สกว
T R F

รายงานวิจัยฉบับสมบูรณ์

โครงการส่งเสริมกลุ่มวิจัยและพัฒนาสรีรวิทยาหลังการเก็บเกี่ยวของผลิตผลพืชสวน

โดย

ศ.ดร.สายชล เกตุษา

31 กรกฎาคม 2548

สัญญาเลขที่ RTA 4580011

รายงานวิจัยฉบับสมบูรณ์

โครงการส่งเสริมกลุ่มวิจัยและพัฒนาสรีรวิทยาหลังการเก็บเกี่ยวของผลิตผลพืชสวน

ศ.ดร.สายชล เกตุษา
ภาควิชาพืชสวน คณะเกษตร
มหาวิทยาลัยเกษตรศาสตร์

สนับสนุนโดย สำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

การดำเนินการวิจัยของโครงการในรอบ 3 ปี ได้ทำกับผัก (ข้าวโพดฝักอ่อน แตงกวา และคะน้า) ผลไม้ (มังคุด มะม่วง และกล้วย) และดอกไม้ (ปทุมมา และกล้วยไม้) โดยการวิจัยเกี่ยวกับการเกิดความเสียหาย เนื่องจากอุณหภูมิต่ำทำกับข้าวโพดฝักอ่อน มะม่วง มังคุด และกล้วย การแข็งของเปลือกผลมังคุดหลังการตกกระทบ และการตกกระของผลกล้วยไข่สุก ผลกระทบของความมืดและความสว่างต่อการเก็บรักษาผลแตงกวาและคะน้า การยืดอายุการปักแจกันของดอกปทุมมา และดอกกล้วยไม้สกุลหวาย และปรากฏการณ์ของดอกกล้วยไม้สกุลหวายหลังการผสมเกสร ผลที่ได้จากการวิจัยของโครงการมีนิสิตปริญญาโท จบการศึกษา 3 คน ปริญญาเอกจบการศึกษา 4 คน มีการตีพิมพ์ผลงานวิจัยในวารสารนานาชาติ 12 เรื่อง ส่งตีพิมพ์และกำลังรอแก้ไข 6 เรื่อง และกำลังดำรอส่งตีพิมพ์ 4 เรื่อง นอกจากนั้น ยังมีการตีพิมพ์บทความทั่วไป 1 เรื่อง และบทความทั่วไปกำลังรอตีพิมพ์ 1 เรื่อง

Abstract

The research of the 3 – year project was carried out with fruits (mango, mangosteen and banana), vegetables (baby corn, cucumber and Chinese kale) and flowers (curcuma and orchid). Chilling injury of baby corn, mango, mangosteen and banana, pericarp hardening of mangosteen pericarp after impact, senescent spotting of ripening banana, effects of light on postharvest changes of cucumber and Chinese kale, prolongation and postharvest changes of curcuma and orchid flowers, and postpollination developmental processes of orchid flowers were intensively conducted. Achievement of the project resulted in 3 students graduated with M.S. and 4 students graduated with Ph.D. Regarding the publication, 12 papers have been already published in international journals, 6 papers have been submitted and being revised, while 4 papers are in the process of being submitted. In addition, one general article has been already published and another one has been submitted and will be published soon.

เนื้อหางานวิจัย

การวิจัยสรีรวิทยาหลังการเก็บเกี่ยวของผลิตผลพืชสวน ทำกับผัก ผลไม้ และดอกไม้ ในส่วนของผักได้ทำวิจัยกับข้าวโพดฝักอ่อน คะน้า และแตงกวา ผลไม้ทำวิจัยกับมะม่วง กล้วย และมังคุด และดอกไม้ทำกับกล้วยไม้ ปทุมมา และกุหลาบ การวิจัยพบว่า ข้าวโพดฝักอ่อนที่ปอกเปลือกออกแล้วมีการเกิดสีน้ำตาล (browning) ที่ยอดรังไข่ (ovule) ไหม (silk) ที่ดึงออกไม่หมดและรอยแผลที่หักก้านฝักอ่อน การเกิดสีน้ำตาลขึ้นอยู่กับพันธุ์และสิ่งแวดล้อม (อุณหภูมิและความชื้นสัมพัทธ์) การเกิดสีน้ำตาลของข้าวโพดฝักอ่อนมีความสัมพันธ์โดยตรงกับการสูญเสียน้ำหนัก และปริมาณสารฟีนอลิก แต่ไม่มีความสัมพันธ์กับกิจกรรมของเอนไซน์ phenylalanine ammonia lyase (PAL) และ polyphenol oxidase (PPO) ซึ่งเป็นเอนไซน์ที่เกี่ยวข้องกับการสังเคราะห์สารฟีนอลิก และการเกิดสีน้ำตาล ตามลำดับ (เอกสารแนบหมายเลข 1 reprint) ผลแตงกวาเก็บรักษาไว้ที่อุณหภูมิต่ำ (10 °ซ.) ภายใต้ความมืดและแสงสว่างตลอดการเก็บรักษา พบว่าผลแตงกวาเก็บรักษาภายใต้ความมืดมีการสูญเสียคลอโรฟิลล์ที่ผิวรวดเร็วกว่าผลแตงกวาที่เก็บรักษาภายใต้แสงสว่าง ขณะเดียวกันผลแตงกวาที่เก็บรักษาภายใต้ภายใต้แสงสว่างมีการสังเคราะห์แคโรทีน และกิจกรรมของเอนไซน์คลอโรฟิลเลสที่ผิวเพิ่มมากกว่าผลแตงกวาเก็บรักษาไว้ในที่มืด (เอกสารแนบหมายเลข 2 manuscript) ผักคะน้าเก็บรักษาที่อุณหภูมิต่ำ (1 °ซ.) ภายใต้ความมืด และแสงสว่าง

(เอกสารแนบหมายเลข 2 manuscript) ผักคะน้าเก็บรักษาที่อุณหภูมิต่ำ (1 °ซ.) ภายใต้ความมืด และแสงสว่างตลอดระยะเวลาการทดลอง พบว่าคะน้าที่เก็บรักษาภายใต้แสงสว่างตลอดเวลามีการเสียน้ำหนัก ปริมาณคลอโรฟิลล์ทั้งหมด คลอโรฟิลล์บี กิจกรรมของคลอโรฟิลเลส และวิตามินซี มากกว่าคะน้าเก็บรักษาไว้ในที่มืด ขณะที่คะน้าเก็บรักษาไว้ในที่มืดมีการกระตุ้นการสังเคราะห์แคโรทีนอยด์ คลอโรฟิลล์เอ แป้ง น้ำตาลกลูโคสและฟรุคโตส และกิจกรรมเพอร์ออกซิเดส มากกว่าคะน้าเก็บรักษาไว้ในที่สว่าง คะน้าเก็บรักษาไว้ในที่สว่างมีการเปิดของปากใบมากกว่าคะน้าเก็บรักษาไว้ในที่มืด แต่คะน้าเก็บรักษาไว้ในที่สว่างและมืดไม่มีความแตกต่างกันของปริมาณซูโคสในใบ (เอกสารแนบหมายเลข 3 manuscript)

ผลกล้วยไข่สุกมีการตกกระเป็นจุดสีน้ำตาลที่ผิวของเปลือก เมื่อห่อผลกล้วยไข่สุกในระยะที่ยังไม่มีการตกกระ คือผิวของผลกล้วยเปลี่ยนสี.ไม่มี colour index 3-4 ด้วยพลาสติกฟิล์มพีวีซี (PVC film) หลายยี่ห้อ (brand) สามารถลดการตกกระได้อย่างมีประสิทธิภาพ การเก็บรักษาผลกล้วยไข่สุกที่มีสีผิว colour index 3-4 ในบรรยากาศที่มีออกซิเจน 5 เปอร์เซ็นต์ สามารถควบคุมการตกกระได้เช่นเดียวกับการใช้พลาสติกฟิล์มพีวีซี การเก็บรักษาผลกล้วยไข่สุกที่มีสีผิว colour index 3-4 ไว้ในบรรยากาศที่คาร์บอนไดออกไซด์เป็นปกติ 5 – 15 เปอร์เซ็นต์ ไม่สามารถควบคุมการตกกระได้ ขณะเดียวกันพบว่า ผลกล้วยไข่สุกที่อยู่ในสภาพบรรยากาศที่ออกซิเจนต่ำ มีกิจกรรมเอนไซม์ PAL ลดลง แต่มีกิจกรรมเอนไซม์ PPO เพิ่มขึ้น (เอกสารแนบหมายเลข 4 reprint) ผลกล้วยไข่ที่สุกภายใต้สภาพบรรยากาศที่มีความชื้นสัมพัทธ์สูง (90-95 %) มีการตกกระมากกว่าผลกล้วยที่สุกภายใต้สภาพบรรยากาศที่มีความชื้นสัมพัทธ์ต่ำ (60-70 %) ขณะที่การใช้สารดูดซับเอทิลีนและคาร์บอนไดออกไซด์ในบรรยากาศรอบผลกล้วยไข่ ไม่มีผลต่อการตกกระของผลกล้วยไข่สุก (เอกสารแนบหมายเลข 5 manuscript) ผลกล้วยไข่สุกที่มีสีผิว colour index 3-4 โดยผิวยังไม่ตกกระ นำไปเก็บรักษาไว้ที่อุณหภูมิต่ำ 12 15 และ 18 °ซ. พบว่าอุณหภูมิยิ่งต่ำยิ่งสามารถยับยั้งการตกกระของผลกล้วยไข่ได้ดี แต่เมื่อเคลื่อนย้ายผลกล้วยไข่จากอุณหภูมิต่ำไปที่อุณหภูมิห้อง ทำให้ผลกล้วยไข่มีการตกกระเพิ่มขึ้นเร็ว ผลกล้วยไข่สุกเก็บรักษาไว้ที่อุณหภูมิต่ำมีกิจกรรม PAL และ PPO ลดลง ขณะที่ ปริมาณสารฟีนอลิกเพิ่มขึ้น (เอกสารแนบหมายเลข 6 manuscript) ผลกล้วยไข่สุกที่มีสีผิว colour index 3-4 เคลือบผิวด้วยสารเคลือบผิว (surface coating material) ทำให้สามารถลดการตกกระของผลกล้วยไข่สุกได้ ผลกล้วยไข่ที่เคลือบผิวมีกิจกรรม PAL เพิ่มขึ้น และ PPO ลดลง และปริมาณสารฟีนอลิกเพิ่มขึ้น ผลกล้วยไข่ที่เคลือบผิวมีสารฟีนอลิกประเภทโดปามีน (dopamine) ลดลงช้ากว่าผลกล้วยไข่ที่ไม่ได้เคลือบผิว (เอกสารแนบหมายเลข 7 manuscript)

ผลกล้วยสุกมีการอ่อนตัวของเปลือก และเนื้อระหว่างการสุก หรือมีความแน่นเนื้อของเปลือกและเนื้อลดลงระหว่างการสุก การสุกของผลกล้วยไข่มี soluble pectin เพิ่มขึ้นในเนื้อแต่ไม่ได้เพิ่มในเปลือกระหว่างการสุก กิจกรรม pectinesterase (PE) เพิ่มในเนื้อแต่ลดลงในเปลือก ขณะที่กิจกรรม polygalacturonase (PG) และ β-galactosidase (GAL) เพิ่มขึ้นในเนื้อและเปลือก แต่กิจกรรม GAL เพิ่มขึ้นในเปลือกมากกว่าในเนื้อ กิจกรรมของ cellulase ไม่มีการเปลี่ยนแปลงทั้งในเปลือกและเนื้อของผลกล้วยระหว่างการสุก ข้อมูลดังกล่าวแสดงให้เห็นว่าการเปลี่ยนแปลงองค์ประกอบทางเคมีของเปลือกและเนื้อของผลกล้วยไข่ระหว่างสุกที่นำไปสู่การอ่อนตัวของเปลือกและเนื้อของผลกล้วยไข่สุกที่มีกลไกแตกต่างกัน (เอกสารแนบหมายเลข 8 reprint) ผลกล้วยระหว่างการสุกนอกจากมีการอ่อนตัวเกิดขึ้นแล้ว ยังพบว่ามีการหลุดร่วง (finger drop) ของผลกล้วยจากหวีกล้วยอีกด้วย การศึกษาเปรียบเทียบการหลุดร่วงของผลกล้วยระหว่างการสุกของกล้วยหอมทองและน้ำว้า พบว่าผลกล้วยหอมทองมีการหลุดร่วงของผล 100 % ขณะที่กล้วยน้ำว้าไม่มีการหลุดร่วงของผลระหว่างการสุก การตรวจสอบกิจกรรมของเอนไซม์ PE, PG, GAL และ pectin lyase (PL) ในส่วนที่เป็นเปลือกตรงกลางผลและก้านผลหรือขั้วผลบริเวณที่เกิดการหลุดร่วงของผลระหว่างการสุก พบว่าบริเวณขั้วผลของกล้วยทั้งสองชนิดมีกิจกรรมของ PE, PG และ GAL มากกว่าบริเวณเปลือกตรงกลางผล แต่กล้วยหอมทองที่มีการหลุดร่วงของผลมากระหว่างการสุก ไม่พบความสัมพันธ์โดยตรงกับกิจกรรมของ PE, PG และ GAL ในบริเวณขั้วของผลบริเวณที่มีการหลุดร่วงของผล ยกเว้นกิจกรรมของ PL ซึ่ง

มีแนวโน้มของความสัมพันธ์กับการหลุดร่วงของผลกล้วยระหว่างการสุกของกล้วยหอมทองและกล้วยน้ำว้า (เอกสารแนบหมายเลข 9 manuscript)

การเก็บรักษาผลกล้วยทั้งดิบและสุก ไว้ที่อุณหภูมิต่ำเหนือจุดเยือกแข็ง ทำให้ผลกล้วยได้รับความเสียหายเนื่องจากอุณหภูมิต่ำ (chilling injury) อาการของความเสียหายที่เกิดขึ้นเห็นได้ชัดเจนคือ การเกิดสีเทา น้ำตาล และดำ (browning) ของเปลือก การศึกษาพบว่าทั้งผลกล้วยหอมทองและผลกล้วยไข่ดิบ เมื่อเก็บรักษาไว้ที่อุณหภูมิ 6 และ 10 $^{\circ}$ซ. พบว่ามีการเปลี่ยนสีผิวของเปลือกอย่างรวดเร็วในผลกล้วยทั้งสองพันธุ์ซึ่งไม่มีความแตกต่างกัน กิจกรรมของ PAL และ PPO เพิ่มขึ้น ขณะที่ปริมาณสารฟีนอลิกลดลง แม้ว่าผลกล้วยทั้งสองชนิดมีความแตกต่างกันของกิจกรรม PAL และ PPO และปริมาณสารฟีนอลิกในเปลือกผล แต่ไม่มีความแตกต่างของสีผิวเปลือกเมื่อเกิด browning (เอกสารแนบหมายเลข 10 reprint) การเก็บรักษาผลกล้วยในสภาพบรรยากาศดัดแปลง (modified atmosphere packaging, MAP) โดยเก็บรักษาผลกล้วยไว้ในถุงพลาสติก ก่อนเก็บรักษาไว้ที่อุณหภูมิต่ำ สามารถลดความเสียหายที่เกิดขึ้นเนื่องจากอุณหภูมิต่ำ คือชะลอหรือลดการเปลี่ยนสีผิวผิดปกติของผลกล้วย และยังพบว่า MAP ชะลอการลดลงของปริมาณสารฟีนอลิกในเปลือกผลกล้วย ขณะเดียวกัน MAP ลดกิจกรรม PAL และ PPO ในเปลือกผลกล้วยด้วย (เอกสารแนบหมายเลข 11 reprint) การเก็บรักษาผลมะม่วงพันธุ์ต่าง ๆ คือ แรด อกร่อง ทองดำ น้ำดอกไม้ หนังกลางวัน และแก้ว ไว้ที่อุณหภูมิ 4, 8 และ 12 $^{\circ}$ซ. ทำให้ผลมะม่วงได้รับความเสียหายเนื่องจากอุณหภูมิต่ำ เช่นเดียวกับผลกล้วย และอาการที่เห็นชัดเจนคือ การเกิดสีผิดปกติที่ผิวของเปลือก เนื้อ และเมล็ด โดยเปลี่ยนเป็นสีเทา ม่วง หรือน้ำตาลเข้มและไหม้ (browning) ผลมะม่วงพันธุ์ต่าง ๆ มีความไวหรือตอบสนองต่ออุณหภูมิต่ำแตกต่างกัน โดยพันธุ์ที่ไวต่ออุณหภูมิต่ำคือ ทองดำ น้ำดอกไม้ แก้ว และหนังกลางวัน ขณะที่ผลมะม่วงพันธุ์แรด และอกร่อง ตอบสนองน้อยต่ออุณหภูมิต่ำ จากข้อมูลนี้จะนำไปสู่การศึกษากลไกของความแตกต่างในการเกิด chilling injury ของผลมะม่วงพันธุ์ต่าง ๆ และสามารถใช้เชื้อพันธุ์เหล่านี้ในการปรับปรุงพันธุ์มะม่วงให้ทนต่ออุณหภูมิต่ำระหว่างการเก็บรักษาได้อีกด้วย (เอกสารแนบหมายเลข 12 reprint)

การเก็บรักษาผลมังคุดวัยสีแดงและสีม่วง ไว้ที่อุณหภูมิ 4 8 และ 12 $^{\circ}$ซ. พบว่าผลมังคุดเก็บรักษาไว้ที่อุณหภูมิ 4 และ 8 $^{\circ}$ซ. มีการแข็งของเปลือกผลมังคุดภายในเวลา 9-12 วัน ขณะที่ผลมังคุดเก็บรักษาไว้ที่อุณหภูมิ 12 $^{\circ}$ซ. สามารถเก็บรักษาไว้นาน 21 วัน โดยยังไม่มีการแข็งของเปลือกผล ผลมังคุดที่นำออกจากอุณหภูมิต่ำหลังการเก็บรักษาไปไว้ที่อุณหภูมิห้องทำให้เปลือกผลมังคุดที่มีการแข็งมากขึ้น การจุ่มผลมังคุดในสารละลาย GA (gibberellic acid) และ NAA 1-naphthaleneacetic acid) ก่อนเก็บรักษาไว้ที่อุณหภูมิต่ำไม่สามารถลดการเหี่ยวของขั้วผลและกลีบเลี้ยง ขณะที่การจุ่มผลในสารละลาย BA (benzyladenine) ชะลอการเหี่ยวของขั้วผลและกลีบเลี้ยงได้ (เอกสารแนบหมายเลข 13 reprint) ผลมังคุดหล่นจากที่สูงกระทบพื้นคอนกรีต ทำให้เปลือกผลมังคุดบริเวณที่ตกกระทบมีการแข็งอย่างรวดเร็วภายในเวลา 1-3 ชั่วโมง ผลมังคุดวัยสีม่วงมีการแข็งของเปลือกผลเร็วและรุนแรงมากกว่าผลมังคุดวัยสีแดง การตกกระทบที่สูงเพิ่มขึ้น ยิ่งทำให้เปลือกผลมังคุดบริเวณตกกระทบมีการแข็งเพิ่มมากขึ้น เนื้อเยื่อบริเวณที่ตกกระทบของเปลือกผลมังคุดมีปริมาณสารฟีนอลิกลดลง และปริมาณสารลิกนินเพิ่มขึ้นอย่างรวดเร็ว ผลมังคุดที่ตกกระทบและอยู่ในสภาพที่มีออกซิเจนต่ำ สามารถชะลอการแข็งของเปลือกผลมังคุดบริเวณที่ตกกระทบ (เอกสารแนบหมายเลข 14 reprint) จากการศึกษาโดยการย้อมสีเนื้อเยื่อของเปลือกผลมังคุดบริเวณที่ตกกระทบกับสารเคมีที่ทำปฏิกิริยาเคมีกับสารลิกนินและให้สีเฉพาะ โดยการใช้สาร phloroglucinol, $KMnO_4$ และ toluidine blue O ทำให้สามารถยืนยันได้อีกว่าเปลือกผลมังคุดบริเวณตกกระทบมีการสังเคราะห์ลิกนินเพิ่มขึ้นอย่างรวดเร็ว (เอกสารแนบหมายเลข 15 reprint)

ดอกปทุมมาหลังการเก็บเกี่ยวที่ปักแจกันในน้ำกลั่นมีการเกิดสีน้ำตาลที่ปลายกลีบเลี้ยง (bract) อย่างรวดเร็ว การให้เอทิลีนจากภายนอกทำให้ปลายกลีบเลี้ยงเกิดสีน้ำตาลรุนแรงมากขึ้น การปักแจกันในสารละลายที่มีน้ำตาลซูโครสและสารฆ่าจุลินทรีย์ในน้ำ คือ 8-hydroxyquinoline sulfate และ dichloroisocyanuric acid ไม่สามารถยืดอายุการปักแจกันของปทุมมาได้ และการเก็บรักษาดอกปทุมมาไว้ที่อุณหภูมิ 5-7 $^{\circ}$ซ. นาน 3 วัน และนำมาปัก

แจกันทำให้อายุการปักแจกันลดลง ยิ่งการเก็บรักษาที่อุณหภูมิต่ำมากยิ่งและนานขึ้น ทำให้อายุการปักแจกันลดลงมาก การเก็บรักษาแบบเปียกสามารถเก็บรักษาได้นานกว่าการเก็บรักษาแบบแห้ง ปทุมมาเป็นดอกไม้ที่ไวต่ออุณหภูมิต่ำมาก โดยทำให้กลีบเลี้ยงเป็นสีน้ำตาล (เอกสารแนบหมายเลข 16 reprint)

การแช่โคนก้านช่อดอกกล้วยไม้สกุลหวายในสารละลายน้ำตาล (ซูโคสและกลูโคส) และ aminooxyacetic acid (AOA) ทำให้สามารถยืดอายุการปักแจกันของดอกกล้วยไม้ ลดการร่วงของดอกตูมและบาน ชะลอการเหี่ยวของดอก และเพิ่มการบานของดอกตูม การแช่โคนก้านช่อในสารละลายน้ำตาล หรือ AOA เพียงอย่างเดียว ไม่สามารถยืดอายุการปักแจกันได้ น้ำตาลกลูโคสให้ผลดีกว่าน้ำตาลซูโคส ประสิทธิภาพของสารละลายที่มีน้ำตาลและ AOA ลดลงเมื่อปรับ pH ของสารละลาย (pH 3) ให้สูงขึ้น ประชากรของจุลินทรีย์ในสารละลายที่น้ำตาลและ AOA และปักแจกันดอกกล้วยไม้มีน้อยกว่าในน้ำที่ไม่มีสารเคมีระหว่างการปักแจกัน ดอกกล้วยไม้ที่ปักแจกันในสารละลายที่มีน้ำตาลและ AOA มีการดูดน้ำเพิ่มมากขึ้นและน้ำหนักสดลดลงช้า (เอกสารแนบหมายเลข 17 reprint) ดอกกล้วยไม้ที่ลดอุณหภูมิ (precooling) ก่อนการบรรจุในกล่องกระดาษเลียนแบบการส่งออก โดยให้รับอุณหภูมิต่ำที่ 10 °ซ. นาน 1 ชั่วโมง ทำให้ความเข้มข้นของเอทิลีนภายในกล่องกระดาษที่บรรจุกล่องกล้วยไม้ลดลง และลดปริมาณสารตัวกลางในการสร้างเอทิลีน (1-aminocyclopropane-1-carboxylic acid, ACC) กิจกรรมของเอนไซน์ที่กระตุ้นปฏิกิริยาการสร้าง ACC ในดอกกล้วยไม้ การลดอุณหภูมิของดอกกล้วยไม้นานกว่า 1 ชั่วโมง ทำให้ดอกกล้วยไม้ได้รับความเสียหายเนื่องจากอุณหภูมิต่ำ การใส่สารดูดซับเอทิลีนในการบรรจุดอกกล้วยไม้เลียนแบบการส่งออกร่วมกับการลดอุณหภูมิเพิ่มประสิทธิภาพในการลดความเข้มข้นของเอทิลีนภายในกล่องบรรจุดอกกล้วยไม้และชะลอการเหี่ยวของดอกไม้ขณะที่ปักแจกัน (เอกสารแนบหมายเลข 18 reprint) ดอกกล้วยไม้สกุลหวายที่ได้รับ 1-methylcyclopropene (1-MCP) 100-500 ppb ก่อนบบรจุเลียนแบบการส่งออกทำให้สามารถปรับปรุงคุณภาพของดอกกล้วยไม้ระหว่างการปักแจกันโดย 1-MCP เพิ่มอายุการปักแจกันและลดการร่วงของดอกตูมและดอกบาน ความเข้มข้นของเอทิลีนภายในกล่องกระดาษที่บรรจุดอกกล้วยไม้ไม่ได้ 1-MCP มีความเข้มข้นน้อยกว่าในกล่องกระดาษบรรจุดอกกล้วยไม้ที่ไม่ได้รับ 1-MCP พบว่า 1-MCP ลดการสร้างเอทิลีน ปริมาณสารตัวกลางในการสร้างเอทิลีน (ACC) กิจกรรมของเอนไซน์ที่กระตุ้นปฏิกิริยาการสร้าง ACC (ACC synthase) และประสิทธิภาพการเปลี่ยน ACC ไปเป็นเอทิลีน (ACC oxidase) (เอกสารแนบหมายเลข 19 manuscript) ดอกกล้วยไม้สกุลหวายหลังการผสมเกสรมีการเหี่ยวของกลีบดอกอย่างรวดเร็ว พร้อมกับการเจริญเติบโตของรังไข่โดยการเพิ่มขนาด และมีการสร้างเอทิลีนเพิ่มขึ้น การให้สารสังเคราะห์ออกซิน (NAA) กับดอกกล้วยไม้สามารถที่ไม่ผสมเกสรทำให้ดอกกล้วยไม้มีพฤติกรรมคล้ายกับดอกกล้วยไม้ที่ได้รับการผสมเกสร การผสมเกสรของดอกกล้วยไม้สกุลหวายมีลักษณะที่เป็น compatible และ incompatible pollination การผสมเกสรของดอกกล้วยไม้บางพันธุ์ไม่สามารถทำให้มีการเจริญเติบโตของรังไข่ได้หลังการผสมเกสร แม้ว่าสามารถกระตุ้นการสร้างเอทิลีนเพิ่มขึ้นและมีการเหี่ยวของกลีบดอก (incompatible pollination) การให้สารยับยั้งการสร้างเอทิลีน (AOA, Co) หรือสารยับยั้งการทำงานของเอทิลีน (1-MCP, Ag) กับดอกกล้วยไม้ก่อนการผสม ทำให้การสร้างเอทิลีนลดลงและชะลอการเหี่ยวของกลีบดอกกล้วยไม้ที่ได้รับการผสมเกสร และชะลอการเจริญเติบโตของรังไข่ ดอกกล้วยไม้ที่มีการผสมเกสรเป็น compatible pollination มีการสร้างเอทิลีน การงอกของเกสร และการเจริญเติบโตอง pollen tube มากกว่าดอกกล้วยไม้ที่มีการผสมเกสรเป็น incompatible pollination (เอกสารแนบหมายเลข 20 21 และ 22 manuscript)

นอกจากนั้น ยังมีการตีพิมพ์บทความทั่วไปเพื่อเผยแพร่ผลงานวิจัยที่เกิดจากโครงการอีกด้วย คือเรื่อง การตกกระ ของผลกล้วยไข่สุก (เอกสารแนบหมายเลข 23 reprint) และการแข็งของเปลือกผลมังคุดหลังการตกกระทบ เอกสารแนบหมายเลข 24 manuscript)

Output

ผลงาน	รวม
1. จำนวนนักวิจัยรุ่นใหม่ที่สร้างจากโครงการ	
1. 1 จำนวนนักศึกษาระดับปริญญาเอกที่จบ	4
1. 2 จำนวนนักศึกษาระดับปริญญาโทที่จบ	3
2. จำนวนผลงานตีที่พิมพ์ในวารสารวิชาการนานาชาติ	
2.1 ผลงานวิจัยที่ตีพิมพ์แล้ว	12
2.2 ผลงานวิจัยที่ส่งตีพิมพ์และกำลังรอการแก้ไข	6
2.3 ผลงานวิจัยที่กำลังรอส่งตีพิมพ์	4
3. จำนวนบทความทั่วไปที่ตีพิมพ์	
3.1 บทความทั่วไปที่ตีพิมพ์แล้ว	1
3.1 บทความทั่วไปที่กำลังรอตีพิมพ์	1

เอกสารแนบหมายเลข 1

Browning of Baby Corn after Harvest

A. Meenaphan
Department of Agricultural Extension
Ministry of Agriculture and Cooperatives
Bangkok 10900, Thailand

S. Ketsa
Department of Horticulture
Faculty of Agriculture
Kasetsart University
Bangkok 10900, Thailand

Keywords: Weight loss, total phenolics, phenylalanine ammonia lyase, polyphenol oxidase

Abstract

Browning development of three baby corn cultivars was studied in relation to their weight loss, total phenolics and polyphenol oxidase activity after harvest. 'Chiang Mai 90' baby corn had a greater browning development during storage than 'CP 45' and 'Pacific #5' baby corn. Browning development of baby corn correlated with weight loss and total phenolics but not with phenylalanine ammonia lyase and polyphenol oxidase activities. The role of desiccation in browning development of baby corn after harvest was discussed.

INTRODUCTION

Baby corn or young-ear corn is an important vegetable crop growing in Thailand, as tonnage and export value increase every year. Baby corn is the entire young cob, which has a high nutritional value. One of the major postharvest problems in baby corn is the development of browning (Somboonsarn, 1993). Browning occurs in many fruit and vegetable species and the mechanisms underlying the process may be either enzymatic or non-enzymatic (Martinez and Whitaker, 1995). Polyphenol oxidase is widely distributed in plant tissues and catalyzes the oxidation of phenolic compounds to quinones that in turn polymerize to form brown pigments (Mayer and Harel, 1979). Preliminary data showed that some cultivars of baby corn were less susceptible to browning than others. The aim of this study was to monitor the development of browning and postharvest changes in three different baby corn cultivars stored at room temperature.

MATERIALS AND METHODS

Baby corn (*Zea mays*) cultivars used in this study were 'Chiang Mai 90', 'Pacific #5' and 'CP 50'. Young-ear corns were individually harvested as soon as silk showed approximately one inch above the husk. Husk was removed by knife while silk was removed manually. Young-ear corns were selected by uniformity of size and colour. They were then placed on polystyrene trays (12.5 x 12.5 cm) and wrapped with a PVC film. Each tray contained 10 young-ear corns that were kept continuously at room temperature (27 °C and 70% RH) for 6 days. Browning development, water loss, total phenolics, phenylalanine ammonia lyase (PAL), and polyphenol oxidase (PPO) activities of young-ear corns were monitored during the study period. Browning of young-ear corns was assessed on a 0-4 scale, where 0 was no browning development and 4 was severe browning development.

Weight loss of the young-ear corns was calculated as % of initial fresh weight, from the weight measured at harvest, and after 2, 4 and 6 days in storage.

Extraction and assay of PAL and PPO were carried out as described by Camm and Towers (1975) and Luh and Phithakpol (1972), respectively. Protein content was determined according to Bradford (1976). Total phenolics were determined using the method described by Singleton and Rossi (1965). Each treatment had five (biological) replications, and each replication consisted of the whole young-ear corn from 10 young ear-corns, pooled together.

RESULTS

Weight losses of all three cultivars of baby corn increased rapidly during storage (Fig. 1). 'Chiang Mai 90' baby corn had the greatest weight loss, while 'Pacific #5' baby corn had the least. Average daily weight loss as a proportion of the original weight of 'Chiang Mai 90', 'CP 50' and 'Pacific #5' baby corn over the six-day period was 14.5, 11.7 and 10.8%, respectively.

Browning of baby corn occurred at the tip of ovules, cut surface of the cob and silk which remained attached to the cob. Development of browning increased rapidly for all three cultivars during the first two days of storage and increased slightly thereafter, except for 'Chiang Mai 90' baby corn in which browning increased rapidly on day 6 (Fig. 2). Total phenolics of all three cultivars increased gradually during storage at ambient temperature (Fig. 3).

PAL activity of 'Chiang Mai 90' baby corn was very high on day 0 and rapidly decreased thereafter, while PAL activities of 'CP 50' and 'Pacific #5' baby corn were very low on day 0 and decreased slightly thereafter (Fig. 4). PPO activity of 'CP 45' baby corn was the least on day 0 and gradually increased thereafter, while PPO activity of 'Pacific #5' baby corn was the greatest on day 0 and slightly increased thereafter. PPO activity of 'Chiang Mai 90' baby corn was also high on day 0 and decreased gradually throughout the study period (Fig. 5).

DISCUSSION

The weight loss of three baby corn cultivars studied increased steadily during the six days. The daily average weight loss, mainly due to the loss of water, on baby corn was high compared with other commodities (Robinson, et al., 1975). The surface of young-ear corns is covered with very small ovules resulting in a large surface area unit per volume, which enhances weight loss (Burton, 1982). Moreover, 'Chiang Mai 90' baby corn had the smallest size with a fresh weight of 60 g per young-ear corn, while 'CP 50' and Pacific #5' baby corn had a fresh weight of 100 g per young-ear corn. Thus, 'Chiang Mai 90' baby corn had a greater ratio of surface area unit per volume than 'CP 50' and 'Pacific #5' baby corn, contributing to a greatest weight loss during storage .

Browning of baby corn occurred at the tip of the immature ovules, cut surface of the cob, and silks which remained attached to the cob. But, browning of immature ovules is the main limiting factor of baby corn quality, which results in rejection by the consumer. Oxidation of phenolic compounds is the cause of browning in plant tissues (Mayer and Harel, 1975; Martinez and Whitaker, 1995). However, changes in PAL and PPO activities did not show a good correlation with browning development of young-ear corn. This suggested that browning of young-ear corn might not depend on de novo synthesis of PAL and PPO activities. All three cultivars of baby corn exhibited a greater weight loss concomitant with an increase in browning and total phenolics. Desiccation has also been reported to be one of main factors causing browning on lychee fruit (Scott et al., 1982; Underhill, 1992). Desiccation increases the breakdown of vacuoles, causes the leakage of phenolic compounds, and destroys the compartmentation of browning-related enzymes and their substrates (Chen and Hong et al., 1992; Underhill and Critchley, 1994). Thus, severe weight loss of young-ear corn may cause the breakdown of vacuoles and release phenolic compounds, which come into contact with preexisting PPO, resulting in oxidation of phenolic compounds to quinones and finally polymerize to form browning pigments (Martinez and Whitaker, 1975).

CONCLUSION

All three cultivars of baby corn exhibited a great weight loss and browning development after removal of husk and silk. Browning correlated with water loss and total phenolics but not with PAL and PPO activities.

ACKNOWLEDGEMENTS

This research was financially supported by the Thailand Research Fund (TRF).

Literature Cited

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

Burton, W.G. 1982. Post-Harvest Physiology of Food Crops. Longman Group Ltd., London.

Camm, E.C. and Towers, G.H.N. 1973. A review to Phenylalanine ammonia lyase. Phytochem. 12:961-973.

Chen, W.J. and Hong, Q.Z. 1992. Studies on senescence and browning of litchi fruit pericarp during storage. Acta Hort. Sin. 19:227-232.

Luh, B.S. and Phitakpol, B. 1972. Characteristics of polyphenol oxidase related to browning in cling peaches. J. Food Sci. 37: 264-268.

Martinez, M.V. and Whitaker, J.R. 1995. The biochemistry and control of enzymatic browning. Trends in Food Sci. Technol. 6:195-200.

Mayer, A.M. and Harel, E. 1979. Polyphenol oxidases in plants. Phytochem. 18:192-215.

Robinson, J.E., Browne, K.M. and Burton, W.G. 1975. Storage characteristics of some vegetables and soft fruits. Ann. Appl. Biol. 81:399-408.

Scott, K.J., Brown, B.I., Chaplin, G.R., Wilcox, M.E. and Bain, J.M. 1982. The control of rotting and browning of lychee fruit by hot benomyl and plastic film. Scientia Hort. 16:253-262.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Vitic. 16:144-157.

Somboonsarn, N. 1993. Harvesting Indices, Storage and Postharvest Physiology of Baby Corn. Ph.D. Thesis. Kasetsart University, Bangkok.

Underhill, S.J.R., Bagshaw, J., Prasad, A., Zauberman, G., Romen, R. and Fuchs, Y. 1992. The control of litchi postharvest skin browning using sulphur dioxide and low pH. Acta Hort. 321:732-741.

Underhill, S.J.R. and Critchley, C. 1994. Anthocyanin decolorisation and its role in lychee pericarp browning. Aust. J. Exp. Agri. 34:115-122.

Figures

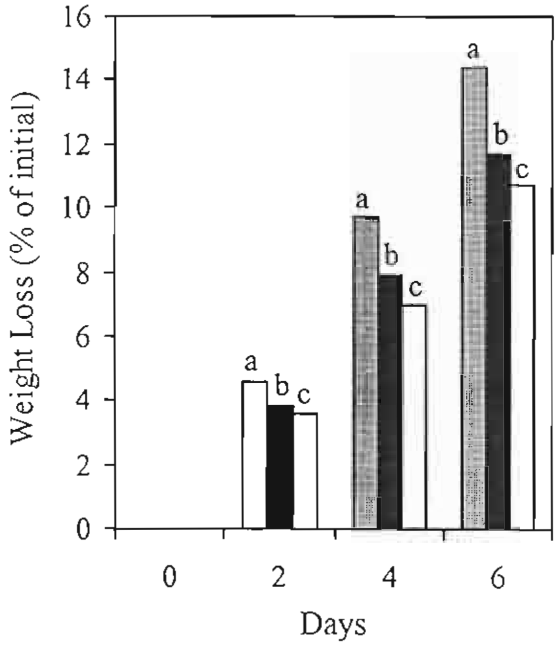


Fig. 1. Weight loss of baby corn cultivars. Chiang Mai 90(▨), CP 45 (■) and Pacific#5(□) packed in foam trays wrapped with PVC film and kept at room temperature (27 °C, 70%RH) for 6 days. Mean separation within each day by DMRT at $P = 0.05$ level.

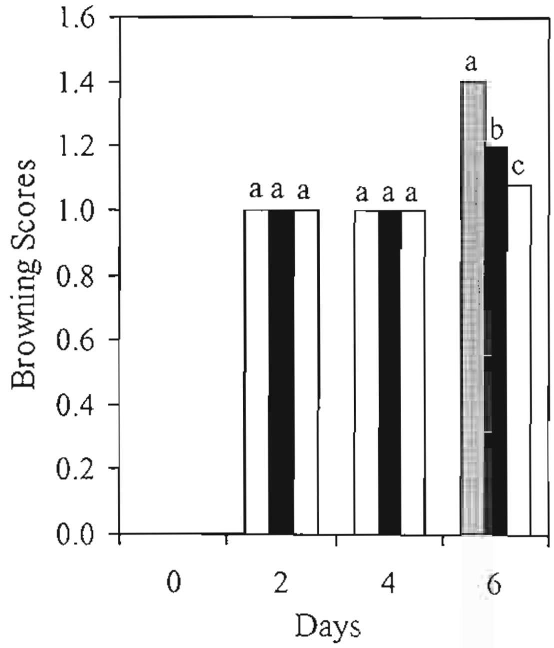


Fig. 2. Browning (0 = no browning and 4 = severe browning) of baby corn cultivars. Chiang Mai 90 (▨), CP 45 (■) and Pacific #5 (□) packed in foam trays wrapped with PVC film and kept at room temperature (27 °C, 70%RH) for 6 days. Mean separation within each day by DMRT at $P = 0.05$ level.

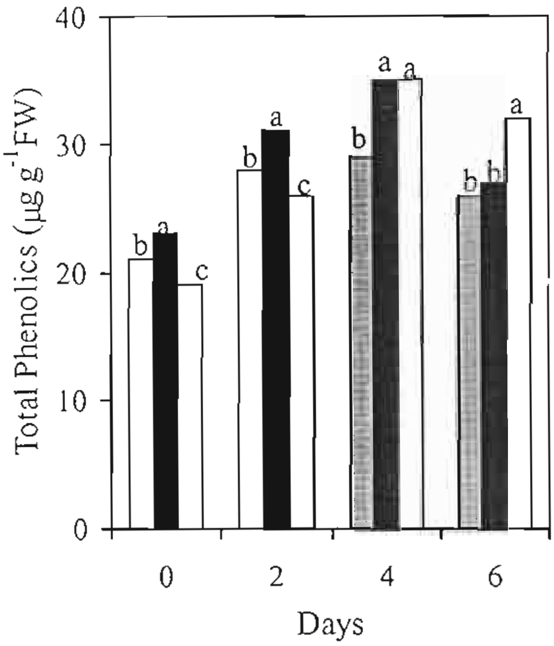


Fig. 3. Total phenolics of baby corn cvs. Chiang Mai 90 (▧), CP 45 (■) and Pacific #5 (□) packed in foam trays wrapped with PVC film and kept at room temperature (27 °C, 70% RH) for 6 days. Mean separation within each day by DMRT at $P = 0.05$ level.

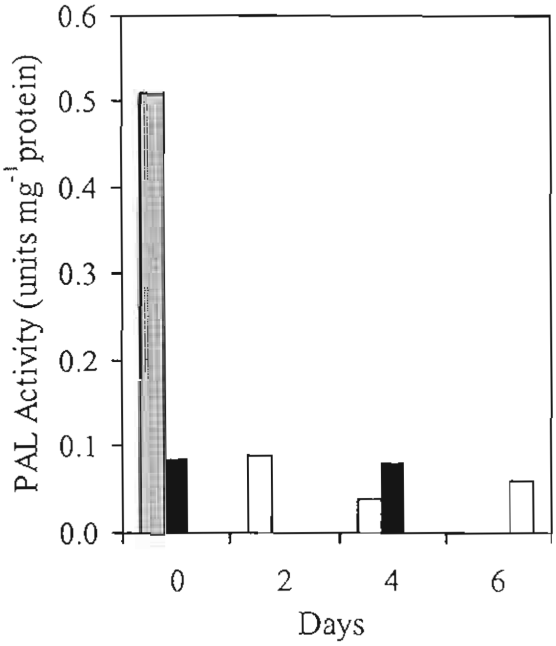


Fig. 4. Phenylalanine ammonia lyase (PAL) of baby corn cvs. Chiang Mai 90 (▧), CP 45 (■) and Pacific #5 (□) packed in foam trays wrapped with PVC film and kept at room temperature (27 °C, 70% RH) for 6 days.

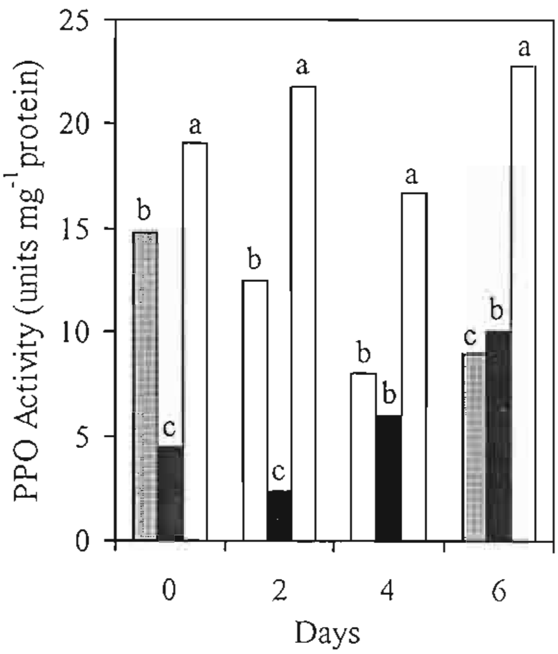


Fig. 5. Polyphenol oxidase (PPO) of baby corn cvs. Chiang Mai 90 (▧), CP 45 (■) and Pacific #5 (□) packed in foam trays wrapped with PVC film and kept at room temperature (27 °C, 70% RH) for 6 days. Mean separation within each day by DMRT at $P = 0.05$ level.

เอกสารแนบหมายเลข 2

Effect of light and darkness on the colour of stored cucumber fruit

Sompoch Noichinda[1], Kitti Bodhipadma[1], Wipa Saosumran[1], Attcharawan Rodses[1], Saichol Ketsa[2]* and Wouter G. van Doorn[3]

[1]Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's Institute of Technology North Bangkok, Bangsue, Bangkok 10800 Thailand

[2]Department of Horticulture, Faculty of Agriculture, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

[3]Wageningen University and Research Centre, P.O. Box 17, 6700AA Wageningen, The Netherlands

Abstract

Storage of cv. Micro-C cucumbers in darkness, at 13 °C and 95 % RH, induced rapid peel yellowing. The presence of fluorescent light (16 μmol m^{-2} s^{-1}) during storage prevented this yellowing. The chlorophyll content in the cucumber fruit peel stored in darkness decreased much more rapidly than that of fruit stored in the light. Although the chlorophyllase activity in the cucumber peel increased under both conditions, it increased much earlier, and immediately, in fruits placed in darkness. The carotenoid content of cucumber peel stored in darkness increased more than that of fruit stored in the light. The data indicate that the peel colour, both in darkness and in the light, is mainly due to the chlorophyll concentration. Light prevention of chlorophyll loss was correlated with lower chlorophyll degradation by chlorophyllase. As the light level seems below the compensation point, the effect of darkness on chlorophyll breakdown may relate to a phytochrome effect.

---

*Corresponding author. E-mail address: agrsck@ku.ac.th (S. Ketsa). Fax: 66 2 5791951

Keywords: Carotenoid; Chlorophyll, Chlorophyllase, *Cucumis sativus* ; Light; Dark

1. Introduction

Peel yellowing is a main problem, and an indicator, for cucumber quality. A more yellow peel is correlated with a decrease in fruit firmness (Schouten et al. 2002).

Light has been reported to affect the synthesis and degradation of pigments in many freshly harvested plant commodities (Gross, 1987). In the present research, we report that very low light levels can prevent cucumber peel yellowing. We also assessed chlorophyll and carotenoid levels, and the peel chlorophyllase activity.

2. Materials and Methods

Plant material

Fruits of cucumber (Cucumis sativus L. cv. Micro-C, a local Thai cultivar) were harvested at the commercial stage based on size and peel colour. They were obtained from a local grower in the morning and brought to the laboratory within 2 h of harvest. Fruits were further selected for uniformity of colour and size. Fruits were then separated into two groups and each group was placed into plastic containers (30x30x30 cm). At the upper side of container covered with a polyethylene plastic film (1 micron thickness) and exposed continuously to light (at 16 μmol m-2 s-1) from the Philips E27 (60w, 12 lm./w) lamp over the upper side (PE film side) of plastic container and without lamp for control. The RH inside plastic container was about 90%. All treatments were done in the refrigerator at 13°C. The RH in side the refrigerator was about 80%

Pigment analysis

Chlorophyll content in the cucumber fruit peel was both extracted and measured (spectrophotometrically) according to the method of Whitham et al. (1971). Carotenoid in the cucumber fruit peel was extracted with petroleum ether and measured using the method of Georges and Olivier (1993).

Chlorophyllase assay

Chlorophyllase in the cucumber fruit peel was extracted and the activity was determined according to the method of Shimokawa et al. (1978). The protein concentration in the homogenate was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Statistical analysis

Experiments were carried out using 3 replications and each replication had 12 fruits. Results were the average of 36 fruits. Experiments were repeated twice at later dates.

3. Results

3.1. Colour changes during storage

The Micro-C cultivar is a small cucumber. When mature it is about 10 cm long and has a diameter of 3 cm. The colour is dark green. Just as other cucumbers, it is chilling sensitive; the optimum temperature for storage being about 13°C. During storage in darkness at 13°C and 90% RH, the peel of the cv. Micro-C cucumbers changed rapidly from green to yellow (Fig. 1). So the fruit cannot be stored at all at these conditions, as it is unacceptably yellow within 3 days of storage. However, a low light level by fluorescent lamps, providing as little as 16 μmol m^{-2} s^{-1} largely prevented the colour change during the nine days of the tests (Fig. 1). Further experiments showed that the fruit can be stored under such light conditions for up to nine days, after which they have an acceptable shelf life (results not shown).

3.2. Chlorophyll level and chlorophyllase activity

Although the chlorophyll content in the cucumber peel decreased under both light and dark conditions, it decreased much faster in cucumbers held in darkness (Fig. 1). Chlorophyllase activity in the cucumber peel gradually increased under both conditions, but much more rapidly in fruits held in darkness (Fig. 2).

3.3. Caroteinoid levels

In the peel of cucumber that were held in darkness the total carotenoid level increased very little during the first 6 days of the experiments, and increased somewhat more from day 6 to 9. A similar large increase was observed in the peel of fruit stored in the light, but here it occurred from day 3 to 6 (Fig. 3).

4. Discussion

In cucumber, colour is a major criterion by consumers to determine whether the fruit fresh or not. The green colour seems mainly due to the presence of chlorophyll. If the chlorophyll is only slightly altered, its green colour is lost (Matile and Hortensteiner, 1999). Chlorophyll degradation shows at least two stages, an early one, before cleavage of the tetrapyrrole ring, and a late one, after this cleavage. The products of the early stage are greenish, whereas those of the late stage are essentially colourless (Takamiya et al., 2000). Chlorophyll degradation occurs both by non-enzymatic and buy enzymatic reactions (Gross, 1987). Chlorophyllase cleaves chlorophyll to chlorophyllide (Hortensteiner, 1999).

Chlorophyll content of cucumber peel stored under light and dark conditions decreased, concomitant with a roughly proportional increase in chlorophyllase activity. However, in the light conditions some chlorophyll was degraded without an apparent increase in chlorophyllase (Fig. 1). An increase of chlorophyllase activity has also been reported in stored citrus fruit (Brandis et al., 1996) and in radish roots (Akiyama and Yamauchi, 2001). Chlorophyllase been reported to have a much lower activity in plant parts in darkness than in parts growing in the light (Ellsworth 1971).

Leaf yellowing in many species is not only affected by light quantity, but also by light quality. Red light prevents chlorophyll degradation and far red light promotes it, through the action of phytochrome (van Doorn and van Lieberg, 1993). In the present tests the light levels (16 μmol m^{-2} s^{-1}) was lower than the compensation point for the photosynthesis of many green plant tissues, even those adapted to low light levels. So it may be light quality rather than light quantity that affects cucumber peel yellowing.

Disappearance of chlorophyll is often associated with the synthesis of pigments ranging from yellow to red. Many of these are carotenoids (Gross, 1987). In cucumber fruit, carotenoid content increased under both light and dark storage, although earlier in the light than in darkness. Research on tomato also shows that light is not an absolute requirement for increased caretonoid synthesis, although light stimulated the synthesis (Raymundo et al., 1976). Light also stimulated carotenoid levels in apple (Proctor and Creasy, 1971).

It is concluded that the highly sensitive cucumber cultivar stored at lower temperature cucumber peel turned yellowing more rapidly in the darkness than in the light. Cucumber fruit stored in the darkness had more carotenoid content and chlorophyllase activity and less chlorophyllase activity than those stored in the light. Appearance of yellowing of cucumber peel was probably the result of a combination of the loss of chlorophyll pigments combined with carotenoid synthesis.

Acknowledgements

The research was financially supported by the Thailand Research Fund (TRF).

References

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Brandis, A., Vainstein, A., Goldschmidt, E.E. 1996. Distribution of chlorophyllase among components of chloroplast membrane in *Citrus sinensis* organs. Plant Physiol. Biochem. 34: 49-54.

Ellsworth, R.K. 1971. Studies on chorophyllase I.. Hydrolytic and esterification activities of chlorophyllase from wheat seedlings. Photosynthetica 5:226-232.

Georges, C. and Olivier, H., 1993. Carotenoid pigment of the algae *Haematococcus pluvialis* assay on Rainbow trout. Aqua. Culture 112: 217-226.

Gross, J., 1987. Pigments in Fruits. Academic Press, London.

Hortensteiner, S., 1999. Chlorophyll breakdown in higher plants and algae. Cell. Mol. Life Sci. 56: 330-347.

Matile, P., Hortensteiner, S., 1996. Chlorophyll degradation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50: 67-95

Proctor, J.T.A., Creasy, L.L. 1971. Effect of supplementary light on anthocyanin synthesis in 'McIntosh' apples. J. Amer. Soc. Hort. Sci. 96: 523-526.

Raymundo, L.C., Chichester, C.O., Simpson, K.L., 1976. Light dependent carotenoid synthesis in the tomato. J. Agric. Food Chem. 24: 59-64.

Shimokawa, K., Shimada, S. and Yaeo, K. 1978. Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unchin* Marc. Sci. Hort. 80: 129-135.

Schouten R.E., Tijskens L.M.M., van Kooten O. 2002. Predicting keeping quality of batches of cucumber fruit based on a physiological mechanism. Postharvest Biol. Technol. 26: 209-220

Takamiya, K.I., Tsuchiya, T., Ohta, H., 2000. Degradation pathway(s) of chlorophyll: what has gene cloning revealed? Trends in Plant Sci. 5: 426-431.

van Doorn W.G, van Lieburg, M.J. 1993. Interaction between the effects of phytochrome and gibberellic acid on the senescence of *Alstroemeria pelegrina* leaves. Physiologia Plantarum 89: 182-186.

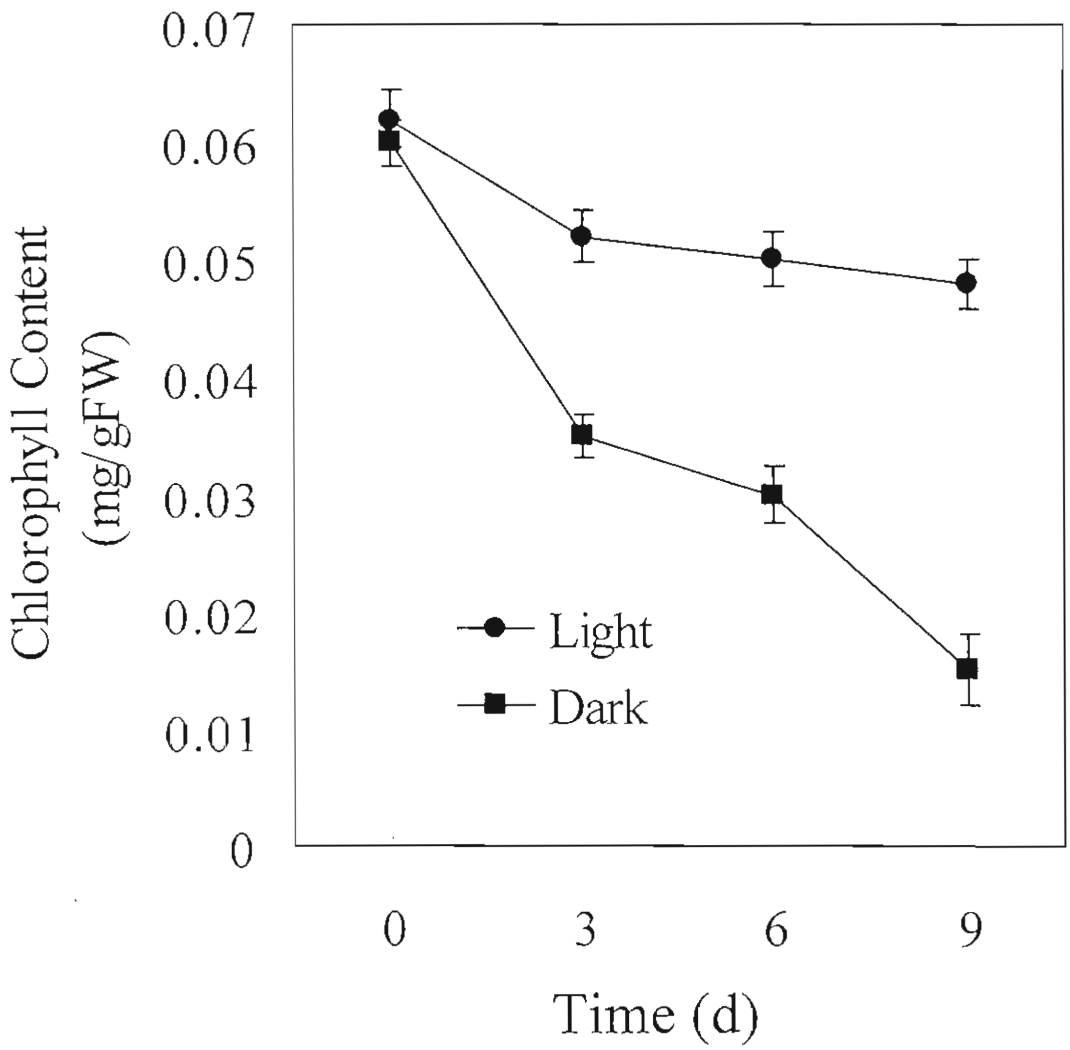
Legends to Figures

Fig. 1 **Peel colour and peel** chlorophyll content in cucumber cv. Micro-C during storage at 13° C under light (—○—) and dark (—●—) conditions. Data are means of 3 replications, each consisiting of 12 fruits, ± SD.

Fig. 2 Chlorophyllase activity in the cucumber fruit peel during storagre at 13° C under light (—○—) and dark (—●—) conditions. Data are means of 3 replications, each consisting of 12 fruits, ± SD.

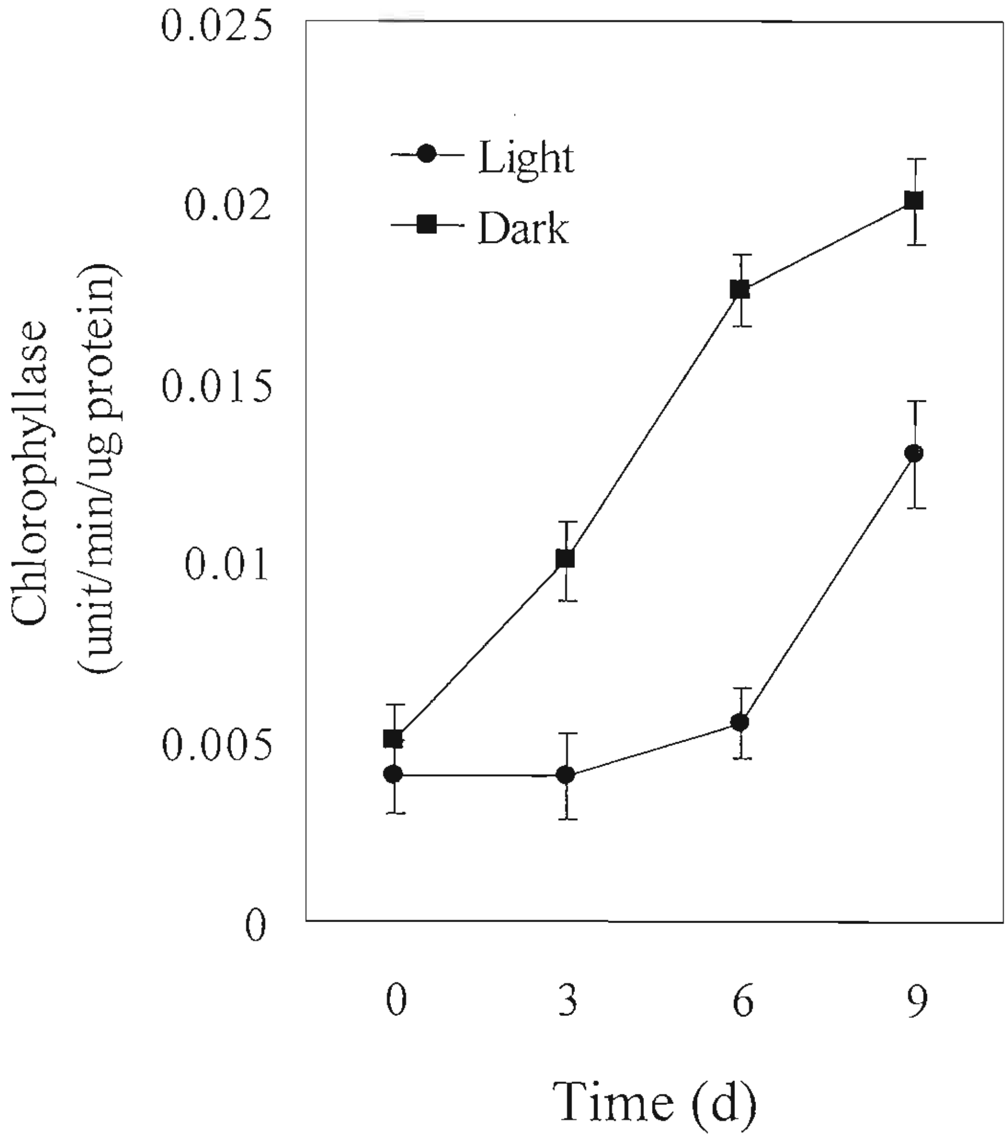
Fig. 3 Carotenoid content in the cucumber fruit peel during storage at 13° C under light (—○—) and dark (—●—) conditions. Data are means of 3 replications, each consisting of 12 fruits, ± SD.

Figure 1



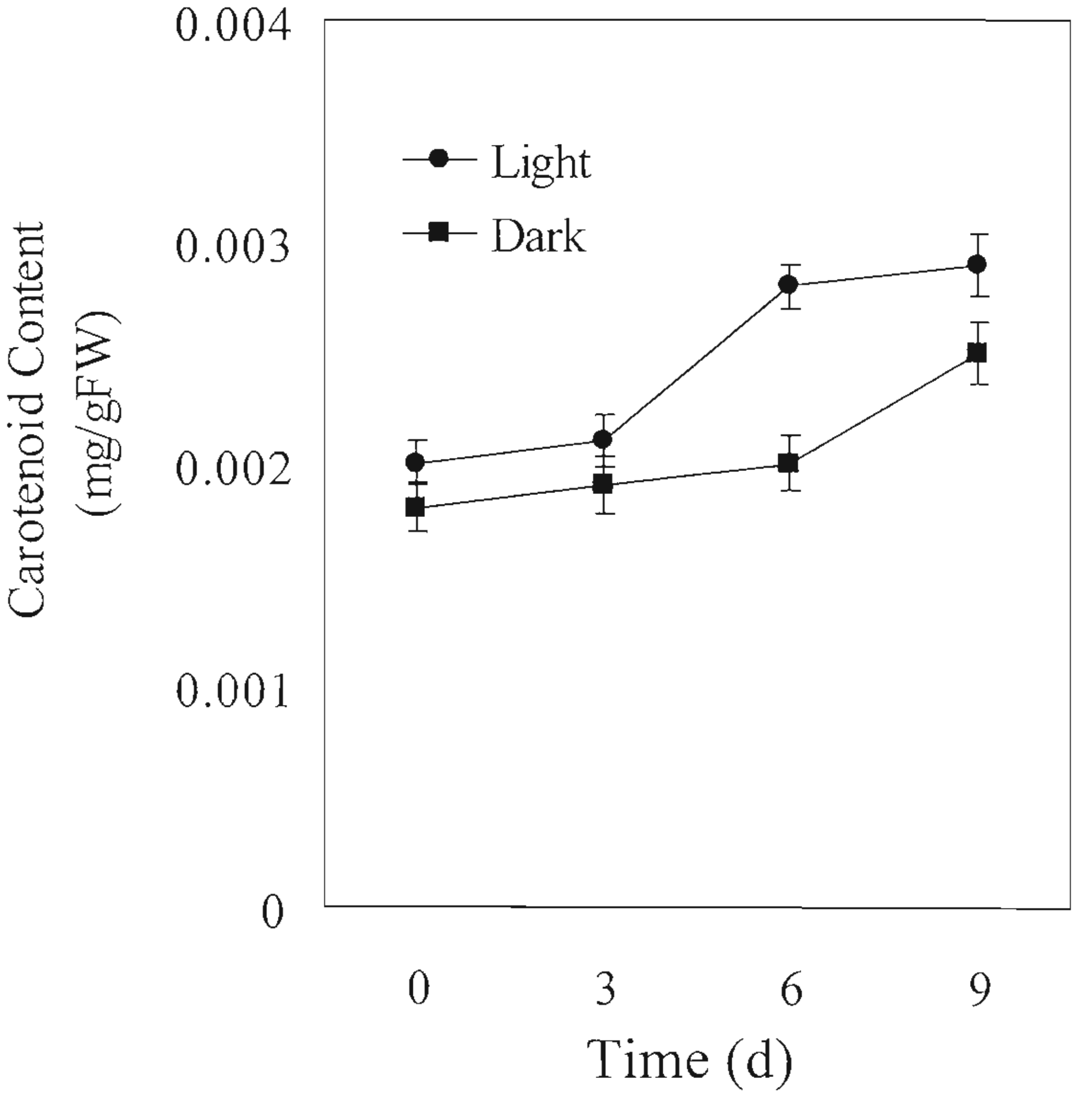
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Chlorophyll Content (mg/gFW)
Light
Dark
0
3
6
9
Time (d)

Figure 2



0.025
0.02
0.015
0.01
0.005
0
Chlorophyllase (unit/min/ug protein)
Light
Dark
0
3
6
9
Time (d)

Figure 3


0.004
0.003
0.002
0.001
0
Carotenoid Content (mg/gFW)
Light
Dark
0
3
6
9
Time (d)

เอกสารแนบหมายเลข 3

Effects of light and darkness on postharvest changes in Chinese kale during storage

Sompoch Noichinda[1], Kitti Bodhipadma[1], Chalida Mahamontri[1], Thitima Narongruk[1]

Saichol Ketsa[2]* and Wouter G. van Doorn[3]

[1]Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's Institute of Technology North Bangkok, Bangsue, Bangkok 10800, Thailand

[2]Department of Horticulture, Faculty of Agriculture, Kasetsart University, Chatuchak Bangkok 10900, Thailand

[3]Agrotechnology and Food Innovations, Wageniugen University and Research Centre P.O.Box 17, 6700AA Wageningen, The Netherlands

Abstract

Chinese kale (*Brassica oleracea* var. *alboglabra*) leaves stored at 1°C (95% RH) under the light and darkness were monitored for quality and postharvest changes. Chinese kale leaves stored under the light had better overall quality than stored indarkness. Weight loss of Chinese kale leaves stored under light increased more rapidly than stored under the darkness while ascorbic acid stored under the darkness decreased more rapidly than stored under the light. Light exposure did not only enhance carotenoid synthesis but also delayed chlorophyll loss in Chinese kale leaves. Chlorophyll a content increased rapidly while chlorophyll b content decreased sharply in Chinese kale leaves stored both under stored both under the light and darkness. Chlorophyllase activity of Chinese kale

---

*Corresponding author. E-mail address: agrsck@ku.ac.th (S.Ketsa). Fax: 66-02-5791951

leaves stored under the light and dark conditions decreased rapidly at initial time storage but chlorophyllase activity of Chinese kale stored under the darkness decreased more rapidly while peroxidase activity of Chinese kale leaves stored under the light and darkness increased rapidly at later of storage. Starch synthesis increased more rapidly under the light than by the darkness. Both fructose and glucose content increased rapidly while sucrose content remained slightly unchanged under the light and darkness.

Keywords: ascorbic acid, carotenoid, Chinese kale, chlorophyll, chlorophyllase, sugar, starch, peroxidase, weight loss

1. Introduction

Chinese kale (*Brassica oleracea* var. *alboglabra*) is an important leafy green vegetable. Chinese kale is a hardy, cool-season vegetable but it can be grown all year-round in the tropical region because it adapts and tolerates summer heat. Chinese kale differs primarily in leaf color and texture among varieties. After harvest, it can be stored for 10 to 14 days at 1°C (90-95% RH). The main problems of Chinese kale after harvest is lignification and yellowing (Poochai et al., 1984; Wilson et al., 1988). Mature leaves of Chinese kale are prone to turn yellow within a few days at ambient temperature after harvest. Yellowing of leaves may involve the degradation of chlorophyll by chlorophyllase and/or peroxidase (Yamauchi and Minamide, 1985). Although cold storage could maintain the quality of several perishable commodities but it is unable to delay the occurrence of yellowing in certain leafy and fruit vegetables (Yamauchi and Hashinaga, 1992; Yamauchi et al., 1997). Light was proved to be useful in activating the synthesis of pigments such as chlorophylls and anthocyanins in certain crop commodities (Perrin, 1982; Tombesi et al., 1993). It seems that light is related to an energy generation process of harvested green plant tissue. Thus, it is quite interesting to see whether light has any effect on postharvest changes of Chinese kale leaves during storage.

This study was aimed to monitor postharvest changes in Chinese kale leaves during storage under the light and darkness.

2. Materials and methods

2.1. Plant material

Mature, medium-green color leaves of Chinese kale, were harvested from a vegetable farm near Bangkok suburban. They were selected for uniformity of size and color. Leaves were then randomly separated into two groups and each group was placed in PE bag (0.0625mm thickness, 20 x 30 cm, Manee Mongkol import export, Thailand) containing 12 holes of 0.5cm in diameter and stored at 1°C (95±1 % RH). Individual PE bags had 5 stems of Chineses kale approximately 300 g. The first group was continuously exposed to the light under fluorescent lamp (light intensity, 2,000 Luxs), while the second groups were stored under the darkness.

2.2. Weight loss

Weight loss was recorded at 2-day intervals and calculated as a percentage of the initial weight.

2.3. Pigment analysis

Carotenoid was determined spectophotomically at 540 nm according to the method of Georges and Olivers (1993). Briefly, 5 g of Chinese kale leaves was ground with mortar and 15mg of ground powder was then added into 10ml ethanol, 1ml 60% potassium hydroxide and incubated in hot water bath (45-50°C) for 5 min. Subsequently, the incubated solution was centrifuged at 3500 rpm for 5 min. The supernatant was collected and the pellet was dissolved in 2ml ethanol and centrifuged with the same method as mentioned above. The supernatant included 15ml petroleum ether, 20ml of 9% NaCl was gently shacked by hand and stayed it until separate into two layers. Carotenoids was then determined spectophotomically at 540 nm.

Chlorophyll content was extracted and determined according to method of Martin et al. (1999). Leaf tissue (0.5g) of Chinese kale was ground with 30ml of 95% acetone and filtered through a Whatman No.2. The filtered solution volume was adjusted into 100ml with 80% acetone. Chlorophyll content was measured spectrophotometically at 645 and 663 nm and calculated as follows: total chlorophyll = 20.2(A645) + 8.02(A663); chlorophyll a = 12.7(A663) – 2.69(A645); chlorophyll b = 22.9(A645) – 4.68(A663).

2.4 Enzyme assay

Chlorophyllase extraction, 20g of leave blade mixed in 40ml of cold acetone and ground with mortar. The ground solution kept under the dark condition for 6 hours and then filtered through filter paper. The pellet (0.8g) consequently soaked in 20ml of 0.03M potassium phosphate buffer (pH 7.0) and kept under the dark condition at 5°C for 12 hours. Afterward, the incubated solution was centrifuged at 12000 rpm for 20 min. The supernatant was used for an enzyme activity assay. Chlorophyllase activity was spectrophotometrically assayed at 663 nm according to method of Shimokawa et al. (1978).

Peroxidase activity was assayed spectrophotometrically at 436 nm according to method described by Putter (1974). Leaf tissue (3g) was ground in extraction buffer including 30mM ethylenediamine, 3mM calcium chloride, 3mM β-mercaptonethanol in 20% glyceral (v/v) with mortar. The extracted solution was filtered through a filter paper No.2 and centrifuged at 15,000 rpm for 30 min. The supernatant was used for an enzyme activity assay.

Peroxidase activity assay, the reaction mixture included 4ml enzyme solution, .1 M potassium phosphate buffer (pH 7.0), 20mM guaiacol and 0.042% H2O2. Peroxidase activity was assayed spectrophotometrically at 436 nm.

Protein content was measured according to method of Bradford (1976) using bovine serum albumin as a standard.

2.5 Ascorbic acid determination

Leaf tissue (5g) was ground in 3% metaphosphoric acid with blender and filtered through a filter paper. The extraction solution was titrated with a standard dye solution (Rangana, 1978).

2.6 Carbohydrate analysis

Leaf tissue (100g) was blended and the blending paste was subsequently squeezed with nylon cloth. Then, the solution (10 ml) was centrifuged at 1,200 rpm for 5 min. Supernatant was filtered to Sepad® filter. For sugar (sucrose, glucose, and fructose) content determination, 20 μl of filtered solution was injected into high-pressure liquid chromatography (HPLC) equipped with a refractive index detector and Lichrocart NH2 column. Acetonenitrite in water (90:10) was used as a mobile phase at the flow rate 2 ml/min (Conard and Palmer, 1976). Starch content was determined with an Anthron reagent as described by McCready et al. (1950).

2.7 Statistical analysis

Experiments were carried out using 3 replications and each replication had 3 PE bags. Experiments were repeated twice.

3. Results

Weight loss

Weight loss of Chinese kale leaves stored in the darkness slightly increased, while weight loss of Chinese kale leaves stored in the light rapidly increased throughout the study period (Fig. 1A). At the end of 10-day experiment weight loss of Chinese kale leaves stored in the darkness was about 1.81% while weight loss of Chinese kale leaves stored in the light was about 3.92%.

Pigment content

Carotenoid contents of Chinese kale leaves stored in the light and darkness gradually increased and carotenoid content of Chinese kale leaves stored in the darkness was more slightly than that stored in the light throughout the study period (Fig.1B).

Total chlorophyll content of Chinese kale leaves rapidly decreased from day 0 to day 2 and slightly decreased thereafter until day 8 and slightly increased to a maximum on day 10 and sharply decreased thereafter. However, chlorophyll content of Chinese kale leaves stored in the darkness was much lower than that stored in the light during day 2 and 10 (Fig. 2A).

Chl a content of Chinese kale leaves stored in the light and darkness rapidly increased from day 0 to day 2 and chl a content slightly increased until day 10 and then sharply decreased thereafter. While chl a content of Chinese kale leaves stored in the darkness slightly increased from day 2 to day 4 and decreased to a minimum on day 6, then slightly increased until day 10 and sharply decreased thereafter. Chl a content of Chinese kale leaves stored in the darkness was much lower than that stored in the light during day 2 and day 10 (Fig. 2B).

Chl b content of Chinese kale leaves stored in the light and darkness rapidly decreased from day 0 to day 2 and slightly decreased until day 10 and rapidly decreased thereafter. However, chl b content of Chinese kale leaves stored in the darkness was more slightly lower than that of stored in the light (Fig. 2C).

Enzyme activity

Chlorophyllase activity of Chinese kale leaves stored in the light and darkness rapidly decreased from day 0 to day 4 and chlorophyllase activity of Chinese kale leaves stored in the remained unchanged from day 4 to day 8 and gradually increased thereafter. While chlorophyllase activity of Chinese kale leaves stored in the light slightly increased from day 4 to day 12 (Fig. 3A).

Peroxidase activity of Chinese kale leaves stored in the light and darkness slightly increased from day 4 to day 10 and rapidly increased from day 10 to day 12. Peroxidase activity of Chinese kale leaves store (Fig. 3B).

Carbohydrate content

Glucose content of Chinese kale leaves stored in the light slightly increased from day 0 to day 2 and rapidly decreased to a minimum on day 4 and rapidly increased to a maximum on day 8 and sharply decreased thereafter. Glucose content of Chinese kale leaves stored in the darkness fluctuated up and down and reached a minimum on day 6 and rapidly increased to a maximum on day 8 and rapidly decrease thereafter (Fig. 4A).

Fructose content of Chinese kale leaves stored in the light rapidly increased from day 0 to a maximum on day 6 and sharply decreased thereafter, while fructose content of Chinese kale leaves stored in the darkness increased from day 0 to day 4 and sharply decreased thereafter to a minimum on day 6 and rapidly increased to a maximum on day 8, then rapidly decreased thereafter (Fig. 4B). Sucrose contents of Chinese kale leaves stored in the light and darkness were relatively low and indifferent and slightly changed throughout the study period (Fig. 4C). Regardless to light and darkness, sucrose content of Chinese kale leaves was much lower than fructose and glucose contents.

Starch content of Chinese kale leaves stored in the light and darkness rapidly increased from day 0 throughout day 12 but starch content of Chinese kale leaves stored in the light increased more rapidly than that stored in the darkness (Fig. 5A).

Ascorbic acid content

Ascorbic acid content of Chinese kale leaves stored in the light and darkness steadily decreased but ascorbic acid content of Chinese kale leaves stored in the light decreased more rapidly than that of stored in the darkness (Fig. 5B).

4. Discussion

Weight loss is a problem of mostly leafy vegetables after harvest due to transpiration resulting in wilting (Ref). Temperature and relative humidity are main factor environmental factors controlling weight loss of commodities during storage (Ref). Weight loss of Chinese kale leaves stored in the light was significantly greater than that stored in the darkness. This should not be due to temperature and relative humidity because Chinese kale leaves stored in the same temperature and relative humidity under this experimental condition. Weight loss of leafy vegetables occurs mostly via stomata (Ref). Number of opening stomata of Chinese kale leaves stored in the light was significantly greater than that stored in the darkness resulting in more transpiration via opening stomata and thus increasing greater weight loss. This also indicated that light stimulated opening of stomata of Chinese kale leaves (Ref). Especially, blue light caused greater stomatal opening than red light (Lurie, 1978).

Total chlorophylls decreased concomitant with an increase in chl a and a decrease in chl b. This suggested that chl b was more degradable than chlorophyll a. The breakdown of chl b requires conversion of chlorophyll b to chlorophyll a before breakdown to colorless products. This conversion has been demonstrated convincingly in barley leaves (Scheumann et al., 1999) and cucumber cotyledons (Tanaka et al., 1995). Therefore, increased chl a was not only due to the light stimulation but also due to the conversion of chlorophyll b to chl a. Chl a increased and the amount was many times higher than chlorophyll b while total chlorophyll still decreased, suggesting that decreased total chlorophylls was mainly due to a breakdown of chl b. This indicated that most total chlorophyll remaining was largely shared by chl a. Chl a increased more rapidly in the light than in the darkness. This indicated that light stimulated chlorophyll a synthesis while the darkness stimulated chl b breakdown. Total carotenoids of Chinese kale leaves increased in the presence of light and darkness. This indicated that light is not essential for their accumulation. However, it was found that carotenoid biosynthesis in pepper leaves was completely stalled under dark condition (Simkin et al., 2003).

Chl b decreased concomitant with a decrease in chlorophyllase activity. This suggested chlorophyllase may not involve in the breakdown of chlorophyll b. Peroxidase activity has been reported that can catalyse the breakdown of chlorophylls (Yamauchi and Minamide, 1985; Yamauchi and Hashinaga, 1992). But peroxidae activity started to decrease on day 6 while chl b rapidly decreased on day 0 to day 2. This indicated early breakdown of chlorophyll b was not due to peroxidase. In addition to chlorophyllase and peroxidase, other systems such as Mg-dechelatase have been reported (Kunieda et al., 2005). Therefore, chlorophyll breakdown of Chinese kale leaves may involve with systems other than early chlorophyllase and peroxidase. Chl a and b rapidly decreased on day 10 to day 12 concomitant with a rapid increase of peroxidase activity on day 10 to day 12 while clorophyllase activity remained unchanged. This indicated that breakdown of chl a and b during from day 10 to day 12 was mainly due to peroxidase.

Green leafy vegetable like Chinese kale is a rich source of ascorbic acid (Gupta, et al., 2005) and ascorbic acid is perhaps only a vitamin in leafy vegetable easily lost after harvest. Ascorbic acid in Chinese kale stored in the light and darkness continued to rapidly decrease throughout the study period even though stored at 1°C but more drastical decline in the light, suggesting that light catalized more oxidation of ascorbic acid than the darkness. Peroxidase can catalize oxidation of ascorbic acid (Chinoy et al.,1984). However, peroxidase activity in Chinese kale did not increase until day 6 and rapidly increased on day 10 to day 12, while ascorbic acid started to decrease on day 0 until the end of study period. Yet peroxidase activity in Chinese kale stored in the darkness was higher than that of stored in the light. This suggested that peroxidase is not involved in the ascorbic acid loss of Chinese kale stored in the light and darkness.

Since ascorbic acid is synthesized from a hexose precursor (Smirnoff and Pallanca, 1996). There has been reported light enhancing ascorbic acid biosnythesis in spinach leaves and the role of light related to the control of ascorbic acid pool size due to the increased availability of carbohydrates, especially that of glucose, could then contribute to the control of ascorbic acid pool. Indeed, starch and glucose contents in Chinese kale were higher in the light than in the darkness. But ascorbic acid in Chinese kale decreased more rapidly in the light than in the darkness. This suggested that higher carbohydrate content can not delay the loss of ascorbic acid.

The main enzyme for the oxidation of ascorbic acid is ascorbate oxidase (Fatibello-Filho and Vieira, 2000). Light probably increase this enzyme resulting oxidation of ascorbic acid, thus reducing more loss of ascorbic acid in Chinese kale. This need further study.

Starch accumulation rapidly increased in the presence of light and darkness. This indicated that light is not essential for its accumulation even though light stimulated the accumulation. Light probably increased starch accumulation via photosynthesis. Starch can be degraded into sugars. Glucose and fructose contents increased and were greater in the light than in the darkness while sucrose contents were comparable in the light and darkness. This suggested that turnover of starch was slow because its accumulation was greater than its degraded.

Acknowledgements

This research was financially supported by the Thailand Research Fund (TRF).

References

Bradford, M.M. 1976. A rapid and sensitive method for the quantisation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Chinoy, N.J., Dave, I.C., Singh, Y.D., Saxena, O.P., and Vyas, A.V. 1984. The Role of Ascorbic Acid in Growth, Differentiation and Metabolism of Plants. Martinus Nijhoff/Dr W.Junk Publishers, The Hague.

Conrad, E.C. and Palmer J.K. Rapid analysis of carbohydrate by high-pressure liquid chromatogarphy. Food Technol. 30: 84-92.

Decoteau, D.R. 2000.Vegetable Crops. Pentice Hall. Upper Saddle River, New Jucrsey. 464P.

Delvin, M.D. and Witham, C.W. 1983. Plant Physiology. 4th edition, Willard Grant Press, Boston.

Fatibello-Filho, O. and I.C. Vieira. 2000. L-ascorbic acid determination in pharmaceutical formulations using a biosensor based on carbon paste modified with crude extract of zucchini (*Cucurbita pepo*). J. Braz. Chem. Soc. 11: 412-418.

Georges, C. and Olivier, H. 1993. Carotenoid pigment of the algae *Haematococcus pluvialis* assay on Rainbow trout. Aqua. Culture. 112: 217-226.

Guiamet, J.J. and Giannibelli, C. 1994. Inhibition of the degradation the chloroplast membranes during senescence in nuclear "stay green" mutants of soybean. Physiol. Plant. 91: 395-402.

Gupta, S., A.J. Lakshmi, M.N. Manjunath and J. Prakash. 2005. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT 38: 339-345.

Heaton, J.W. and Mrangoni, A.G. 1996. Chlorophyll degradation in processed foods and senescent plant tissures. Trends in Food Sci. Technol. 7: 8-15.

Hendry, G.A.F., Houghton, J.D. and Brown, S.B. 1987. The degradation of chlorophyll a biological enigma. New Phytol. 107: 255-302.

Kunieda, T., T. Amano and Y. Shioi. 2005. Search for chlorophyll degradation enzyme, Mg-dechelatase, from extracts of *Chenopodium album* with native and artificial substrates. Plant Sci. 169: 177-183.

Laurie, S. 1978. The effect of wavelength of light on stomatal opening. Planta 140: 245-249.

Martin, S., Ihi, M. and Bifani, V. 1999. Preliminary report on colour quality measured as chlorophyllase in strawberries at different stage of maturity. Acta Hort.251: 203-207.

McCredy, R.R., J. Guggolz, V. Silverna and H.S. Owens. 1950. An improved method for starch determination in banana fruit. Anal Chem. 22: 1156-1164.

Okada, K., Inue, Y., Satoh, K. and Katoh, S. 1992. Effects of light on degradation chlorophyll and proteins during senescence of deteched rice leaves. Plant Cell Physiol. 33: 1183-1191.

Perrin, 1982; Putter, J. 1974. Peroxidase, pp. 685-690. *In* H.U. Bergmeyer (ed.) Methods of Enzymatic Analysis Vol. 2. Verlag Chemic, Weinheim.

Poochai, S., Ketsa, S. and Kosiyachinda, S. 1984. Effects of temperatures and packaging materials on quality and storage life of Chiness kale (*Brassica oleracea* var. *acephala* D.C.). Kasetsart J. (Sci.) 18: 1-6.

Rangana, S. 1978. Manual of Analysis of Fruit and Vegetable Products. Tata McGraw-Hill Publishing Co., New Delhi. 340 P.

Scheumann, V., S. Schoch and W. Rudiger. 1999. Chlorophyll b reduction during senescence of barley seedlings. Planta 209: 364-370.

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E. and Duncan, G. 1995. Effects of environment on the composition of epicuticular wax from kale and swede. Phytochem. 40: 407-417.

Shimokawa, K., Shimada, S. and Yaeo, K. 1978. Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unchin* Marc. Sci. Hort. 80: 129-135.

Simkin, A.J., C. Zhu, M. Kuntz and G. Sandmann. 2003. Light-dark regulation of carotenoid biosynthesis in pepper (*Capsicum annuum*) leaves. J. Plant Physiol. 160: 439-443.

Smirnoff, N. and J.E. Pallanca 1996. Ascorbate metabolism in relation to oxidative stress. Biochem. Soc. Trans. 24: 472-478.

Tanaka, A., Y. Tanaka, T. Takabe and H. Tsuji. 1995. Calcium-induced accumulation of apoproteins of the light-harvesting chlorophyll a/b-protein complex in cucumber cotyledons in the dark. Plant Sci. 105: 189-194.

Tayor, A.O. and Craig, A.S. 1971. Plants under climatic stress, II. Low temperature, high light effects on chloroplast ultrastructure. Plant Physiol. 47: 719-725.

Thimann, K.V. 1980. The senescence of leaves, pp. 85-115. *In* K.V. Thimann (ed.). Senescence in Plants. CRC Press, Boca Raton.

Tombesi, A., Antognozzi, E. and Palliotti, A. 1993. Influence of light exposure on characteristics and storage life of kiwifruit. New Zealand J. Crop Hort. Sci. 21: 87-92.

Wilson, D.W., Barwick, J.M., Lomax, J.A., Jarvis, M.C. and Duncan, H.J. 1988. Lignified and non-lignified cell walls from kale. Plant Sci. 57: 83-90.

Yamauchi, N. and Minamide, T. 1985. Chlorophyll degradation by peroxidase in parsley leaves. J. Japan. Soc. Hort. Sci. 54: 265-271.

Yamauchi, N. and Hashinaga, F. 1992. Chlorophyll degradation by peroxidase in ethylene-treated satsuma mandarin fruits. J. Japan. Soc. Cold Preserv. Food 18: 167-172.

Yamauchi, N., Harada, K. and Watada, A.E. 1997. In vitro chlorophyll degradation in stored broccoli (*Brassica oleracea* L. var. *italica* Plen.) florets. Postharvest Biol. Technol. 12: 239-245.

Francisco J. Pérez, Daniel Villegas and Nilo Mejia Ascorbic acid and flavonoid-peroxidase reaction as a detoxifying system of H2O2 in grapevine leaves. Phytochemistry, Volume 60, Issue 6, July 2002, Pages 573-580

Yoko Morimura, Koji Iwamoto, Toshihide Ohya, Takao Igarashi, Yoshiko Nakamura, Akihiro Kubo, Kiyoshi Tanaka and Tomoyoshi Ikawa Light-enhanced induction of ascorbate peroxidase in Japanese radish roots during postgerminative growth. Plant Science, Volume 142, Issue 2, 29 March 1999, Pages 123-132.

Table 1. Number of opened and closed stomata of Chinese kale leaves under light and darkness conditions during storage

Day	Light		Dark	
	Number of stomata		Number of stomata	
	Opened	Closed	Opened	Closed
0	16.33	1.67	16.33	2
2	15.33	1.67	1	15.33
4	13.67	0	1.33	10
6	26.33	0	1	10.33
8	21.33	0.33	1	11
10	25.67	0	2	12.37

Note: Number of stomata counted from 0.24 X 0.32 mm^2 leaves area under microscope (data were collected from 3 replications)

Legends to Figures

Fig. 1 Weight loss (A) and carotenoid content (B) of Chinese kale leaves stored under light (-●-) and darkness (-■-) conditions at low temperature. Means are the average of 3 replications ± SE.

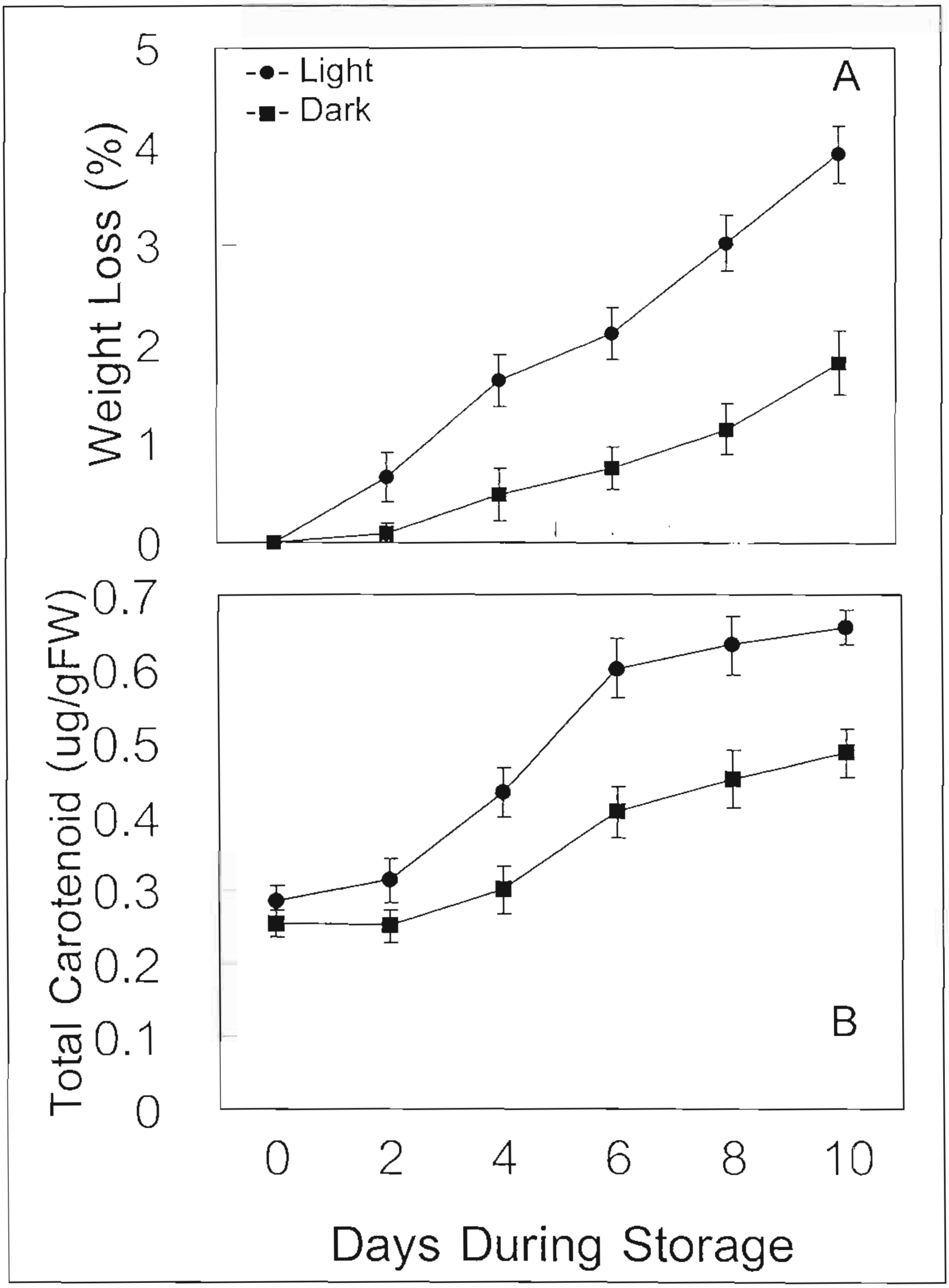
Fig. 2 Total chlorophyll (A), chlorophyll a (B) and chlorophyll b (B) of Chinese kale leaves stored under light (-●-) and darkness (-■-) conditions at low temperature. Means are the average of 3 replications ± SE.

Fig. 3 Activities of chlorophyllase (A) and peroxidaes (B) of Chinese kale leaves stored under light (-●-) and darkness (-■-) conditions at low temperature. Means are the average of 3 replications ± SE.

Fig. 4 Sucrose (A), fructose (B) and glucose (C) content of Chinese kale leaves stored under light (-●-) and darkness (-■-) conditions at low temperature. Means are the average of 3 replications ± SE.

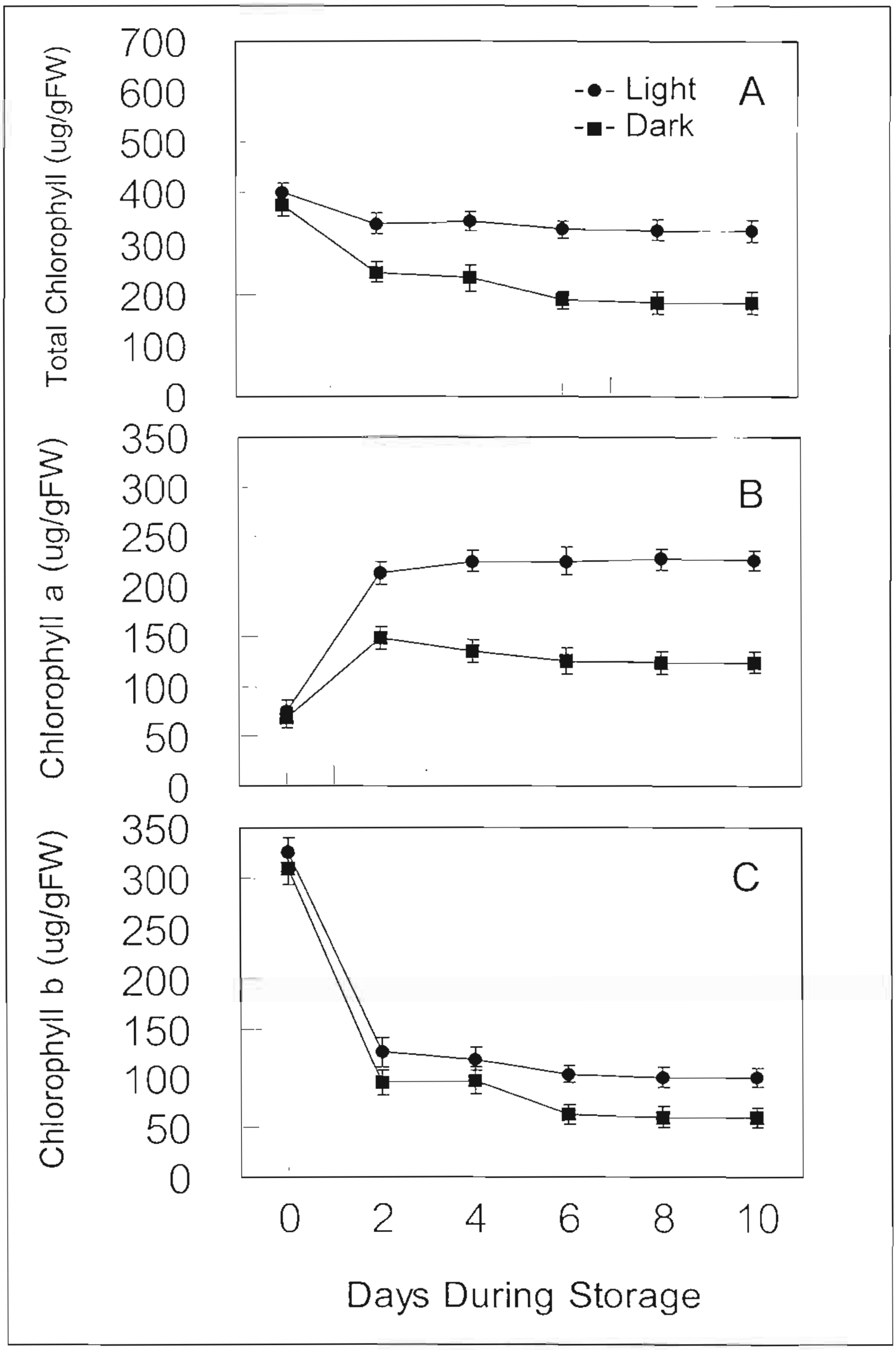
Fig. 5 Starch(A) and ascorbic acid (B) content of Chinese kale leaves stored under light (-●-) and darkness (-■-) conditions at low temperature. Means are the average of 3 replications ± SE.

Figure 1



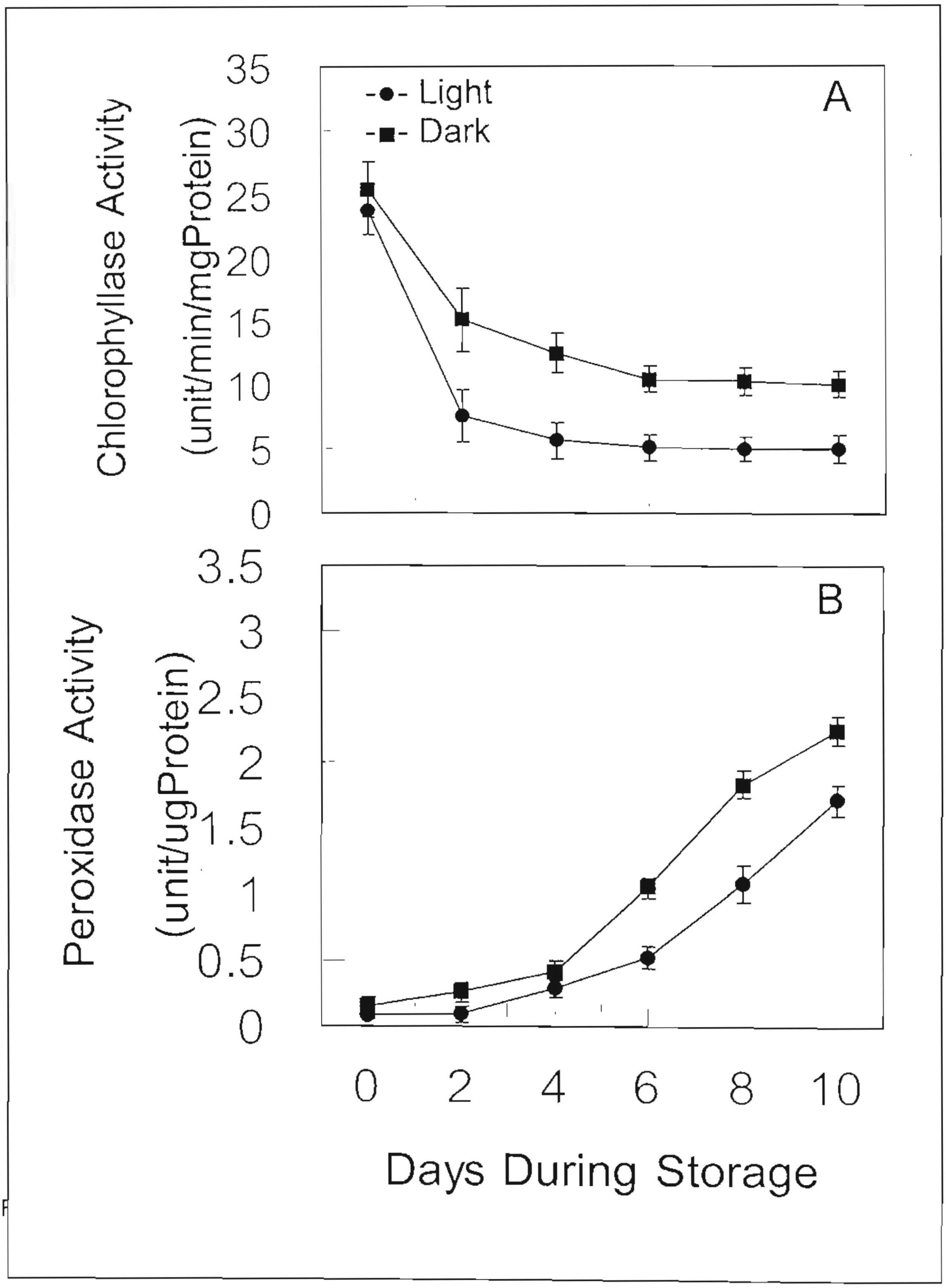
-●- Light
-■- Dark
A
Weight Loss (%)
5
4
3
2
1
0
Total Carotenoid (ug/gFW)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
B
0
2
4
6
8
10
Days During Storage

Figure 2



Total Chlorophyll (ug/gFW)
700
600
500
400
300
200
100
0
-●- Light
-■- Dark
A
Chlorophyll a (ug/gFW)
350
300
250
200
150
100
50
0
B
Chlorophyll b (ug/gFW)
350
300
250
200
150
100
50
0
C
0
2
4
6
8
10
Days During Storage

Figure 3



-●- Light
-■- Dark
A
B
Chlorophyllase Activity
(unit/min/mgProtein)
0
5
10
15
20
25
30
35
Peroxidase Activity
(unit/ugProtein)
0
0.5
1
1.5
2
2.5
3
3.5
0
2
4
6
8
10
Days During Storage

Figure 4

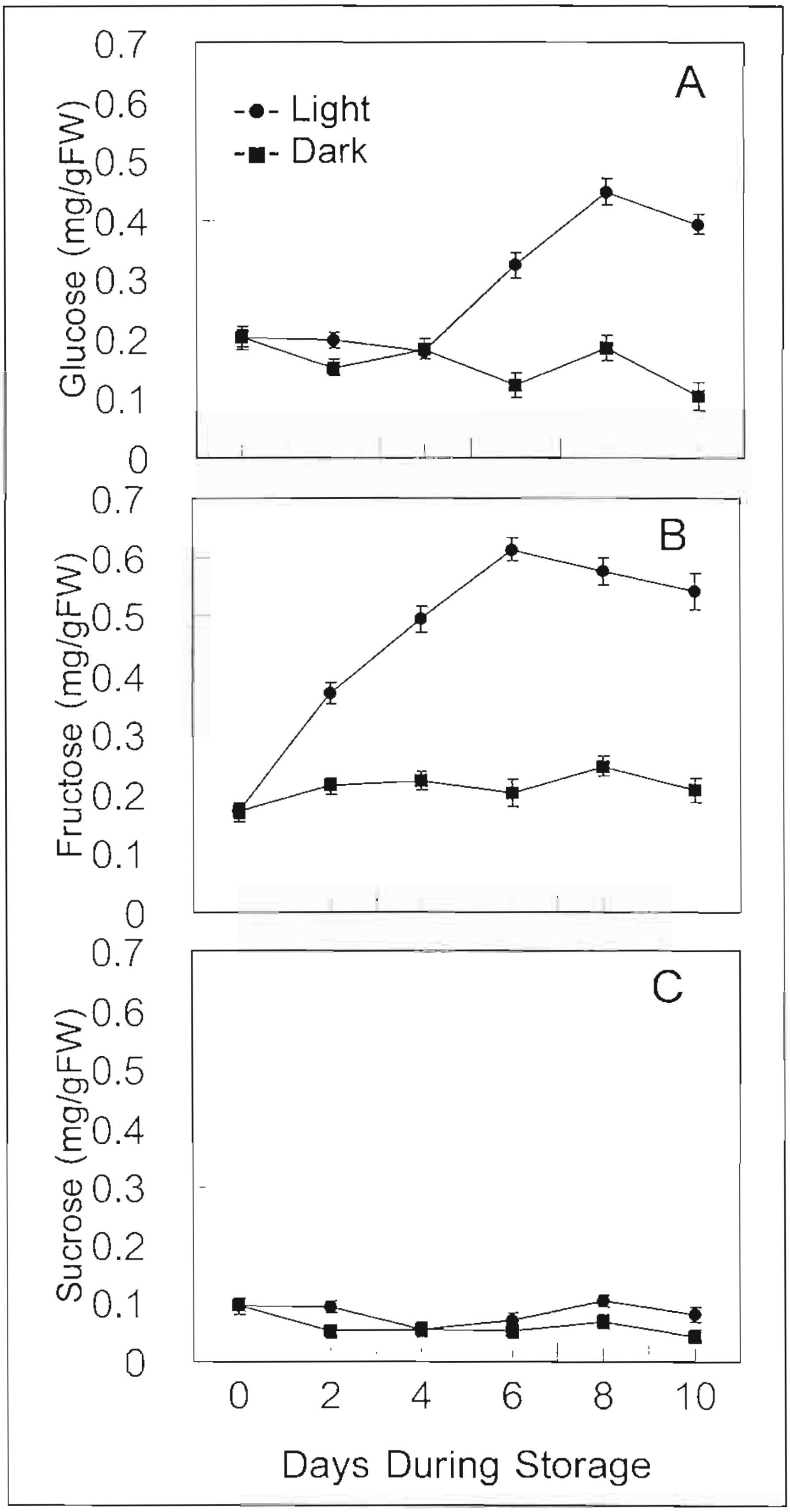
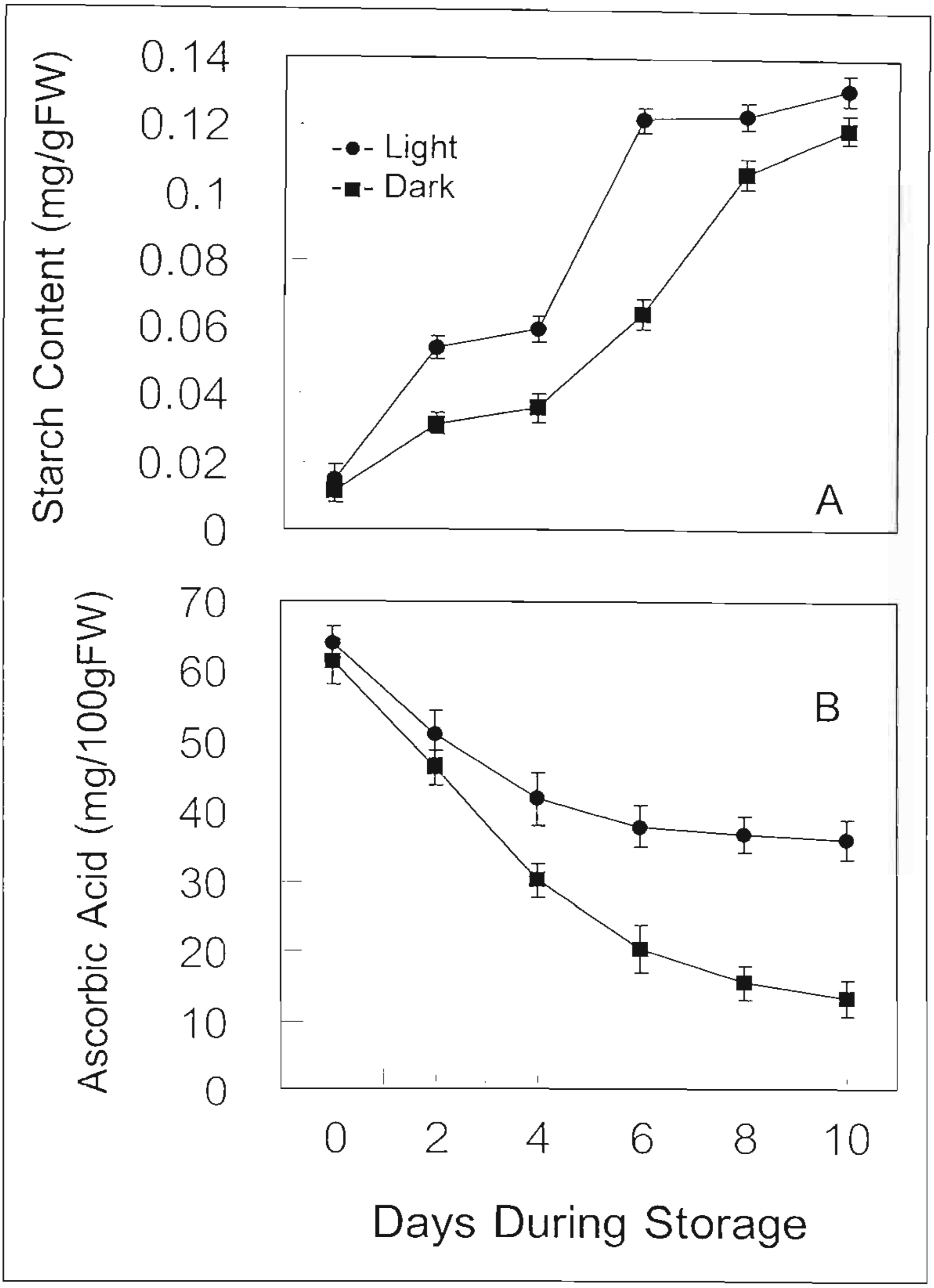

A
-●- Light
-■- Dark
Glucose (mg/gFW)
B
Fructose (mg/gFW)
C
Sucrose (mg/gFW)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
2
4
6
8
10
Days During Storage

Figure 5


Starch Content (mg/gFW)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-●- Light
-■- Dark
A
Ascorbic Acid (mg/100gFW)
70
60
50
40
30
20
10
0
B
0
2
4
6
8
10
Days During Storage

เอกสารแนบหมายเลข 4

Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

Postharvest Biology and Technology 31 (2004) 167–175

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Senescent spotting of banana peel is inhibited by modified atmosphere packaging

Rujira Choehom[a,1], Saichol Ketsa[a,*], Wouter G. van Doorn[b]

[a] Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
[b] Agrotechnology and Food Innovations, Wageningen University and Research Centre, P.O. Box 17, 6700 AA Wageningen, The Netherlands

Received 7 February 2003; accepted 31 July 2003

Abstract

Banana fruit (*Musa cavendishii* [*Musa acuminata*] AA Group cv. Sucrier) were placed in trays and held at 29–30 °C. Covering the trays with 'Sun wrap' polyvinyl chloride film prevented the early senescent peel spotting, typical for this cultivar. Carbon dioxide and ethylene concentrations within the packages increased, but inclusion of carbon dioxide scrubbers or ethylene absorbents, which considerably affected gas composition, had no effect on spotting. Experiments with continuous low oxygen concentrations confirmed that the effect of the package was mainly due to low oxygen. Relative humidity was higher in the packages but this had no effect on spotting. The positive effect of modified atmosphere packaging on peel spotting was accompanied by reduced in vitro phenylalanine ammonia lyase (PAL) activity in the peel, and by an increase of in vitro polyphenol oxidase (PPO; catechol oxidase) activity. We conclude that senescent spotting of banana peel requires rather high oxygen levels. It is not known which reaction becomes limiting for spotting, at low oxygen levels. Whatever the mechanism, the increase of in vitro PPO activity apparently shows an increase in potentially active protein.

Keywords: Banana; Catechol oxidase; *Musa*; Phenylalanine ammonia lyase; Polyphenol oxidase; Senescent spotting; Total free phenolics

1. Introduction

Senescent spotting of the banana peel is a physiological postharvest disorder. Initially, some very small brownish spots are found locally. Subsequently, such spots are observed all over the peel, and their number, intensity of browning, and size increase. The spots may then overlap to form larger patches, they become dark brown or even black, and form sunken pits on the surface. Little is known both about the origin of this disorder and about its physiological mechanism (New and Marriott, 1974; Liu, 1976; Marriott, 1980; Valdez and Mendoza, 1988; Ketsa, 1996, 2000). A few experiments showed that film wrapping reduced the disorder (Ketsa, 1996, 2000; Valdez and Mendoza, 1988), but it has remained unclear why this was so.

In most cultivars, senescent spotting occurs late with respect to pulp softening. However, in a few important cultivars, this disorder occurs as soon as the fruit has become more yellow than green, a stage that coincides

* Corresponding author. Fax: +66-2-579195.
E-mail address: agrsck@ku.ac.th (S. Ketsa).
[1] Present address: Laboratory of Postharvest Technology, Thailand Institute of Scientific and Technological Research, Bangkok 10900, Thailand.

0925-5214/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2003.07.001

with good taste and aroma of the fruit flesh. Consumers that are unfamiliar with these cultivars may reject the produce as they believe it is too ripe or infected (Marriott, 1980; Lizada et al., 1990; Ketsa, 1996).

The relations between senescent peel spotting and the concentration of free phenolics in the peel, or between spotting and the activities of phenylalanine ammonium lyase (PAL) and polyphenol oxidase (PPO; catechol oxidase) have apparently not been reported. Browning is common in plant tissues and usually brought about by free phenolic compounds (Mayer and Harel, 1979; Rhodes et al., 1981). The synthesis of free phenolics can start with deamination of phenylalanine by PAL (EC 4.3.1.5), thereby producing trans-cinnamate, a monophenol. Independently, PPO (catechol oxidase; EC 1.14.18.1 and EC 1.10.3.1) can convert monophenols to diphenols (monophenoloxidase activity) and it can further oxidize diphenols (diphenoloxidase activity). PPO, for example, converts monophenols to dopachrome, which is at the beginning of the cascade leading to brown pigments, and it further catalyses the oxidation of dopachrome to quinones. The latter polymerize nonenzymatically into brown pigment (Hanson and Havir, 1979; Macheix et al., 1990). Although this needs to be established, peel spotting in banana may also follow this pathway.

We used 'Sucrier' bananas, a cultivar widely grown in Thailand, since it has a serious problem of early senescent spotting. Preliminary studies showed that senescent spotting in this cultivar was considerably reduced if the fruit were wrapped in plastic. The purpose of this study was, therefore, to examine the effect of film wrapping on the incidence of spotting, and the role of the modified gas composition within the package. We also studied the relationship between peel spotting and the activities of PAL and PPO, and with the levels of total free phenolics in the peel.

2. Materials and methods

2.1. Plant material and assessment of senescent spotting

Each batch in the experiments consisted of three hands of banana fruit, which were the unit of replication. Each hand consisted of two layers of fruit. For analysis only the middle, larger, fruit were selected. From each hand, 10–15 fruit were taken at random for visual inspection and for measurements.

'Sucrier' bananas (*Musa cavendishii* [*Musa acuminata*] AA group; locally known as 'Kluai Khai' (Valmayor et al., 2000)) were harvested at commercial maturity from a plantation in Petchaburi province (Western Thailand). Bunches were cut in hands and transported to the laboratory in corrugated cardboard boxes, within 2 h of harvest. In the laboratory, hands, after selection for uniformity of size and color, were cleaned in a solution of 0.5% $MgSO_4$ to remove latex from the cut surface. Bananas were then dipped for 2–3 min in 500 $\mu g\, ml^{-1}$ thiabendazole solution to control fruit rot, and were allowed to dry before dipping for 1–2 min in 500 $\mu l\, l^{-1}$ ethephon to ripen them uniformly. After this dip, they were dried at ambient temperature (29–30 °C) and left to ripen at that temperature. Bananas were ripened until reaching color index 3–4 (Lizada et al., 1990) for further experimentation. At color index 3, the peel is more green than yellow and at color index 4 it is more yellow than green.

Hands of bananas were randomly sampled for determination of senescent spotting on the banana peel using a scale of 1–4 where 1 means no development of senescent spotting and 4 is severe senescent spotting. In more detail: score 1—peel yellow without spots; score 2—the surface is a little darker yellow, some brownish spots occur, small as the point of a needle; score 3—spots found all over the surface, their number, intensity of browning, and size (now up to 0.5–1 mm) increasing, most spots still separated; and score 4—size of the spots increase, they sometimes overlap into larger patches, spots become darker, or are even black, and form sunken pits on the surface.

2.2. Wrapping

Individual hands of bananas were randomly placed on a polystyrene tray (15 cm × 25 cm × 3 cm) and wrapped with 'Sun wrap' PVC film (Korea Plastic Industry Cooperative, Seoul, South Korea) over the bananas and the tray. The film was 12.5 μm thick, had a permeance to water vapor of 1.33 $nmol\, s^{-1}\, m^{-2}\, Pa^{-1}$ and to oxygen and carbon dioxide of 0.031 and 0.066 $nmol\, s^{-1}\, m^{-2}\, Pa^{-1}$, respectively.

Bananas with and without PVC film were kept at ambient temperature (29–30 °C) and relative humidity (67–69%). Ethylene absorbents were made by soaking pieces of chalk (0.70 cm diameter and 0.5 cm thickness) in saturated potassium permanganate solution; 50 g of the dried material was placed in a perforated polyethylene sachet, and one sachet was used per tray. Small paper bags filled with 30 g of calcium hydroxide served as carbon dioxide scrubbers, using one bag per tray.

2.3. Determination of ethylene, carbon dioxide and oxygen

Concentrations of ethylene, carbon dioxide and oxygen within the packages were measured by sampling through the PVC film with a syringe. The samples were injected into a gas chromatograph equipped with a flame ionization detector (Shimadzu, Kyoto, Japan) for ethylene and a thermal conductivity detector (Shimadzu, Kyoto, Japan) for carbon dioxide and oxygen. Holes in PVC film were sealed with tape between the sampling dates. Gas concentrations are expressed in Pa.

2.4. Oxygen treatment

Banana hands were individually held in sealed plastic containers in air (21 kPa oxygen), 5, 10 and 15 kPa oxygen (balanced by nitrogen), with a flow-through system. These treatments will be referred to as 5, 10, 15 and 21% oxygen. Bananas were randomly sampled for determination of senescent spotting, activities of PPO and PAL and total phenolics, which was done as mentioned below.

2.5. Effect of relative humidity

Control bananas were held in open plastic baskets at room temperature. Other hands of bananas were placed on foam trays wrapped with 'Sun wrap' PVC film. A comparison was made using 0 and 500 g of silica gel inside the packages, using three replications. The silica gel was renewed daily. To compare low and high relative humidity (RH) in unwrapped produce, two hands of bananas were placed in a 20 litters closed plastic container, which was connected with a flow of normal air at 125 ml/min. Data loggers were placed in the PVC wraps and plastic containers to monitor both RH and temperature over the 7 days study period.

2.6. Extraction and assay of PAL and PPO; total free phenolic content

Extraction and assay of PAL was carried out as described by Camm and Towers (1973). Briefly, frozen tissue (1:10, w/v) was homogenized in 95% cold acetone, filtered, extracted again in cold ethanol, and filtered again. Acetone powder was dried in a desiccator, and 500 mg was added to 50 ml of cold 0.2 M sodium borate buffer at pH 8.8. The beaker was shaken for 30 min in a cold room. The suspension was filtered and centrifuged. During the preparation of the enzyme extract, the temperature was kept at 4 °C. The assay medium contained 1.5 ml of enzyme extract, 2 ml distilled water and 1 ml of 10 mg ml^{-1} phenylalanine. The mixture was incubated at 37 °C for 1 h. The reaction was stopped by adding 0.5 ml of 5 N HCl. PAL activity was determined by measuring absorbance at 290 nm. One unit of PAL activity was defined as the change in absorbance per ml enzyme extract.

PPO was extracted and assayed using the method of Luh and Phithakpol (1972). Briefly, the extraction method was the same as that of PAL, except that 0.1 M citric buffer at pH 6.2 was used instead of the borate buffer. The assay medium contained 10 ml of enzyme extract and 5 ml of 0.1 M catechol. PPO activity was determined by measuring absorbance at 420 nm. One unit of PPO activity was defined as the change in absorbance after 1 min of measurement, per ml enzyme extract.

Protein content in the enzyme extracts was estimated using the Bradford (1976) method. Specific activity of the enzyme was expressed as units per mg protein.

Total free phenolic content was estimated colorimetrically, using the method described by Singleton and Rossi (1965). Briefly, frozen tissue was homogenized in ethanol, filtered and centrifuged. The compounds reacted with Folin Ciocalteau reagent, and were determined photometrically.

Each treatment had five replications, and each replication consisted of the whole peel from 10–15 fruit, pooled together. Determinations of enzyme activity were made twice in each extract.

2.7. Statistical analysis

In the tests on visible scores, three replications (each consisting of a hand of fruit) were used in each treatment. Individual fruit were evaluated daily for senescent spotting. Means were compared using Duncan's multiple range test (DMRT).

3. Results

3.1. Development of senescent spotting

At the onset of the packaging experiments the fruit had ripened to stage 3–4 (i.e. they were about 50% green and 50% yellow) and showed no senescent spotting. In control bananas (no PVC film) senescent spotting rapidly increased from day 2 to day 4. The 'Sun wrap' film completely prevented peel spotting during the 6 days of the experiments (Fig. 1A), and in a repeat experiment it almost completely did so. Upon removal of the 'Sun wrap' film, following 3 days of holding the bananas in the package, senescent spotting developed more rapidly than in fruit that was kept in the package. The increase was largest on day one after film removal (results not shown).

Inclusion of absorbers for carbon dioxide and ethylene in the packages did not affect senescent spotting (Fig. 1A). Holding the bananas in an atmosphere containing 5% oxygen effectively reduced senescent spotting, while an atmosphere containing 15% oxygen was not significantly different from the control bananas held in air (Fig. 2).

3.2. Atmospheric composition within the packages

Oxygen concentrations within packages containing bananas and covered with 'Sun wrap' rapidly decreased from about 18% on day 0 to about 5% on day 2 (Fig. 1B). Carbon dioxide concentrations within the packages increased from about 1% on day 0 to a maximum of about 6% on day 2, and slowly decreased thereafter (Fig. 1C). Ethylene concentrations within the packages increased to about 0.08 Pa on days 0–2 (Fig. 1D).

The inclusion of a scrubber (calcium hydroxide) for carbon dioxide or an absorbent (potassium permanganate) for ethylene did not affect the oxygen concentrations within the 'Sun wrap' package (Fig. 1B). The carbon dioxide scrubber prevented the build up of carbon dioxide within the packages, at least until day 4 (Fig. 1C), whilst the ethylene absorber considerably reduced the ethylene concentrations within the package (Fig. 1D). The carbon dioxide scrubber reduced

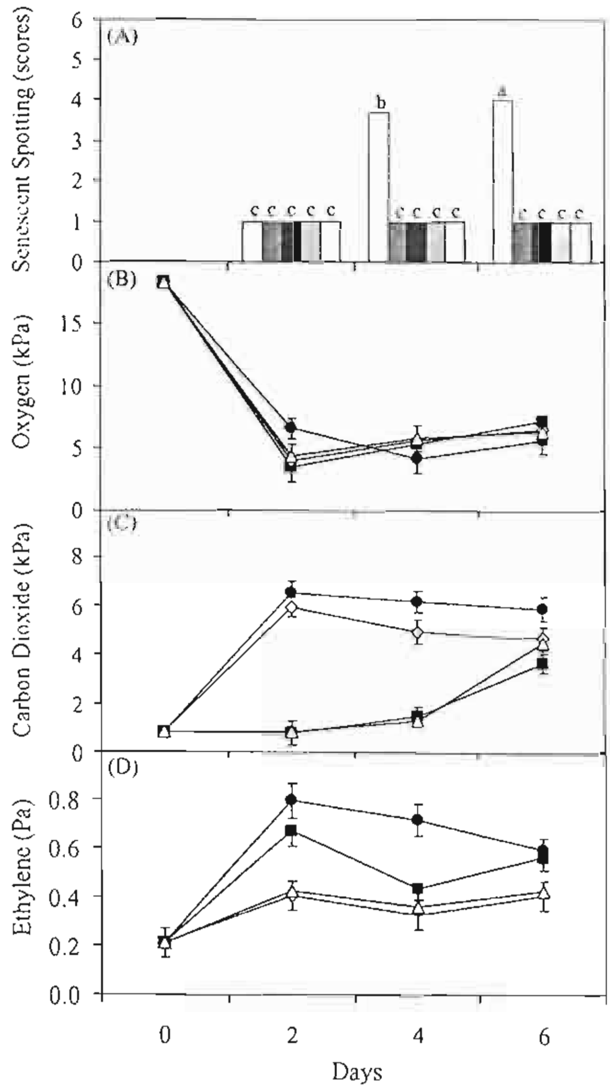


Fig. 1. Senescent spotting (A) of bananas without PVC film (□), and with 'Sun wrap' (▩), 'Sun wrap' + carbon dioxide scrubber (CS) (■), 'Sun wrap' + ethylene absorbent (EA) (□), or 'Sun wrap' + CS + EA (□). Oxygen (B), carbon dioxide (C) and ethylene (D) within 'Sun wrap' packages containing banana without CS + EA (●) 'Sun wrap' with CS (■), 'Sun wrap' with EA (◇) and 'Sun wrap' with CS + EA (△). Data are means of three replications ± S.E.

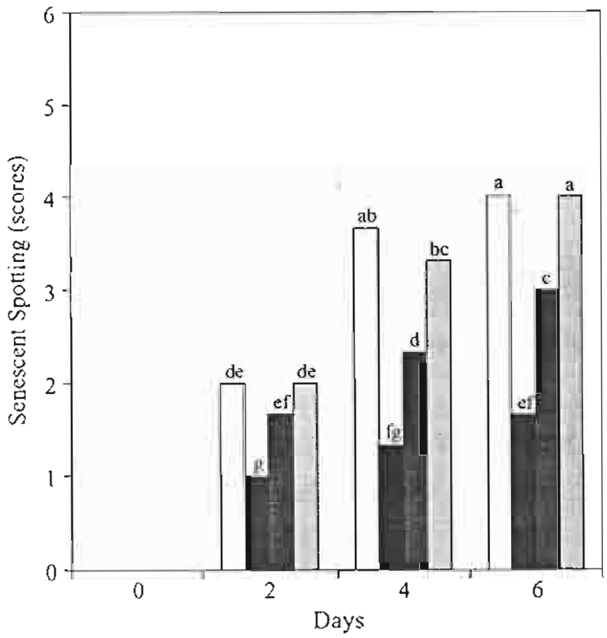


Fig. 2. Senescent spotting of bananas held in air (□), 5 (■), 10 (■), and 15% (□) O_2. Data are means of three replications ± S.E.

the ethylene concentrations on day 4, but this may be an anomalous result (Fig. 1D).

3.3. *Effect of relative humidity*

As compared to controls, PVC wrapping will increase the RH, depending on the permeability for water vapor of the film. We tested the effect of RH on senescent spotting by adding silica gel within the packages wrapped with 'Sun wrap' PVC. This reduced the RH to levels similar to that around unwrapped produce. The average RH at room temperature was 75%. RH within PVC wrap with and without silica gel was 77 and 93%, respectively. Unwrapped fruit showed serious senescent spotting, whereas almost no spotting was observed in PVC-wrapped fruit (results not shown). We also increased the RH around unwrapped produce. Fruit was placed in a closed container connected with ambient airflow. RH in the container was 99–100%. Bananas in the container had serious senescent spotting. The brown spots on these fruit seemed larger than in bananas held at lower RH (results not shown).

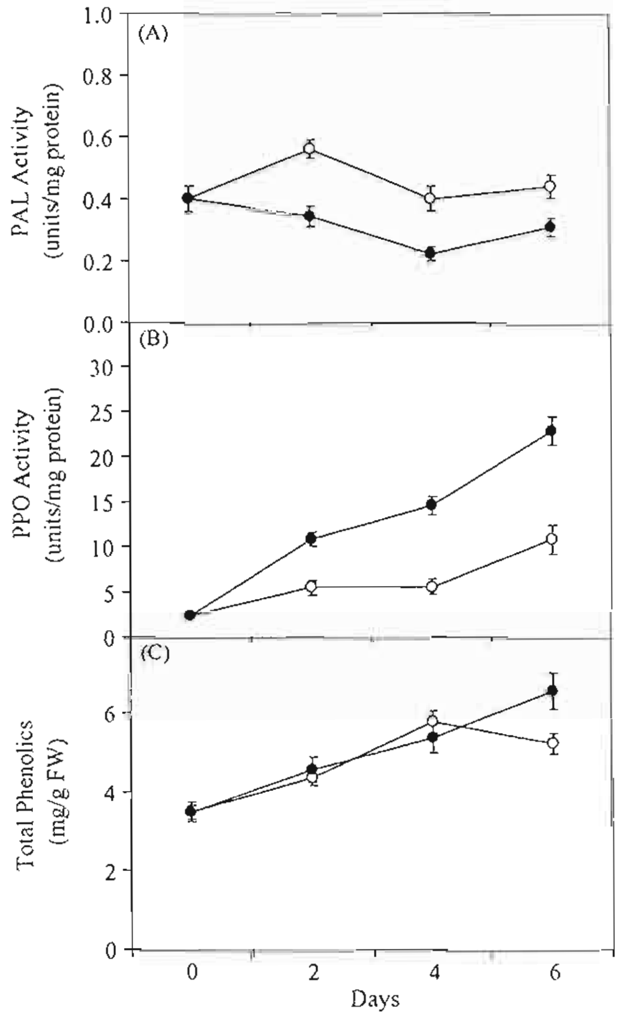


Fig. 3. Activities of phenylalanine ammonia lyase (A), and polyphenol oxidase (B), and levels of total phenolic compounds (C) in banana peel without PVC film (○), with 'Sun wrap' film (●). Data are means of five replications ± S.E.

3.4. *Activities of PAL and PPO, and levels of total free phenolics*

The PAL activity in the peel of bananas in 'Sun wrap' PVC was lower than that in the peel of the control (Fig. 3A; all data points except day 0 were statistically different from the control; $P > 0.05$). The peel PPO activity of control bananas was variable, and gradually increased (Fig. 3B) or, in a repeat experiment, stayed about constant (results not shown). The PPO activity in the peel of bananas wrapped with film increased more rapidly than that in control fruit (Fig. 3B; all data points except day 0 were statistically different from the control; $P > 0.05$). The level of total free phenolic compounds in the peel of control fruit increased, just the same as in peel of bananas wrapped in film (Fig. 3C). Upon removal of the film, PAL activities of banana peel increased again to values that were similar to the ones found in unpacked controls. In contrast, removal of the film resulted in a decrease of PPO activity (results not shown).

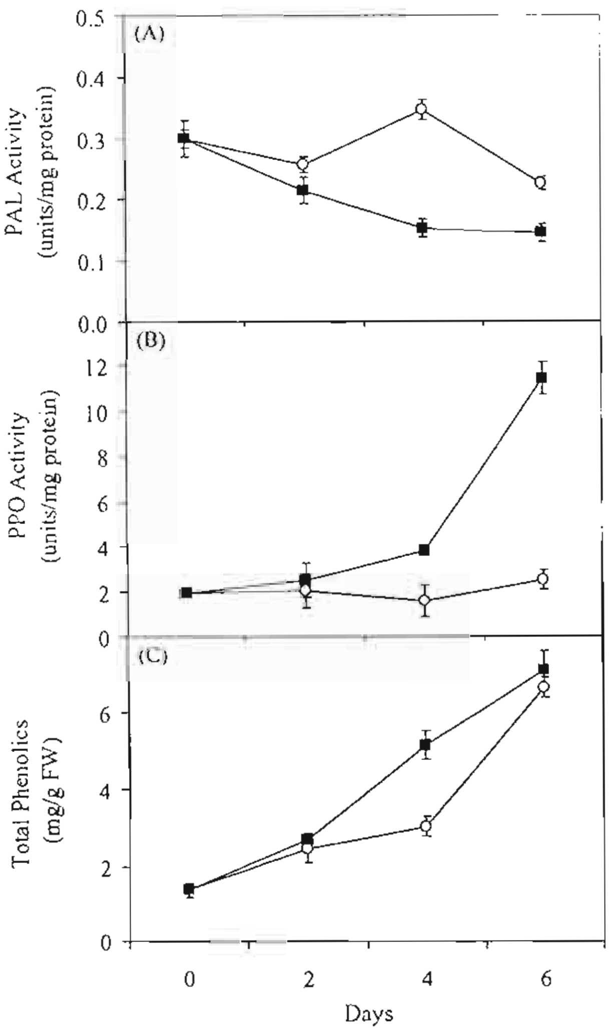


Fig. 4. Activities of phenylalanine ammonia lyase (A) and polyphenol oxidase (B), and levels of total phenolics (C) in banana peel held in air (○) and 5% O_2 (■). Data are means of five replications ± S.E.

PAL activity of banana peel held under 5% oxygen decreased throughout the study period and remained lower than that in controls held in normal air (Fig. 4A; all data points except day 0 were statistically different from the control; $P > 0.05$). PPO activity in peel of bananas held at 21% oxygen did not change whilst that at 5% oxygen increased (Fig. 4B; data points of days 4 and 6 were statistically different from the control; $P > 0.05$). Oxygen at 15% had a small effect on PAL and no effect on PPO activity, and 10% oxygen had an effect on PAL similar to that of 15%, but it stimulated PPO activity relative to controls (results not shown). Total free phenolics in peel of bananas held in normal air and low oxygen levels increased, and differences between the treatments were (with the exception of day 4) not significantly different (Fig. 4C).

4. Discussion

We observed that 'Sucrier' banana that were stored in trays wrapped with 'Sun wrap' PVC film had much less senescent spotting. Since this effect was found at various concentrations of ethylene and carbon dioxide, it was apparently mainly due to the decrease in oxygen levels. Results from fruit held in controlled atmospheres confirmed the requirement of more than about 5% oxygen for the development of senescent spotting.

Our results are reminiscent of 'Bungulan' bananas, where senescent spotting covered about 50% of the peel after 4 days of storage at 21% oxygen, whereas after 4 days of storage at 5% oxygen spotting covered about 30% (Valdez and Mendoza, 1988). Senescent spotting in 'Sucrier' bananas thus seemed even more sensitive to decreased oxygen levels, since it was completely suppressed when oxygen reached about 5% on day 2 (Fig. 2).

The oxygen requirement of browning may lie with several enzyme reactions. Both PAL and PPO reactions require oxygen, and some of these may have a relatively high Km for oxygen (Penalver et al., 2002). PAL is a regulatory enzyme at the onset of reactions that produce free phenolics. Shirsat and Nair (1981) showed that 5% oxygen in excised potato parenchyma tissue inhibited PAL activity, due to an inhibition of de novo PAL synthesis. The low oxygen concentration apparently first resulted in inhibition of cinnamic acid-4-hydrolase, (a mixed-function oxidase) which resulted in accumulation of cinnamic acid. The latter accumulation seemed the signal for low PAL synthesis. The present inhibition of PAL activity, under low oxygen levels, may be due to a similar mechanism. Whether this would be adequate for the inhibition of browning is, however, unclear. Besides cinnamic acid, other phenolics in the phenylpropanoid pathway can also inhibit PAL activity (Blount et al., 2000; Camm and Towers, 1973; Shirsat and Nair, 1981). Although we found no effect of low oxygen on total free phenolics, it is unclear if cinnamic acid or another phenolic did accumulate in the present tests.

PPO (catechol oxidase) is required in least at two late reactions that lead to browning: the conversion of dopachrome to 5,6-dihydroxyindole, and the conversion of the latter to indole-5,6-quinone. Both are diphenoloxidase reactions of PPO. The enzyme is also involved in several reactions leading to dopachrome, most of which are diphenoloxidase reactions. In mushrooms, the Km of PPO for oxygen was low (about 2 μM) for the monophenoloxidase reactions, but high (40–100 μM) for diphenoloxidase reactions (Espin et al., 1999). Browning of cut potatoes and apples is inhibited by placement of the parts in water, in which the diffusion rate of oxygen is slower than in air, but where the oxygen concentration is considerably higher than 0. The effect of low oxygen on browning in such systems is often attributed mainly to PPO (Hyodo and Uritani, 1966; Martinez and Whitaker, 1995). Finally, oxygen is required at the (nonenzymatic) polymerization of indole-5,6-quinone to melanin. The oxygen concentration required for this reaction has apparently not been reported.

The measured in vitro PPO activity was not positively related with senescent spotting in 'Sucrier' banana, but rather was inversely proportional to spotting (Figs. 3 and 4). Removal of the PVC film from banana trays after 3 days in the packages, and transferring them to normal air, resulted in an increase in senescent spotting. The increase was highest on the first day following film removal. This was related to a decrease of in vitro PPO activity, again showing an inverse relation between browning and in vitro PPO activity.

The lack of a positive relation between spotting and in vitro PPO activity can be interpreted along two lines. First, assuming that PPO is involved in the browning reaction, its activity may not be rate limiting. PAL, peroxidase, or other enzymes may limit the reaction rate. In lychee pericarp, for example, browning was related to the activity of both PPO and peroxidase (Underhill and Critchley, 1995). However, this interpretation does not account for the *increase* of in vitro PPO activity when spotting was reduced by low oxygen tension. The second interpretation might account for this effect. On this interpretation, the measured in vitro PPO activity does not reflect in vivo activity. PPO may well be inhibited, in vivo, by low oxygen concentrations, but in vitro measurements, where ample oxygen is present, will not show this. If this is true, the increase of in vitro activity, being a measure of potentially active protein, indicates a feedback mechanism whereby more active protein is produced in the face of PPO inhibition.

When 5% oxygen was combined with 3% carbon dioxide, peel spotting in 'Bungulan' bananas was further reduced (Valdez and Mendoza, 1988). Previous research with 'Sucrier' fruit indicated that a combination of about 19% oxygen and 2.5% carbon dioxide inhibited spotting on day 3, if fruit were held at ambient temperatures (Ketsa, 1996). This indicated that elevated carbon dioxide concentrations were effective at oxygen concentrations close to 21%. In the present tests, where spotting was completely prevented by oxygen concentrations of about 5%, the concomitant increase in carbon dioxide concentrations apparently had little effect. This conclusion follows from the absence of an effect of carbon dioxide scrubbing (Fig. 1).

Additionally, continuous high concentrations of ethylene (10%) may reduce banana peel spotting (Liu, 1976). In our tests, ethylene levels increased in the packages to 0.8 Pa (about 8 $\mu l\,l^{-1}$). This apparently had little effect in addition to the low oxygen level, as shown by the absence of an effect of reducing the ethylene concentration (to about 0.4 Pa) in the package after inclusion of an absorbent (Fig. 1).

Our experiments showed that the high RH around the PVC-wrapped product was not the cause of the reduction of senescent spotting. The spotting also occurred when the RH in the packages was held at ambient levels, and was not reduced when the RH was increased under conditions where the gas composition was not changed. Spotting thus occurs irrespective of RH, at the range of RH investigated (75–100%).

The presently investigated senescent spotting eventually covers most of the peel surface, thereby results in rather large pits, and browning of most of the peel.

In several other cultivars, senescent spotting, if it occurs, and the subsequent browning of the whole surface, happens at a much later stage with respect to pulp ripening. When the fruit is overripe, the peel of still other cultivars turns dark brown, at room temperature, without senescent spotting. This browning is not accompanied by the presence of pits on the peel surface. The activity of PAL has apparently not been reported in bananas that show late browning without senescent spotting. The in vitro activity of PPO—when expressed per unit FW or per fruit—has been reported to stay about constant (Gooding et al., 2001), and when expressed per unit protein (taken from our own data) there seems also little change (unpublished results). This is in contrast with the increase in PPO activity during senescent spotting.

Another type of browning occurs in fruit held below 12–14 °C, as a result of chilling injury. At such low temperatures, the peel becomes grey–brown rather than dark brown. We found that chilling-induced browning in banana peel is accompanied by in a concerted increase of the in vitro activities of both PPO and PAL (Nguyen et al., 2003). Senescent spotting, in contrast, was not accompanied by an increase of in vitro PAL activity.

The three types of banana peel discoloration (senescent spotting, normal browning at room temperature, and greyish browning at chilling temperatures) therefore, seem to be accompanied by a different combination of in vitro PAL and PPO activity.

We conclude that senescent peel spotting in 'Scurier' bananas requires relatively high oxygen concentrations, as it is virtually inhibited by 5% oxygen. It is unclear where low oxygen tension exerts its effect. Oxygen is required at least for the final step in the browning reaction, i.e. the nonenzymatic polymerization of indole-5,6-quinone to melanin, and for PPO (catechol oxidase) activity. We hypothesize that, among other enzymes, in vivo PPO activity may be inhibited and that the increase of in vitro PPO activity under low oxygen indicates an increase in potentially active enzyme.

Acknowledgements

The research was financially supported by the Kasetsart University Research and Development Institute (KURDI) and the Thailand Research Fund (TRF).

References

Blount, J.W., Korth, K.L., Masoud, S.A., Rasmussen, S., Lamb, C., Dixon, R.A., 2000. Altering expression of cinnamic acid 4-hydroxylase in transgenic plants provides evidence for a feedback loop at the entry point into the phenylpropanoid pathway. Plant Physiol. 122, 107–116.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle-dye binding. Anal. Biochem. 72, 248–254.

Camm, E.C., Towers, G.H.N., 1973. Phenylalanine ammonia lyase. Phytochemistry 12, 961–973.

Espin, J.C., Jolivet, S., Wichers, H.J., 1999. Kinetic study of the oxidation of gamma-L-glutaminyl-4-hydroxybenzene catalysed by mushroom (*Agaricus bisporus*) tyrosinase. J. Agric. Food Chem. 47, 3495–3502.

Gooding, P.S., Bird, C., Robinson, S.P., 2001. Molecular cloning and characterisation of banana fruit polyphenol oxidase. Planta 213, 748–757.

Hanson, K.R., Havir, E.A., 1979. An introduction to the enzymology of phenylpropanoid biosynthesis. In: Harborne, J.B., Van Sumere C.F. (Eds.), Biochemistry of Plant Phenolics. Plenum Press, NY, pp. 91–138.

Hyodo, H., Uritani, I., 1966. A study on increase in *o*-diphenol oxidase activity during incubation of sliced sweet potato tissue. Plant Cell Physiol. 7, 137–144.

Ketsa, S., 1996. Control of senescent spots in 'Kluai Khai' by modified atmospheres. In: Frisinia, C., Mason, K., Faragher, J. (Eds.), Proceedings of the Australasian Postharvest Horticulture Conference. Institute of Horticultural Development, Victoria, pp. 185–189.

Ketsa, S., 2000. Development and control of senescent spotting in banana. Food Preserv. Sci. 26, 173–178.

Liu, F.W., 1976. Ethylene inhibition of senescent spots on ripe bananas. J. Am. Soc. Hort. Sci. 101, 684–686.

Lizada, M.C.C., Pantastico, E.B., Shukor, A.R.A., Sabari, S.D., 1990. Ripening of banana: changes during ripening in banana. In: Hassan, A., Pantastico, E.B. (Eds.), Banana: Fruit Development, Postharvest Physiology, Handling and Marketing in ASEAN. ASEAN Food Handling Bureau, Kuala Lumpur, pp. 65–72.

Luh, B.S., Phithakpol, B., 1972. Characteristics of polyphenol oxidase related to browning in cling peaches. J. Food Sci. 37, 264–268.

Macheix, J.J., Fleuriet, A., Billot, J., 1990. Fruit Phenolics. CRC Press, Boca Raton, FL, 378 pp.

Marriott, J., 1980. Banana physiology and biochemistry of storage and ripening for optimum quality. CRC Crit. Rev. Food Sci. Nutr. 13, 41–88.

Martinez, M.V., Whitaker, J.R., 1995. The biochemistry and control of enzymatic browning. Trends in Food Sci. Technol. 6, 195–200.

Mayer, A.M., Harel, E., 1979. Polyphenol oxidases in plants. Phytochemistry 18, 193–215.

New, S., Marriott, J., 1974. Post-harvest physiology of tetraploid banana fruit: response to storage and ripening. Ann. Appl. Biol. 78, 193–204.

Nguyen, T.D.B., Ketsa, S., van Doorn, W.G., 2003. Relationship between browning and the activities of polyphenoloxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. Postharvest Biol. Technol. 30, 187–193.

Penalver, M.J., Hiner, A.N.P., Rodriguez-Lopez, J.N., Garcia-Canovas, F., Tudela, J., 2002. Mechanistic implications of variable stoichiometries of oxygen consumption during tyrosinase catalyzed oxidation of monophenols and *o*-diphenols. Biochem. Biophys. Acta 1597, 140–148.

Rhodes, M.J.C., Wooltorton, L.S.C., Hill, A.C., 1981. Changes in phenolic metabolism in fruit and vegetable tissues under stress. In: Friend, J., Rhodes, M.J.C. (Eds.), Recent Advances in the Biochemistry of Fruits and Vegetables. Academic Press, London, pp. 193–220.

Shirsat, S.G., Nair, P.M., 1981. Biochemical mechanism for the inhibition of phenylalanine ammonia-lyase induction in the absence of oxygen in potato tuber tissue. Phytochemistry 20, 2315–2318.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomonolybdic-phosphotungstic acid reagents. Am. J. Enol. Vit. 16, 144–157.

Underhill, S.J.R., Critchley, C., 1995. Cellular localisation of polyphenol oxidase and peroxidase activity in *Litchi chinensis*. Sonn. pericarp. Aust. J. Plant Physiol. 22, 627–632.

Valdez, E.R.T., Mendoza, D.B., 1988. Influence of temperature and gas composition on the development of senescent spotting in banana (*Musa sapientum* L. cv. Bungulan). Phil. Agric. 71, 5–12.

Valmayor, R.V., Jamaluddin, S.H., Silayoi, B., Kusumo, S., Danh, L.D., Pascua, O.C., Espino, R.R.C., 2000. Banana cultivar names and synonyms in Southeast Asia. INIBAP (International Network for the Improvement of Banana and Plantain), Los Banos, Phillippines, 24 pp.

เอกสารแนบหมายเลข 5

Effect of gas composition and relative humidity on senescent spotting in banana fruit

Saichol Ketsa and Narisa Uthaichay
Department of Horticulture
Faculty of Agriculture
Kasetsart University
Bangkok 10900, Thailand

Wouter G.van Doorn
Agrotechnology and Food Innovations
Wageningen University and Research Centre
P.O. Box 17, 6700AA Wageningen
The Netherlands

Keywords: banana, carbon dioxide, ethylene, oxygen, relative humidity, senescent spotting

Abstract

We studied the effect of relative humidity (RH) and gas composition on senescent spotting in banana cv. Sucrier (*Musa acuminata* AA Group). Fruit that had ripened to colour index 3-4 (about as much yellow as green) were placed in polystyrene trays and wrapped with polyvinyl chloride (PVC) film, with and without inclusion of silica gel, and then held at 29-30°C. Senescent spotting of banana fruit in the packages (both with and without silica gel) was absent, whereas much spotting occurred in unwrapped fruit. Oxygen concentrations in the packages were much lower than in outside air, whereas concentrations of carbon dioxide and ethylene were higher than in air. In another experiment the fruit was placed in closed plastic containers connected to an air-flow system, with and without air drying over silica gel. Senescent spotting was much lower at lower RH (about 60% compared to 95-100%). The oxygen concentrations in both systems were similar, but a higher carbon dioxide and ethylene concentration was found in at the low RH. It is concluded that differences in RH in the range of 70-95% are not important if oxygen concnetrations are low enough to prevent spotting, whereas a RH lower than 60% was correlated with reduced spotting when oxygen levels remain at 21%. The reduced spotting at 60% RH also coincided with a somewhat lower carbon dioxide and ethylene concentration, so that it is not completely clear if the effect must be attributed to RH alone.

INTRODUCTION

Senescent spots on the banana peel normally develop during the latter phase of ripening. In most cultivars, the spots appear when virtually the whole peel is yellow (only the tip may still be green). At this stage, senescent spots are very small, like pins, light brown in colour. The spots gradually increase in size and number, and become dark brown as the fruits ripen. Little is known about the origin of this disorder and about its physiological mechanism (New and Marriot, 1974 ; Choehom et al., 2004).

A few cultivars, for example Sucrier, show spotting already when the peel is as much yellow as green, thus earlier than most other cultivars. Film wrapping reduced the disorder (Valdez and Mendoza, 1988; Ketsa, 1996; Choehom et al., 2004). Film wrapping affects gas concetrations as well as relative humidity (RH). We now studied the effects of relative humidity in packed banana fruit, by including silica gel.

MATERIALS AND METHODS

Plant material

'Sucrier' banana (*Musa acuminata* AA Group) fruits; locally known as 'Kluai Khai', were harvested at commercial maturity from a plantation and transported to the laboratory within 2 h of harvest. After selection for uniformity of size and colour, hands were cleaned in 200 $\mu g\ L^{-1}$ chlorine (Clorox) and allowed to dry before dipping for 1-2 min in 500 $\mu L\ L^{-1}$ ethephon for uniform ripening. After this dip, fruit were dried at room temperature (29-30°C) and then held in a ripening at room 25°C (85-90% RH) until reaching a colour index 3-4. At this stage, the fruit showed no spotting.

Silica gel treatment

Experiment I. Individual banana hands were randomly placed on a polystyrene tray (15 cm × 25 cm × 3 cm) and wrapped with PVC film over the fruit and the tray. The trays were held at 25°C. Low RH in the 'Aro' PVC film (Quickpack Pacific, Thailand) packages was maintained by inclusion of silica gel, which was changed every 24 h. Data loggers were placed inside the packages to monitor both RH and temperature during the 7 days of study. The control banana fruits remained unwrapped.

Experiment II. Two banana hands were held in a closed plastic container at 25°C. The containers were connected with a flow of air with 100% RH at 125 mL/min. A lower RH in the containers was maintained by inclusion of silica gel, which was changed daily. Data loggers monitor RH and temperature over the 7-day study period.

Assessment of senescent spotting

Each batch in the experiment consisted of two hands of banana fruit, which were the unit of replication. From each hand, 10-15 fruits were taken at random for visual inspection of senescent spotting using a scale of 1-5 where 1 means no spotting and 5 severe spotting.

Determination of ethylene, carbon dioxide and oxygen

Concentrations of ethylene, carbon dioxide and oxygen in the PVC packages and plastic containers were measured by sampling through the PVC film and a sampling port, respectively. The samples were injected into a gas chromatograph equipped with a flame ionization detector for ethylene and a thermal conductivity detector for carbon dioxide and oxygen.

Statistical analysis

In the tests on visible scores, three replications (each consisting of two banana hands) were used in each treatment. Individual fruits were evaluated daily for senescent spotting. Means were compared using Duncan's multiple range test (DMRT).

RESULTS

Experiment I.

In unwrapped banana senescent spotting rapidly increased from day 1, and reached a maximum on day 5 (Fig. 1A). In contrast, fruit in the PVC packages did not show senescent spotting (Fig. 1A).

RH in the packages without silica gel was initially about 90%, reached about 98% on day 1, and remained at that level until day 7. RH outside the packages and inside the PVC packages with silica gel was initially about 63-64% and gradually increased to about 76-78% at the end of experimentation (Fig. 1B). The temperatures were not different (27-28°C initially, slightly increasing to 29-31°C at the end of experimentation (Fig. 1C).

The oxygen concentrations inside the PVC packages with and without silica gel was about 4-5%, while the oxygen concentration outside the packages was 20-21% (Fig. 2A). The carbon dioxide concentrations in the PVC packages were initially about 7-8%. They slightly decreased to 6.0-6.3% at the end of experiment. Carbon dioxide concentrations outside the PVC film packages were ambient (close to zero on the present scale) (Fig. 2B). Ethylene concentrations in the PVC packages gradually increased from 3-4 to about 6 $\mu L\ L^{-1}$ at the end of experimentation. The ethylene concentrations outside the PVC film packages remained close to zero (Fig. 2C).

Experiment II.

Banana fruit in the plastic containers with silica gel did not show senescent spotting during the first 4 days of the experiments, and slight increased spotting by day 5. In contrast, fruit in containers without silica gel showed a rapid increase in spotting from day 2, to a maximum on day 5 (Fig. 3A).

RH in the containers without silica gel reached 100% within 24 h after the start of the experiment and then remained stable. RH in the containers with silica gel sharply decreased from to 43% within 48 h and slightly increased thereafter (Fig. 3B). the temperatures in the plastic containers were initially about 27°C, reached 30-31°C on day 2, and remained unchanged thereafter (Fig. 3C).

Oxygen concentrations in plastic containers slightly increased and were not much different between the treatments (Fig. 4A). The carbon dioxide (Fig. 4B) and ethylene (Fig. 4C) concentrations in the containers without silica gel were considerably higher than in the containers with silica gel.

DISCUSSION

The senescent spotting was considerably reduced by PVC wrapping. As previously reported (Choehom et al., 2004), this suggests that senescent spotting requires rather elevated oxygen levels. These experiments indicated that oxygen levels needed to be higher than 5%. Carbon dioxide and ethylene levels in the packages increased, but these apparently had no effect, in the presence of low oxygen levels. We previously also demonstrated that both carbon dioxide and ethylene released by banana fruit in PVC packages had little effect on senescent spotting (Choehom et al., 2004).

Low RH in the packages did not affect senescent spotting. In the second experiment the RH varied in the presence of rather high oxygen levels and now a difference in spotting was observed. It was not possible, however, to ascribe this effect to RH alone, as the concentrations of carbon dioxide and ethylene were higher in the treatment with the low RH. From our previous experiments (Choehom et al., 2004) and from the first of the present experiments it may seem that such differences in carbon dioxide and ethylene have no effect on spotting, but this conclusion is unjustified. The tests reported by Choehom et al. (2004) and the present first experiment show high carbon dioxide and ethylene levels in the presence of low oxygen levels. Such concentrations and even lower ones, may have a different effect in the presence of 21% oxygen. The effect of RH and that of these two gases must therefore be further separated.

ACKNOWLEDGEMENTS

The research was financially supported by the Postgraduate Education and Research Development Project in Postharvest Technology and the Thailand Research Fund.

Literature Cited

Choehom,R., Ketsa,S. and van Doorn, W.G. 2004. Senescent spotting of banana peel is inhibited by modified atmosphere packaging. Postharvest Biol. Technol. 31 : 167-175.

Ketsa, S. 1996. Control of senescent spots in 'Kluai Khai' by modified atmosphere, pp. 185-189. In : C. Frisinia, K. Mason and J. Faragher (eds.). Proceedings of the Australasian Postharvest Horticulture conference, Institute of Horticultural Development, Ferntree Gulley, Victoria.

New, S. and Marriot, J. 1974. Post-harvest physiology of tetraploid banana fruit: response to storage and ripening. Ann. Appl. Biol. 78 : 193-204.

Valdez, E.R.T. and Mendoza, D.B. 1988. Influence of temperature and gas composition on the development of senescent spotting in banana (*Musa sapientum* L. cv. Bungulan). Phil. Agric. 71 : 5-12.

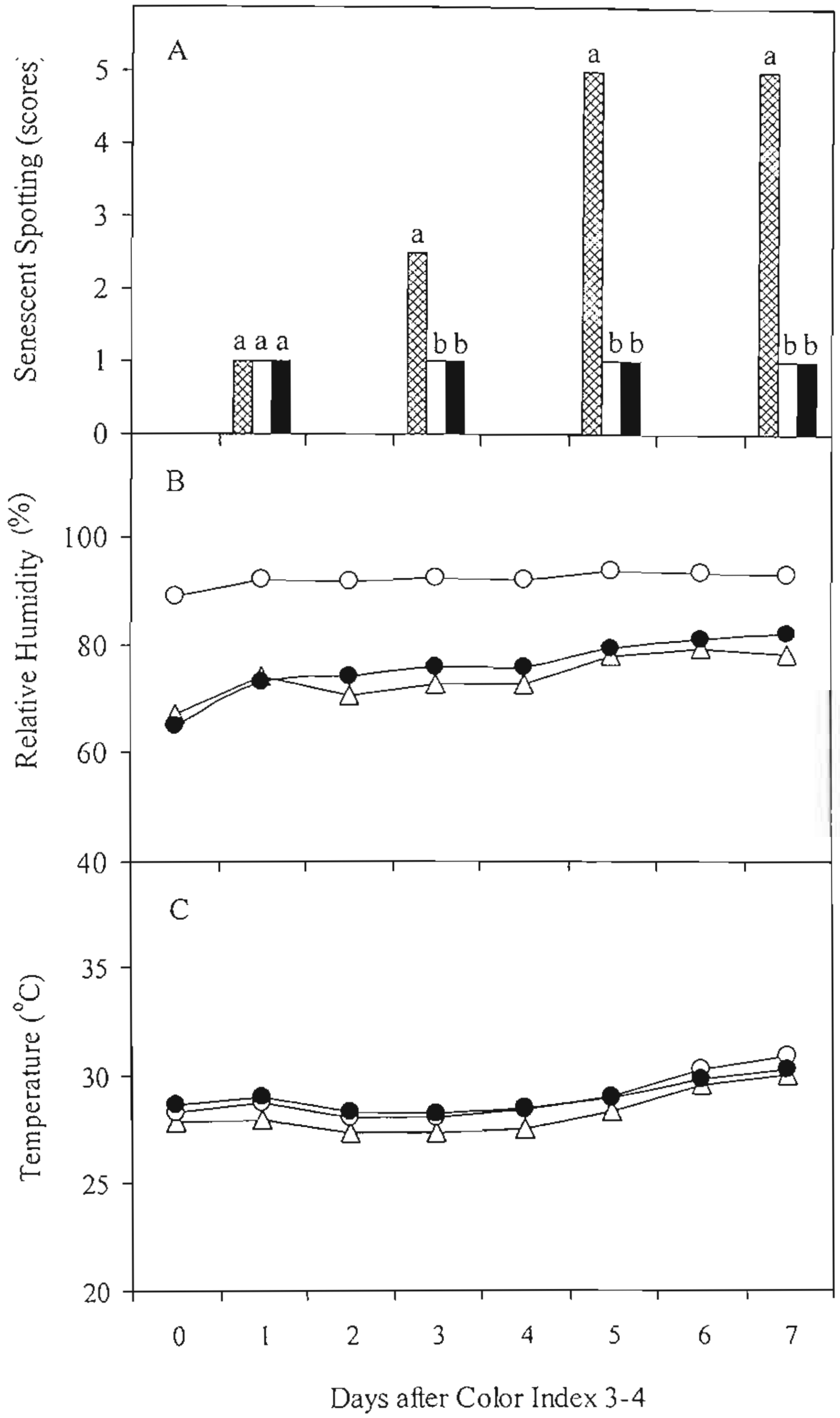


Fig.1. Senescent spotting of banana fruits (A) outside (⊠) and inside the PVC film packages with (■) and without (□) silica gel and relative humidity (B) and temperature (C) outside (△) and inside the PVC film packages with (●) and without (○) silica gel.

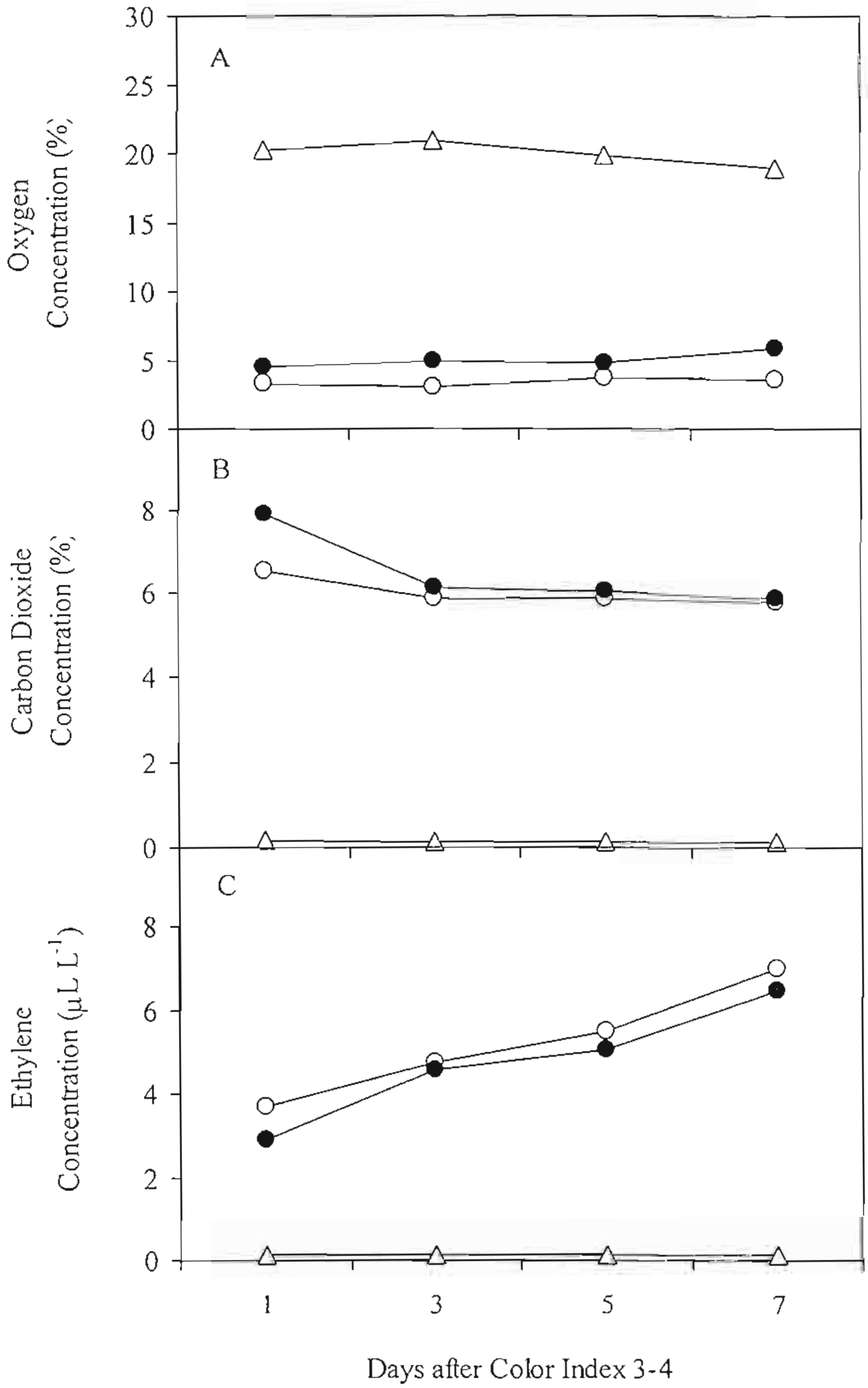


Fig.2. Oxygen (A), carbon dioxide (B) and ethylene (C) concentrations outside (△) and inside the PVC film packages with (●) and without (○) silica gel.

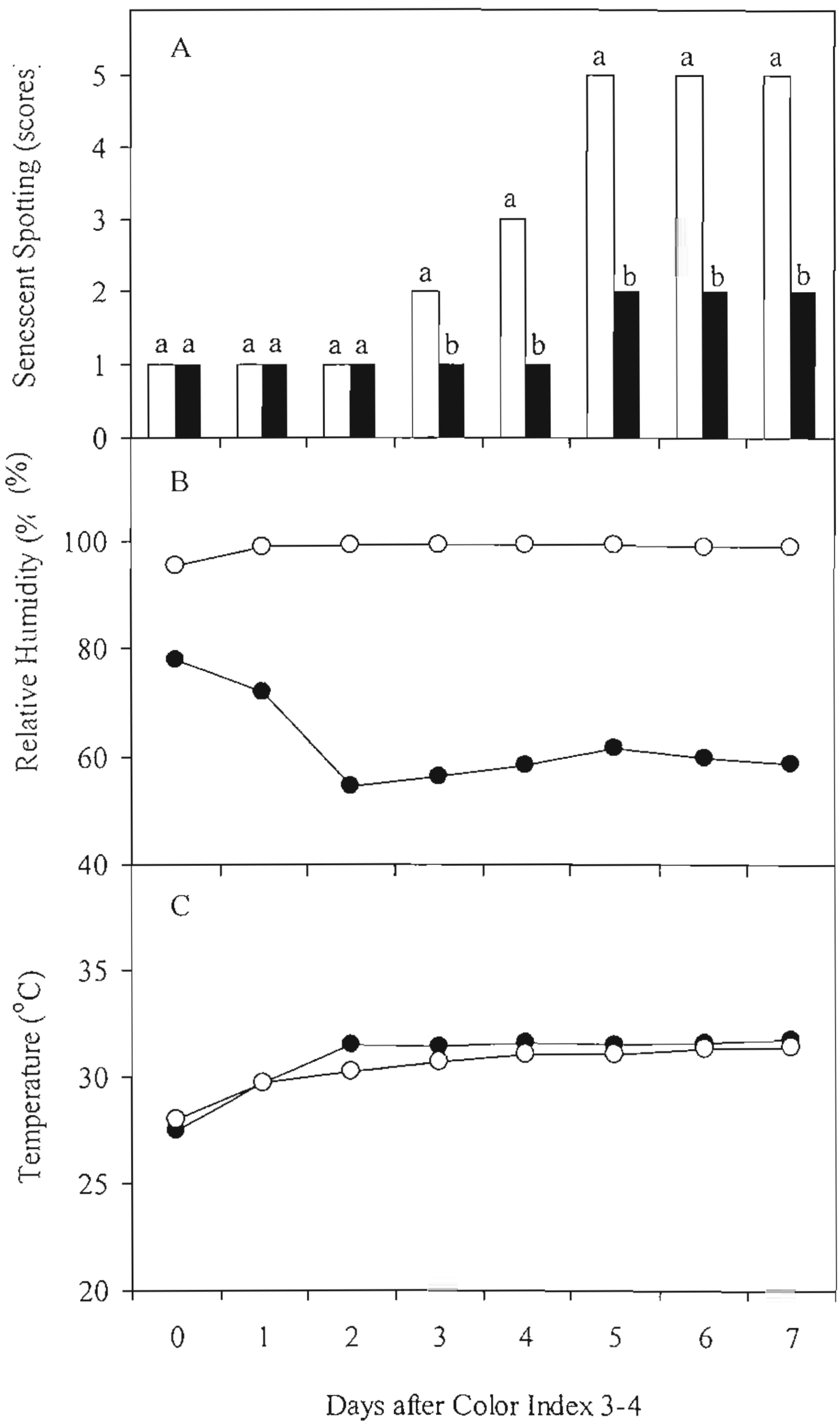


Fig.3. Senescent spotting of banana fruits (A) in the closed plastic containers with (■) and without (□) silica gel, relative humidity (B) and temperature (C) in the closed plastic containers with (●) and without (○) silica gel.

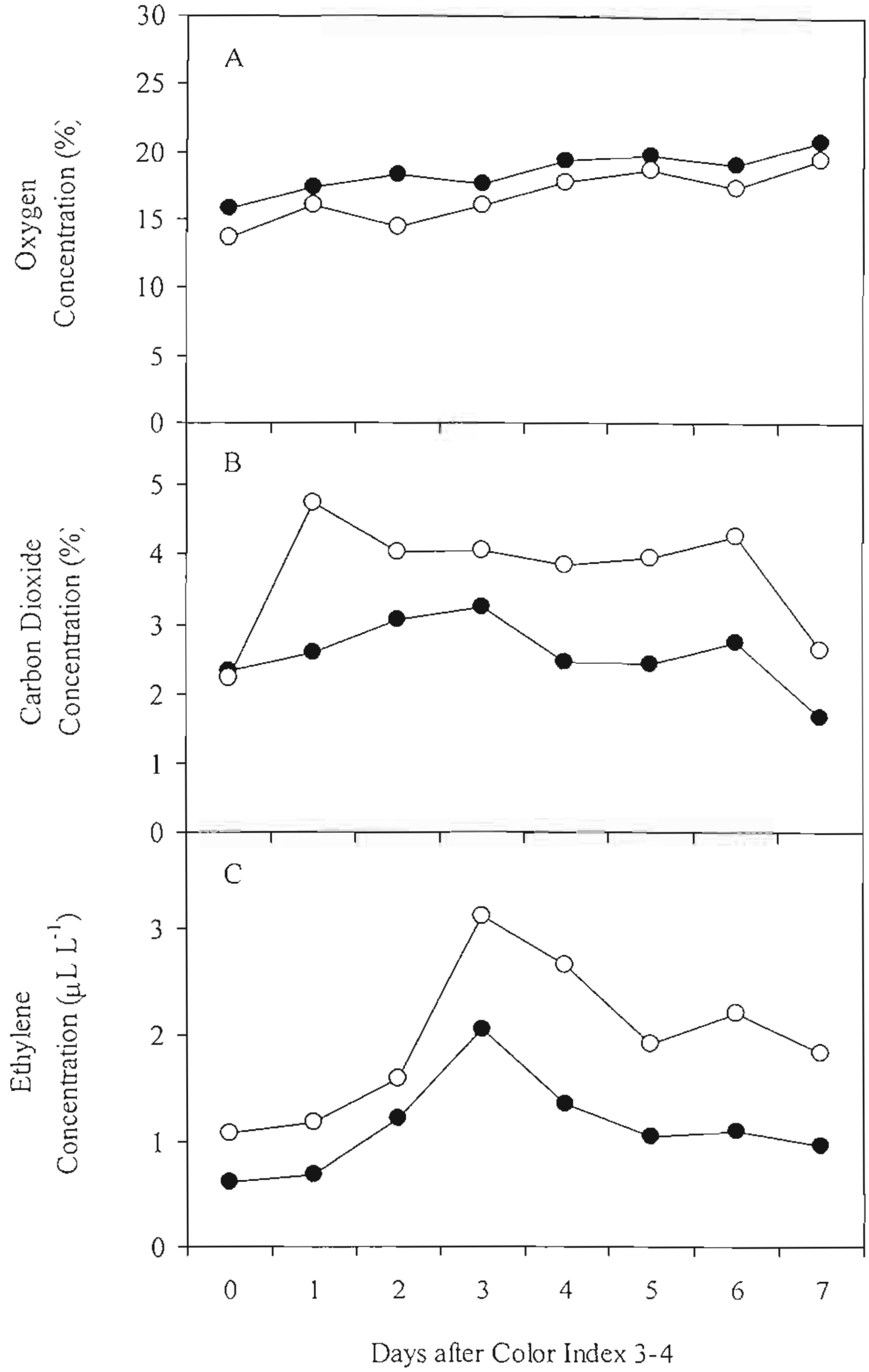


Fig.4. Oxygen (A), carbon dioxide (B) and ethylene (C) concentrations in the closed plastic containers with (●) and without (O) silica gel.

เอกสารแนบหมายเลข 6

Temperature effects on senescent spotting in 'Sucrier' banana peel

Chitra Trakulnaleumsai[1], Saichol Ketsa[2,*]

and Wouter G. van Doorn[3]

[1,2]Department of Horticulture, Faculty of Agriculture

Kasetsart University, Bangkok 10900, Thailand

[3]Agrotechnology and Food Innovations

Wageningen University and Research Centre

P.O. Pox 17, 6700 AA Wageningen, The Netherlands

Abstract

Banana fruit of the cultivar Sucrier (*Musa acuminata* AA Group) develop senescent peel spotting when the peel is about as much yellow as green. This early spotting was accompanied by an increase in total free phenolic compounds and by increased polyphenol oxidase (PPO) activity in the peel. Peel phenylalanine ammonia lyase (PAL) activity remained about constant. Holding ripening bananas at 15 and 18°C instead of room temperature (26-27 °C) only temporarily reduced spotting, but holding the fruit at 12°C completely prevented it. The 12°C treatment resulted in a lower level of total free phenolics, but had no effect on PAL or PPO activity. Transfer of bananas previously held at 12°C to room temperature rapidly increased senescent spotting, while transfer of bananas with some senescent spotting from room temperature to 12°C did not prevent further development of the spotting. It is concluded that holding spotless fruit at 12°C prevents the spotting, although only if they are kept at that temperature, and that PAL and PPO activities seem not rate-limiting.

Keywords : Banana; Low temperature, Phenylalanine ammonia lyase, Polyphenol oxidase, Ripening, Senescent spotting, Total free phenolics

* Corresponding author. E-mail : agrsck@ku.ac.th, Fax : 66-2-579-1951 (S. Ketsa)

1. Introduction

Development of early senescent spotting is typical for Sucrier (local name 'Kluai Khai') bananas. It starts when the fruit is as much yellow as green. The spotting occurs only superficially and does not affect eating quality (Marriot, 1980). The disorder starts with small light brown spots, which become larger and darker brown with advanced ripening. Benomyl, carbendazim and prochloraz are effective fungicides in controlling postharvest diseases of banana fruits (Sapiah et al., 1990). However, preharvest and postharvest application of these three fungicides did not control the senescent spotting in the banana fruits. Similarly, banana bunches covered with polyethylene bags with open-ends at the bottom during their growth and development in the field until harvest did not reduce the development of senescent spotting in bananas (Ketsa, 1995). This indicates that senescent spotting in bananas is a physiological disorder, not a pathogenic disease (Wardlaw, 1972; Marriot, 1980).

Consumers that are familiar with 'Sucrier' bananas see the beginning of senescent spotting as a hallmark of good eating quality. At the stage where spotting starts, the fruit is sweet and has a good taste and aroma. Consumers in East-Asia therefore sometime indicate senescent spotting as 'sweet spot'. In contrast, people that are not familiar with the early spotting think that the fruit is overripe or infected (Marriott, 1980; Lizada et al., 1990; Ketsa, 2000).

Choehom et al. (2004) reported that senescent spotting of 'Sucrier' banana requires relatively high oxygen levels. If placed at 5% oxygen, spotting was absent and this was accompanied by reduced phenylalanine ammonia lyase (PAL) activity and increased polyphenol oxidase (PPO) activity in the peel and by an increase in levels of total free phenolics. The objective of this study was to examine the effect of various temperatures on senescent spotting, and to further explore the relationship between spotting and free phenolics levels and the activity of PAL and PPO.

2. Materials and methods

2.1. Plant material

'Sucrier' bananas (*Musa acuminata* AA Group) were harvested at commercial maturity (80% mature, based on their shape and color development) from a plantation in Petchaburi province in western Thailand. Bunches were dehanded, placed in in corrugated cardboard boxes, and transported in a controlled-temperature truck (25°C) to the laboratory, within 2 h of harvest. Hands were then selected for uniformity of size and color, cleaned in a solution of 0.5% $MgSO_4$ to remove latex from the cut surface. Fruits were dipped for 2-3 min in 500 mg l^{-1} thiabendazole solution to control fruit rot and allowed to air dry before dipping for 1-2 min in 500 mg l^{-1} ethephon solution for uniform ripening. After dipping they were dried at ambient temperature (29-30°C). Bananas were ripened until reaching colour index 3-4 (Lizada et al., 1990), and then used for experiments. At colour index 3 the peel is more green than yellow and at colour index 4 it is more yellow than green. At colour index 3-4 the fruit does not yet show senescent spotting.

2.2. Temperature treatments

Fruits were divided into four groups and each group was placed in plastic baskets and kept continuously at room temperature (26-27°C, 65-70% RH), 18° and 12°C (92-95% RH) until the end of the experiment. In other experiments bananas at colour index 3-4 were held at room temperature for 2 days and then transferred to 12°C where they were held until the end of the experiment. Other fruits were held at 12°C for 4 days and then transferred to room temperature until the end of the experiment. At regular intervals we monitored senescent spotting, quality, total free phenolics content and enzyme activities.

2.3. Assessment of senescent spotting

Hands of bananas were randomly sampled for determination of senescent spotting on the banana peel, using a scale of 1-4 following the procedure described in Choehom et al. (2004). A score of 1 means no senescent spotting and 4 is severe senescent spotting.

Ten to fifteen fingers of individual hands were randomly sampled for determination of peel color, firmness and the content of starch and sugars. Peel color was determined using a color meter (Dr. Lang Tricolor LIM 3), using 'L' value (Hunter scale).

2.4 Peel color, firmness starch and sugar contents

Ten to fifteen fingers of individual hands were randomly sampled for determination of peel color, firmness and content of starch and sugars. Peel color was determined using a color meter (Dr. Lang Tricolor LIM 3) to record 'L' value (Hunter scale). Banana firmness with and without peel was determined with an Effegi firmness tester using a spherical plunger 1.1 cm in diameter. The plunger was inserted to a depth of 5 mm and the necessary force was recorded in newtons (N). Starch and sugar content of the pulp was measured using methods described by Chaitrakulsap (1980) and Hodge and Hofreither (1962), respectively.

2.5. Extraction and assay of total free phenolics and enzyme activity

Extraction procedures and assays of PPO and PAL were carried out as described by Luh and Phitakpol (1972) and Camm and Towers (1973), respectively. Protein content was determined according to Bradford (1976). Total free phenolics were determined using the method described by Singleton and Rossi (1965). Each treatment had five replications, and each replication consisted of the whole peel from 10-15 fruit, pooled together. Determinations of enzyme activity were made twice in each extract.

2.6. Statistical analysis

Three replications each consisting of a hand were used in each treatment. Ten to fifteen fingers from two rows of individual hands were evaluated for senescent spotting, quality, total free phenolic content, and enzyme activity. Scores and other parameters were compared by analysis of variance, using Least Significance Difference (LSD).

3. Results

3.1. Senescent spotting

At the start of experiment, when fruit had ripened to stage colour index 3-4, the peel did not yet show senescent spotting. In the controls held at room temperature (26-27°C) senescent spotting rapidly increased from day 2 to 6 (Fig. 1A). Holding the fruit at 18°C prevented spotting for 2 days, after which it showed the same rate as in fruit that was continuously held at 26-27°C. Little spotting occurred at 15°C and spotting was completely prevented if the fruit was held at 12°C (Fig. 1A). Transfer of bananas to 12°C following two days at room temperature did not prevent the spotting (Fig. 1B). Upon transfer of fruit to room temperature (26°-27°C), following four days at 12°C, senescent spotting increased with a slightly lower rate as in fruit that had been transferred to the high temperature earlier (Fig. 1B).

3.2. Peel colour, firmness and carbohydrate contents

The Hunter 'L' value of the peel (Table 1), firmness of bananas with peel (Table 2) and starch content of the banana pulp (Table 3) steadily decreased during the study period. The decrease was more rapid at higher temperature. In contrast, sugar content of the banana pulp steadily increased. This increase was greater at higher temperature (Table 4).

3.3. Total free phenolics

Total free phenolics level in the peel of the control fruit (26°-27°C) increased rapidly. The total free phenolics level of bananas held at 18°C increased slightly, while that in fruit held at 12° remained rather constant throughout the study period (Fig. 2A). Transfer of bananas to 12°C following two days at room temperature produced a very small increase in total free phenolics (Fig. 2B). Transfer of bananas to room temperature following four days at 12°C also resulted in an increase in the level of total free phenolics, compared with fruit that remained at 12°C (Fig. 2B).

3.4. PAL and PPO activities

PAL activity in the peel of the control bananas held at room temperature tended to increase but the LSD values did not show a statistically significant change. The PAL activity in fruit held at 12°C was not statistically different, on any day, from that in fruit held at room temperature. PAL activity at 18°C rapidly increased to a maximum on day 4 and decreased sharply thereafter (Fig. 3A). The same was found in fruit held at 15°C (results not shown). Transfer of bananas to 12°C following two days at room temperature resulted in a rapid increase in PAL activity, which peaked on day 4, and then gradually decreased (Fig. 3B). Transfer of bananas to room temperature following four days at 12°C had only a small effect on PAL activity (Fig. 3B).

PPO activity in the peel of bananas held at room temperature, 18° and 12°C increased rapidly to a maximum on day 4, and then decreased (Fig. 4A). Transfer of fruit to 12°C following two days at room temperature had no effect, compared with fruit kpet at room temperature (Fig. 4B). Transfer of bananas to room temperature following four days at 12°C resulted in a lower PPO activity, compared to fruit held continuously at 12°C (Fig. 4B).

Correlation coefficients between senescent spotting and the measured parameters are shown in Table 5. Except for total phenolics at 18°C and room temperature, these coefficients were very low.

4. Discussion

Holding the fruit at lower temperature resulted in slower changes in peel colour, firmness and contents of starch and sugar. This shows that ripening is delayed. The effect of temperature on senescent spotting also may be a simple temperature effect on enzymatic activity.

Holding the fruit at 12°C, which is the lowest possible storage temperature for banana, effectively blocked senescent spotting. At lower temperature the peel will become black due to chilling injury. The results indicate that it is possible to prevent most peel spotting in the supermarkets, provided that the product will be stored at about 12°C. At these temperatures, however, the peel will not become more yellow. At the consumer, if no further refrigeration is applied, spotting will then still occur very quickly and the peel may also become more yellow.

Bananas held at room temperature (25-27°C), 12, 15 and 18°C their weight losses were 5, 4, 3 and 2%, respectively at the end of study period (data not shown). Their difference in weight losses should not account for senescent spotting. Our previous experiment showed that weight loss of the banana was not the cause of the reduction of senescent spotting. The spotting of the PVC-wrapped bananas also occurred where the RH in the package was higher at ambient condition (Choehom et al., 2004). Spotting thus occurs irrespective of RH, at the ranged of RH investigated 65-95%

We now did not find evidence for a role of PAL or PPO in early senescent spotting. PPO is the enzyme that usually causes browning in plants. It uses a pool of free phenolic acids. PAL may replete this pool (Mayer and Harel, 1979; Martinez and Whitaker, 1995). Dopamine has been reported to be a major phenolic in the banana peel and it may be a main substrate for PPO oxidation and browning of the banana peel (Griffiths, 1961; Kanazawa and Sakakibara, 2000). However, in this stydy, we used catechol as a substrate to measure PPO activity in vitro and this compound may not be in vitro substrate for PPO in the banana peel. Therefore, PPO activity measured in this study may not represent in vivo activity. However, Weaver and Charley (1974) reported that there was no relationship between PPO activity and browning of the banana pulp. In the present experiments we observed a positive relationship between the level of total free phenolics and senescent spotting. This may mean that accumulation of free phenolics is a prerequisite for spotting.

PAL catalyzes the first step of phenolic compounds in the shikimate pathway (Cam and Towers, 1973). We now observed a poor relationship between senescent spotting and PAL activity in banana peel, indicating that PAL activity is not rate limiting. This is in contrast with our previous data (Choehom et al., 2004), which suggested that PAL might be involved in the browning reaction. We now similarly found a very low correlation coefficient between PPO activity and senescent spotting, which confirms the data of Choehom et al. (2004).

It is concluded that senescent spotting in banana peel is effectively prevented by holding the fruit at 12° C. Since no relationship was observed between spotting and the activities of PAL or PPO, these enzymes seem not rate limiting. Modulating their activity does not seem promising to reduce the disorder.

Acknowledgements

The research was financially supported by the Thailand Research Fund (TRF).