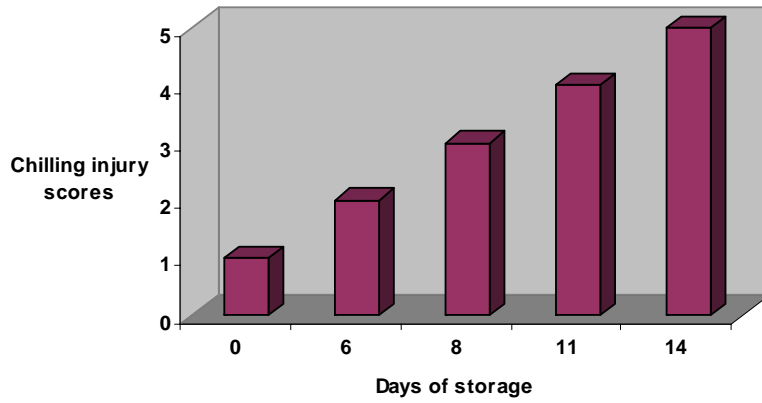


Figure 1 The relationship between chilling injury score and storage time at 5°C

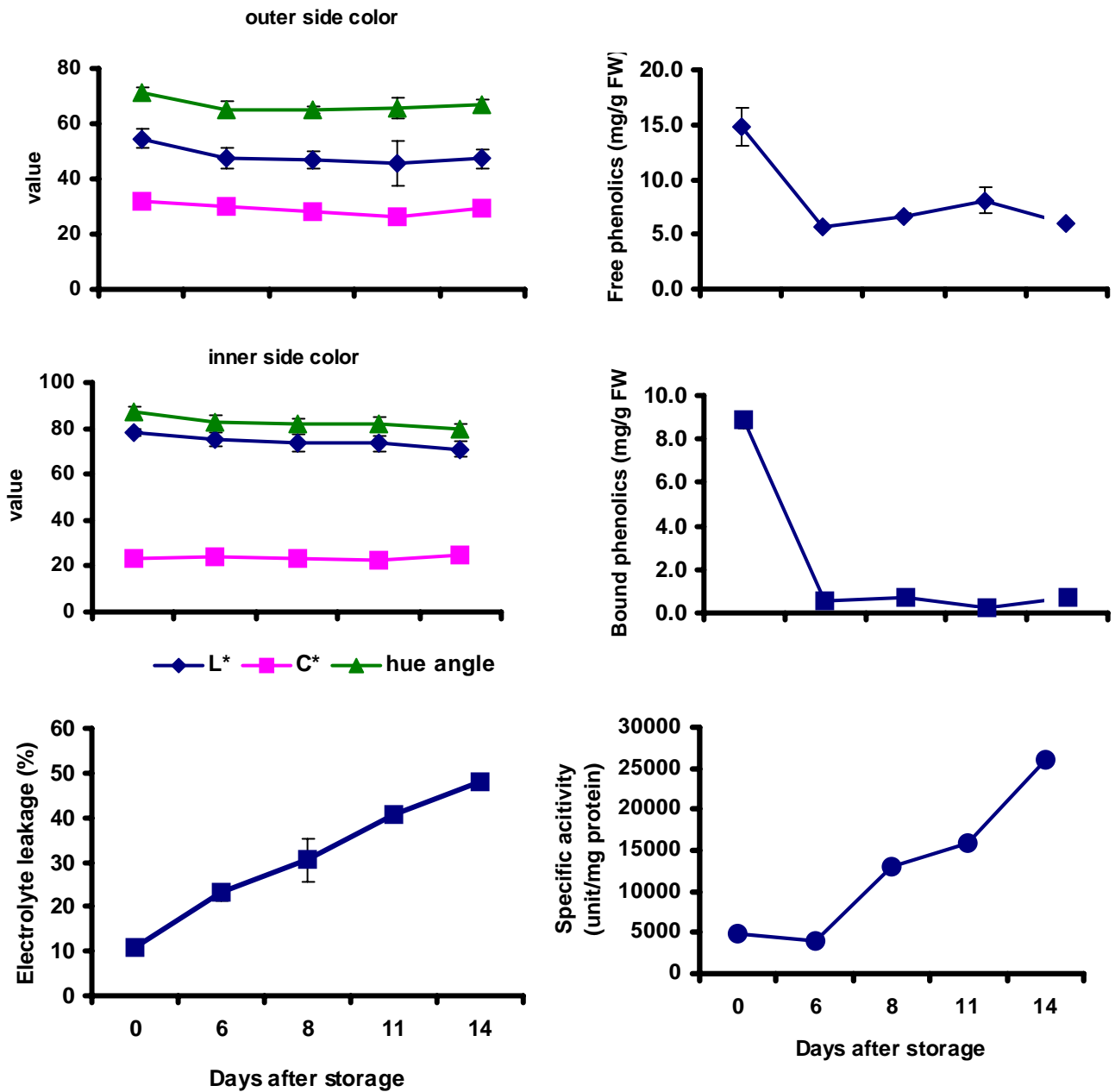


Figure 2 The relationship of outer and inner side skin color (L\*, C\* and H<sup>o</sup>) (2A and 2B) and electrolyte leakage (2C) of longan pericarp during chilling injury (storage at 5°C). Each value is the mean for three replicates, and vertical bars indicate the standard errors.

Figure 3 The relationship between phenolic compounds (free and bound-E) (3A and 3B) and specific activity of PPO (3C).