Improving the Shelf-Life and Safety of Fresh-Cut Mangosteen

by Combined Methods

Choothaweep Palakawong Na Ayudhya

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          by Combined Methods

Author   Mr. Choothaweep Palakawong Na Ayudhya

Major Program   Food Science and Technology

Major Advisor

………………………..………..
(Assoc. Prof. Dr. Pairat Sophanodora)

Co-advisor

………………………………
(Dr. Supachai Pisuchpen)

………………………………
(Assoc. Prof. Dr. Souwalak Phongpaichit)

………………………... ………
(Dr. Pascal Delaquis)

Examining Committee:

………………………..……Chairperson
(Asst. Prof. Dr. Anchalee Sirichote)

……………………….……………….
(Assoc. Prof. Dr. Pairat Sophanodora)

……………………….. …………...
(Dr. Supachai Pisuchpen)

……………………….. …………...
(Dr. Mutita Meenune)

………………………………………..
(Asst. Prof. Dr. Warapa Mahakarnchanakul)

      The Graduate School, Prince of Songkla University, has approved this
thesis as partial fulfillment of the requirement for the Doctor of Philosophy Degree in
Food Science and Technology.

……………………………………….
(Prof. Dr. Amornrat Phongdara)
Dean of Graduate School

ชื่อวิทยานิพนธ์	การปรับปรุงอายุการเก็บรักษาและความปลอดภัยของมังคุดสดตัดแต่งพร้อมบริโภคด้วยการใช้หลายวิธีร่วมกัน
ผู้เขียน	นายชูทวีป ปาลกะวงศ์ ณ อยุธยา
สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีอาหาร
ปีการศึกษา	2554

บทคัดย่อ

การศึกษาการเปลี่ยนแปลงคุณภาพของมังคุดสดตัดแต่งพร้อมบริโภค ที่บรรจุใน ถาดพอลีพอพิลีน (PP) ปิดด้วยฟิล์ม OPP/LLDPE ระหว่างการเก็บรักษาที่อุณหภูมิ 5 องศาเซลเซียส ความชื้นสัมพัทธ์ร้อยละ 85 เป็นเวลา 15 วัน จากการศึกษาพบว่ามังคุดสดตัดแต่งมีอัตราการหายใจ เริ่มต้นสูงสุดที่ 10.7 ml CO_2/(kg h) แล้วลดลงอย่างรวดเร็วภายใน 7 ชั่วโมง หลังจากนั้นลดลงอย่าง ช้าๆ และคงที่ตลอดเวลาการเก็บรักษา นอกจากนี้ยังพบว่ามังคุดสดตัดแต่งมีการผลิตเอทิลีนและ คาร์บอนไดออกไซด์สูงในช่วงเริ่มต้นแล้วลดลงในที่สุด ไม่พบว่าปริมาณออกซิเจน คาร์บอนไดออกไซด์และเอทิลีนในถาดบรรจุมังคุดเข้าสู่สภาวะสมดุล ทั้งนี้อาจเนื่องมาจากฟิล์มที่ ใช้ปิดถาดบรรจุไม่มีความเหมาะสม ความแน่นเนื้อของมังคุดสดตัดแต่งลดลงจากเริ่มต้น 11.10 ถึง 8.36 g/mm^2 ในวันสุดท้ายของการเก็บรักษา ซึ่งมีความสอดคล้องกับน้ำหนักที่สูญเสียไป การ เปลี่ยนแปลงของค่าดัชนีสีน้ำตาล (BI) ที่ค่อนข้างต่ำ อาจเป็นผลมาจากการเก็บรักษาที่อุณหภูมิต่ำที่ อาจส่งผลต่อการชะลอการทำงานของเอนไซม์พอลีฟีนอลออกซิเดส (PPO) ที่วันสุดท้ายของการ เก็บรักษาพบว่ามังคุดสดตัดแต่งมีความส่องสว่างลดลง สำหรับการเปลี่ยนแปลงของอะเซตตลดีไฮด์ และเอธานอล มีการเพิ่มขึ้นอย่างช้าๆหลังการตัดแต่ง แล้วเพิ่มขึ้นสูงสุดเป็น 11.40 และ 1,426.43 μl/kg ของน้ำหนักตัวอย่าง ที่วันสุดท้ายของการเก็บรักษา ตามลำดับ ปริมาณจุลินทรีย์ทั้งหมด ยีสต์ รา และ *Escherichia coli* มีการเพิ่มขึ้นเล็กน้อย คะแนนทางประสาทสัมผัสของการเกิดสีน้ำตาล กลิ่นผิดปกติ เนื้อสัมผัส และคะแนนการยอมรับโดยรวมจากการมองเห็น มีค่าเพิ่มขึ้นตามระยะเวลา ในการเก็บรักษา ซึ่งชี้ให้เห็นว่ามังคุดสดตัดแต่งได้รับการยอมรับที่ลดลงเมื่อมีอายุการเก็บรักษาที่ นานขึ้น

การศึกษาผลของ 1-methylcyclopropene (1-MCP) ที่มีต่อคุณภาพของมังคุดสดตัด แต่งพร้อมบริโภค โดยนำผลมังคุดก่อนการตัดแต่งมาเก็บรักษาที่อุณหภูมิ 28±2 องศาเซลเซียส ภายใต้สภาวะที่มี 1-MCP เข้มข้น 0 20 40 และ 80 พีพีเอ็ม เป็นเวลา 12 ชั่วโมง แล้วนำมาตัดแต่ง บรรจุในถาด PP ปิดด้วยฟิล์ม OPP/LLDPE เก็บรักษาที่อุณหภูมิ 5 องศาเซลเซียส ความชื้นสัมพัทธ์

ร้อยละ 85 เป็นเวลา 12 วัน พบว่ามังคุดสดตัดแต่งที่ผ่านการทรีตด้วยสาร 1-MCP มีอัตราการหายใจ และการผลิตกาซเอธีลีนลดลง รวมทั้งมีการสูญเสียน้ำหนัก และความแน่นเนื้อลดลงเมื่อเทียบกับ ตัวอย่างควบคุม หลังจากนั้นจึงนำผลมังคุดสดที่ทรีตด้วย 1-MCP เข้มข้น 40 พีพีเอ็มที่ได้จากผลการ ทดลองที่เหมาะสมมาเตรียมเป็นมังคุดสดตัดแต่ง แล้วล้างด้วยสารละลายโซเดียมคลอไรต์ในสภาพ ที่เป็นกรด (ASC) ความเข้มข้น 0 (ชุดควบคุม) 500 และ 1,000 พีพีเอ็ม เป็นเวลา 1 นาที ก่อนการ บรรจุและเก็บรักษาที่อุณหภูมิ 5 องศาเซลเซียส ความชื้นสัมพัทธ์ร้อยละ 85 เป็นเวลา 12 วัน พบว่า ASC ไม่ได้มีผลต่อปริมาณจุลินทรีย์เริ่มต้นในมังคุดตัดแต่ง แต่ช่วยลดการเกิดสีน้ำตาลได้ โดย พิจารณาจากค่าดัชนีสีน้ำตาล (BI) ที่ลดลงตลอดเวลาการเก็บรักษา ($p{\leq}0.05$)

การศึกษาคุณสมบัติการเป็นสารต้านอนุมูลอิสระและต้านจุลินทรีย์ ของสารสกัด จากเปลือกผล ใบ และเปลือกต้นมังคุดด้วยเอธานอล และน้ำมันหอมระเหยจากอบเชยและส้ม พบว่าสารสกัดจากเปลือกผล ใบ และเปลือกต้น มีค่า IC_{50} (ด้วยวิธี DPPH) เท่ากับ 5.94, 9.44 และ 6.46 µg/ml ตามลำดับ ส่วนน้ำมันหอมระเหยทั้งสองชนิดไม่มีคุณสมบัติดังกล่าว ในการศึกษา คุณสมบัติการเป็นสารยับยั้งแบคทีเรียแกรมบวก (*Listeria monocytogenes* และ *Staphylococcus aureus*) และแบคทีเรียแกรมลบ (*Escherichia coli* และ *Salmonella* sp.) โดยวิธีเจือจางอาหารเหลว พบว่าสารสกัดจากเปลือกผล ใบ และเปลือกต้นมีค่าความเข้มข้นต่ำสุดในการยับยั้ง (MIC) และการ ทำลาย (MBC) แบคทีเรียแกรมบวกอยู่ในช่วง 0.025-0.78 และ 0.05-0.39 mg/ml ตามลำดับ ส่วน น้ำมันหอมระเหยจากอบเชยมีค่า MIC และ MBC ต่อ *S. aureus*, *E. coli* และ *Salmonella* sp. คือ 3.13 และ 6.25 mg/ml ตามลำดับ ในขณะที่น้ำมันหอมระเหยจากส้มสามารถยับยั้งและทำลายได้ เฉพาะ *S. aureus* โดยมีค่า MIC และ MBC เท่ากับ 6.25 และ 12.50 mg/ml ตามลำดับ

การศึกษาเพิ่มเติมถึงคุณสมบัติในการเป็นสารยับยั้งจุลินทรีย์ ของสารสกัดจาก เปลือกผล ใบ และเปลือกต้นมังคุด โดยใช้วิธีการสกัดด้วยเมธานอลและน้ำร้อน พบว่าสารสกัด ทั้งหมดไม่สามารถยับยั้งเชื้อรา (*Botrytis cinerea* และ *Penicllium expansum*) ยีสต์ (*Saccharomyces cerevisiae*) รวมทั้งแบคทีเรียแกรมลบ (*E. coli* และ *Salmonella* Typhimurium) ได้ แต่สามารถยับยั้ง การเจริญของแบคทีเรียแกรมบวก (*L. monocytogenes* และ *S. aureus*) ได้เป็นอย่างดี นอกจากนี้ยัง พบว่าการลดค่าพีเอชของอาหารจาก 7-4 ช่วยเพิ่มประสิทธิภาพในการยับยั้งและทำลาย *L. monocytogenes, L. grayi, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* และ *S. aureus* ได้ดี ยิ่งขึ้น เมื่อเปรียบเทียบวิธีการสกัดพบว่า สารสกัดที่ได้จากการสกัดด้วยน้ำร้อนมีประสิทธิภาพใน การยับยั้งและทำลายด้อยกว่าสารสกัดที่ได้จากการสกัดด้วยเมธานอล ทั้งนี้เนื่องจากมีปริมาณ tartaric acid และ flavonols น้อยกว่า จากการวัดค่า propidium iodide uptake และ ATP leakage ทำ ให้ทราบถึงกลไกการยับยั้งหรือทำลายเซลล์แบคทีเรีย โดยสารสกัดดังกล่าวมีผลที่เมมเบรนของ

แบคทีเรีย เมื่อนำสารสกัดจากเปลือกผลของมังคุดที่สกัดด้วยเมธานอล มาศึกษาผลที่อาจมีต่อ
คุณภาพของมังคุดสดตัดแต่งพร้อมบริโภคที่บรรจุในถาด PP หุ้มด้วยฟิล์ม OPP/LLDPE พบว่าสาร
สกัดดังกล่าว ไม่มีผลต่อปริมาณออกซิเจนและคาร์บอนไดออกไซด์ในบรรจุภัณฑ์ รวมทั้งความ
แน่นเนื้อ น้ำหนักที่สูญเสีย ปริมาณอะเซตตัลดีไฮด์และเอธานอล แต่มีผลในการรักษาค่าความสว่าง
และค่า hue โดยมังคุดสดตัดแต่งที่จุ่มในสารสกัดจากเปลือกผลมังคุด 0.25 g/ml ให้ค่าความสว่าง
ดีกว่าตัวอย่างชุดควบคุม อย่างไรก็ตามสารสกัดไม่ได้มีผลต่อ *S. aureus* และ *E. coli* ที่เติมลงไป

การศึกษาผลของฟิล์มที่มีค่าการซึมผ่านของออกซิเจน และคาร์บอนไดออกไซด์
ต่างกัน 3 ชนิด คือ OPP/LLDPE, PET และ LDPE ต่อปริมาณออกซิเจนและคาร์บอนไดออกไซด์
และคุณภาพของมังคุดสดตัดแต่งพร้อมบริโภคที่ผ่านการจุ่มในสารสกัดจากเปลือกผลมังคุด 0.25
g/l และบรรจุในถาด PP ปิดด้วยฟิล์มต่างๆ พบว่าฟิล์ม LDPE ซึ่งมีค่าการซึมผ่านของออกซิเจน
และคาร์บอนไดออกไซด์สูงสุด คือ 2,795 และ 10,500 cm^3/m^2 day ตามลำดับ มีปริมาณออกซิเจน
เอธิลีน อะเซตตัลดีไฮด์สะสมในบรรจุภัณฑ์มากที่สุด ส่วนคาร์บอนไดออกไซด์มีสะสมอยู่น้อยที่สุด
นอกจากนี้มังคุดสดตัดแต่งที่บรรจุในถาดที่ปิดด้วยฟิล์มนี้ยังมีการสูญเสียความแน่นเนื้อและน้ำหนัก
มากกว่าฟิล์ม OPP/LLDPE หรือ PET อีกด้วย จากการทดสอบทางประสาทสัมผัสพบว่ามังคุดที่
บรรจุในถาดปิดด้วยฟิล์ม LDPE นี้ไม่เป็นที่ยอมรับ ในด้านของคุณภาพทางจุลินทรีย์นั้นไม่พบว่า
ชนิดของฟิล์มมีผลกระทบต่อจำนวนจุลินทรีย์ เมื่อคัดเลือกฟิล์มที่มีความเหมาะสมเพื่อใช้ปิดถาด
PP สำหรับมังคุดสดตัดแต่งพร้อมบริโภค โดยอาศัยกลไก Michaelis-Menten พบว่ามังคุดสดตัดแต่ง
มีค่าอัตราการหายใจสูงสุดเป็น 714.29 ml CO_2 /(kg h) อาจเนื่องจากการตอบสนองต่อการบาดเจ็บ
จากการตัดแต่ง ซึ่งเพิ่มพื้นที่ผิวสัมผัสกับอากาศรอบด้าน รวมทั้งค่า RQ ที่ต่ำกว่า 1 คือ 0.64 ทำให้
ตัวแบบที่สร้างขึ้นไม่สามารถอธิบายค่าจากการทดลองได้ จึงได้มีการปรับปรุงตัวแบบดังกล่าวด้วย
การเขียนกราฟความสัมพันธ์ระหว่างค่า R_{CO_2} ของข้อมูลจากการทดลองและจากการคำนวณโดยใช้
ค่า RQ จริงจากการทดลอง ทำให้ได้ตัวแบบที่ปรับปรุงแล้วที่สามารถอธิบายค่าจากการทดลองได้
จากนั้นจึงนำตัวแบบที่ได้จากการปรับปรุงนี้มาศึกษาผลของการปรับเปลี่ยน ขนาดของบรรจุภัณฑ์
ความหนาของฟิล์ม ค่า β ratio และน้ำหนักของมังคุด จากการศึกษาพบว่า เฉพาะค่าน้ำหนักของ
มังคุดเท่านั้นที่เป็นค่าที่ต้องคำนึงถึงเพื่อการคัดเลือกฟิล์มที่เหมาะสมที่จะใช้ปิดบรรจุภัณฑ์สำหรับ
มังคุดสดตัดแต่งพร้อมบริโภคนี้

Thesis Title Improving the Shelf-Life and Safety of Fresh-Cut Mangosteen by Combined Methods

Author Mr. Choothaweep Palakawong Na Ayudhya

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ABSTRACT

A study was carried out on quality changes of fresh-cut mangosteen packed in polypropylene (PP) trays sealed with OPP/LLDPE film during storage at 5 $^{\circ}$C and 85% RH. The initial respiration rate of fresh-cut mangosteen (10.7 ml CO_2 /kg h) declined rapidly within 7 h then gradually decreased and remained relatively stable until the end of storage. Ethylene (C_2H_4) and CO_2 production were raped at the beginning of storage but gradually decreased with time. Gas composition and ethylene inside the packages did not reach equilibrium because the transmission rate of the film was excessive. Flesh firmness decreased from 11.10 to 8.36 g/mm^2 as storage time increased and was correlated to increasing weight losses. Browning index (BI) changed at a slower rate than in controls, indicating that low temperature plays an important role in browning control through the inhibition of polyphenoloxidase (PPO) enzyme activity. At the end of storage, the fresh-cut mangosteen became darker with lower L* and higher a* values. Acetaldehyde and ethanol content increased slightly after cutting and then rose sharply, reaching 11.40 and 1,426.43 µl/kg fruit, respectively at the end of storage. Microbial populations (total viable count, yeast and mold and *Escherichia coli*) increased slightly during storage although levels were deemed acceptable. Scores for sensory attributes including browning, off-odor, texture and overall acceptability increased with time, which revealed that the acceptance of fresh-cut mangosteen decreased during storage.

Mangosteen fruit treated with 0, 20, 40 or 80 ppm 1-MCP for 12 h at 28$\pm$2 $^{\circ}$C was processed and packed in PP trays sealed with OPP/LLDPE film. After 12 days storage at 5 $^{\circ}$C and 85% RH fresh-cut mangosteen prepared from fruit without 1-MCP treatment exhibited rapid softening and increases in weight loss. Fresh-cut mangosteen prepared from 1-MCP treated fruit showed a decrease in

ethylene production and respiration rate as well as delayed softening and weight losses. Therefore, the treatment led to improved quality retention in packaged fresh-cut mangosteen. Fruit treated with 40 ppm 1-MCP were used to study the effect of acidified sodium chlorite (ASC) on quality changes of fresh-cut mangosteen. The cut fruit was dipped in 0 (control), 500 or 1,000 ppm ASC for 1 min before packing in PP trays sealed with OPP/LLDPE film. After storage at 5 $^{\circ}$C and 85% RH for 12 days, the results indicated that ASC application did not significantly reduce initial microbial populations. However, ASC reduced browning of the product as indicated by significantly reduced browning index (BI) value throughout the storage period ($p{\leq}0.05$).

The antioxidant and antimicrobial activities of the extracts from pericarp, leaf and bark of mangosteen (*Garcinia mangostana* L.) and some essential oils such as cinnamon and citrus were investigated. The antioxidant activities (IC$_{50}$) of pericarp, leaf and bark extracts, which were evaluated by DPPH method, were 5.94, 9.44 and 6.46 µg/ml, respectively. Both cinnamon and citrus essential oil showed no antioxidant activities with DPPH. A broth dilution method was employed to evaluate the antimicrobial activity against some Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*E. coli* and *Salmonella* sp.). The minimum inhibitory concentration (MIC) values of pericarp, leaf and bark extracts against Gram-positive bacteria were ranged from 0.025-0.78 mg/ml. While the minimum bactericidal concentration (MBC) values were between 0.05-0.39 mg/ml. MIC and MBC values of cinnamon oil against *S. aureus*, *E. coli* and *Salmonella* sp. were 3.13 and 6.25 mg/ml, respectively. Citrus oil showed antibacterial effect on only *S. aureus* with MIC and MBC values of 6.25 and 12.50 mg/ml, respectively.

Further study on antimicrobial activity of mangosteen bark, leaf and fruit pericarp extracts prepared in methanol and in hot water was carried out. None of the extracts could inhibit growth of the fungi *Botrytis cinerea* and *Penicllium expansum,* the yeast *Saccharomyces cerevisiae* or the Gram negative bacteria *E. coli* and *Salmonella* Typhimurium. In contrast, both methanolic and aqueous extracts prepared from bark, leaf or fruit pericarp exhibited strong bacteriostatic and bactericidal effects against *L. monocytogenes* and *S. aureus*. Further examination of

activity against *Listeria* species including *L. monocytogenes, L. grayi, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* and *S. aureus* revealed that this activity was greatly enhanced by lowering test pH from pH 7 to pH 4. The strength of inhibition was lower in aqueous extracts and chemical analysis indicated lesser concentrations of tartaric acid and flavonols. Measurement of propidium iodide uptake and ATP leakage indicated that all the extracts induced damage to the cell membrane of Gram-positive bacteria.

The effect of a mangosteen fruit pericarp extract on the physical, chemical and microbiological qualities of fresh-cut mangosteen dipped in 0.25 g/l of the fruit pericarp extract, packed in PP trays and sealed with OPP/LLDPE film was investigated. The extract did not affect the headspace gas composition (O_2 and CO_2), fruit firmness, weight loss, acetaldehyde or ethanol contents, but the lightness and hue value of the tissue were altered. Fresh-cut mangosteen dipped in 0.25 g/l of the fruit pericarp extract retained lightness and hue values better than the control. The extract could not inhibit *S. aureus* or *E. coli* inoculated onto fresh-cut mangosteen.

Three types of sealing film (OPP/LLDPE, PET and LDPE) were used to examine the influence of films on the composition of the headspace and the quality of fresh-cut mangosteen during storage in PP trays. LDPE film, which has the highest OTR and CTR (2,795 and 10,500 cm^3/m^2 day, respectively), showed the highest O_2, C_2H_4, ethanol and acetaldehyde accumulation and the lowest residual CO_2 in the package. Furthermore, firmness and weight losses were higher compared with OPP/LLDPE and PET films but the latter improved retention of color. Film type did not affect the microbiology of fresh-cut mangosteen. Overall visual quality (OVQ) was influenced by type of film. Samples packed under OPP/LLDPE and PET film maintained better overall visual quality than those packed under LDPE film. Film type was selected on the basis of a mathematic model based on Michaelis-Menten kinetics. The maximum respiration rate (Vm) of fresh-cut mangosteen in a closed system was found to be very high (714.29 ml CO_2 /(kg h)), probably because of wounding response from cutting leading to be exposed to gasses on all sides with increasing surface area. The first model derived from an ordinary differential equation (ODE) did not fit to the experimented data because of low RQ (0.64). The model was modified by plotting experimental R_{CO_2} data and calculated data based on an actual

RQ. The modified model was also used to estimate the effect of package dimension, product weight, film thickness and β ratio on the model. The package dimension, film thickness and β ratio did not affect the model, only product weight did because of the high respiration rate of the product. Changing product weight is the parameter that should be considered to select film lid for fresh-cut mangosteen.

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CHAPTER 1

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) belongs to the family Clusiaceae. It is one of the most famous fruit in Thailand. It has been described as the "Queen of Tropical Fruits" because of its instant visual and taste appeal. The mangosteen fruit is dark purple or reddish, with white, soft and juicy edible pulp with a slightly acid and sweet flavor and pleasant aroma (Jung *et al*., 2006). Mangosteen is a rich source of phenolic compounds such as xanthones, phenolic acids, tannins and anthocyanins (Fu *et al*., 2007). However, xanthone is the only phenolic compound in mangosteen that has been widely studied (Terry, 2011).

Phenolic compounds are secondary metabolites found in some higher plant families, fungi and lichens (Peres *et al*., 2000) and can be isolated from the pericarp, whole fruit, bark and leaf of mangosteen. Several studies have shown that phenolic compounds from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergenic, antibacterial, antifungal and antiviral (Suksamrarn *et al*., 2006; Pedraza-Chaverri *et al*., 2008).

Mangosteen is a seasonal fruit and its production is dependent on the weather. Market prices are therefore subjected to availability, particularly in years where yields are high. Processing into preserved products can alleviate fluctuations in demand and contribute to price stabilization. The literature describes a range of preserved foods made from mangosteen, including canned (Khantee, 1997), dried (Sophanodora *et al*., 2002), frozen (Sripongpunkul, 1990; Sutthirak, 1996) and ready-to-eat or fresh-cut products (Kitpipit, 2005; Manurakchinakorn *et al*., 2005; Ngarmsak, 2007). Fresh-cut mangosteen is an increasingly valuable commodity with excellent marketability. The minimally processed product retains the appearance and freshness of whole fruit and contains higher vitamin and mineral content than other processed products. However, processing mangosteen into fresh-cut products is technically challenging, notably the maintenance of fresh-like quality (color, odor, flavor) and shelf-life. A minimal process meant to prolong the shelf-life of fresh-cut unripe mangosteen (maturity stage

1

1) was developed by Kitpipit (2005). It involves cutting and dipping in a solution containing 0.5% citric acid plus 0.25% (w/v) calcium chloride for 30 minutes, packaging under modified atmosphere ($15\%O_2$ and $10\%CO_2$) and storage at 10^oC. In contrast, little is known about fresh-cut processing of mangosteen at the early ripe stage. Furthermore, there is presently no data pertaining to factors that affect shelf-life, the performance of different packaging systems or preservation strategies. The use of natural antimicrobial agents, including those that can potentially be extracted from the mangosteen plant, has not been explored. A demonstration of their value in fresh-cut fruit processing could provide important added value to the crop.

There is clearly a need to further investigate the performance of processing treatments, the use of natural antimicrobial compounds and the effect of packaging strategies on fresh-cut mangosteen at the early ripe stage (maturity stage 2-3). New knowledge derived from such research would provide information needed to optimize the marketable shelf-life of the fresh-cut product. Hence this research will provide basic knowledge relevant to the quality of fresh-cut mangosteen and information needed to enhance the utilization of an important Thai crop.

Objectives

1. To study quality changes of fresh-cut mangosteen.

2. To study the effect of 1-methylcycropropene (1-MCP), acidified sodium chlorite (ASC) and mangosteen tissue extracts on the quality of fresh-cut mangosteen.

3. To study the antimicrobial activity of extracts prepared from mangosteen tissues.

4. To investigate the influence of packaging film on the quality of fresh-cut mangosteen and to provide appropriate criteria for the selection of a film.

Expected outcome

1. Information about quality changes of packaged fresh-cut mangosteen.

2. Information about the effect of 1-methylcycropropene and acidified sodium chlorite on fresh-cut mangosteen.

3. Information about the effect of mangosteen tissue extracts on the quality of fresh-cut mangosteen.

4. Optimal packaging systems to extend the shelf-life of fresh-cut mangosteen.

Scope of study

Mangosteens at the early ripe stage were processed into a fresh-cut product. The effect of 1-MCP, ASC, crude extracts from mangosteen tissues and some essential oils on quality changes in packaged fresh-cut mangosteen were investigated. Antioxidant and antimicrobial activities were examined in crude extracts prepared from various mangosteen tissues. Studies established the influence of packaging film and packaging system on quality retention of fresh-cut mangosteen.

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CHAPTER 2

LITERATURE REVIEW

2.1 General

Fresh-cut fruit is defined as any fruit that has been trimmed, peeled, washed and cut into 100% usable product. It is then bagged or prepackaged and remains in a fresh state (IFPA, 2004). Since no additional preparation is necessary prior to use these products are widely used in the institutional sector (restaurants, fast food outlets) and increasingly in the retail market. Packaging systems can include containers over-wrapped with film, film packages or glass jars (Watada and Qi, 1999).

The basic requirements for preparation of fresh-cut fruits may include: high quality raw material, strict hygiene and good manufacturing practices, low temperatures during processing, careful cleaning and/or washing before and after peeling, use of mild processing aids in wash water for disinfection or prevention of browning and texture loss, minimize damage during peeling, cutting, slicing and shredding operations, gentle draining to remove excess moisture, correct packaging materials and methods and correct temperature during distribution and handling (Kader, 2002).

2.2 Quality Changes of Fresh-Cut Fruits

2.2.1 Nutritional and Chemical Changes

Fruits contain small to significant amounts of several important nutrients such as carbohydrates, vitamins, minerals and other substances such as phytochemicals. Phytochemicals are defined as substances found in edible fruits or vegetables that exhibit a modulating effect on metabolism when ingested (Camire *et al*., 2000). Phytochemicals are classified into eleven groups: carotenoids, dietary fiber, glucocinolates and others, inositol phosphates, polyphenols, phenols and cyclic compounds, phytoestrogens, plant sterols, protease inhibitors, saponins, sulfide and thiol containing compounds. Some of these phytochemicals are found in fruits such as citrus, papaya, pineapple, cherry, strawberry, peach, apricot, watermelon and guava

(Camire *et al*., 2000; Lamikanra *et al*., 2005b) and can play an important role in consumer preference and health. These compounds also influence the appearance, taste, color and aroma of fruit. In addition, pigments such as chlorophyll, carotenoids and anthocyanins responsible for the color have been shown to have a role in disease prevention (Lamikanra *et al*., 2005b).

The major potential loss of nutritional quality of fruit may occur during harvesting, postharvest handling, processing, storage and distribution. Although processing is generally aimed at the maintenance of shelf-life, it may also affect the nutrient content. Nutrient losses can be divided into three categories: intentional, accidental and inevitable. Intentional losses may be due to the removal of parts during peeling, accidental losses result from inadequate handling and inevitable losses occur when the heat labile nutrients are destroyed by heat, blanching for example (Lamikanra *et al*., 2005b).

Quality changes and nutrient retention in fresh-cut versus whole fruits of pineapple, mango, cantaloupe, watermelon, strawberry and kiwifruit have been examined during storage at 5 °C (Gil *et al*., 2006). After 6 days in storage vitamin C losses in mango, strawberry and watermelon pieces, cantaloupe cubes, pineapple pieces and kiwifruit slices were 5%, 2%, 10% and 12%, respectively. There were no losses in carotenoids in kiwifruit slices and watermelons cubes, but losses in pineapple were the highest at 25%, followed by 10-15% in cantaloupe, mango and strawberry pieces. No significant losses in total phenolics were evident in any of the fruit. In all cases, the fresh-cut fruit were visibly spoiled before significant nutrient loss occurred. Perkins and Collins (2004) reported that in red fleshed watermelons, a recognized source of lycopene, the amount of this phytochemical in two cultivars (Summer Flavor 800 and Sugar Shack) decreased by 6 and 11% after 7 days in storage for each cultivar respectively.

2.2.2 Physiological Changes

Ethylene production and respiration

Sliced fruit is expected to behave differently from the whole fruit during storage because of the tissue wounding response. Fresh-cut fruit is living tissue and requires oxygen for respiration, with carbon dioxide, ethylene and water released as

byproducts. In the absence of oxygen, anaerobic respiration or fermentation can occur. Here alcohols and aldehydes are the major byproducts leading to variable quality defects including off-flavors (Camire *et al.*, 2000).

Membrane degradation and oxidative browning

Membrane deterioration in fresh-cut produce results in decompartmentation of cellular structure and organization and loss of normal cellular function. These changes cause secondary effects such as tissue browning, production of off odors, enzymatic degradation of membrane components and enzymatic production of free fatty acids from the released membrane lipids (Lamikanra, 2002).

Discoloration may occur at the surface as substrates and oxidases come into contact as a result of cutting and the disruption of the cells. Oxidative browning at the cut surface is a primary quality limiting factor in storage of many fresh-cut fruits (Brecht, 1995). Oxidative browning is usually caused by the polyphenol oxidase (PPO) enzyme, which, in the presence of O_2, converts phenolic compounds in fruits and vegetables into dark colored pigments. Enzymatic browning can be catalyzed by the PPO enzyme in two steps: hydroxylation of monophenols to *o*-diphenols and oxidation of *o*-diphenols to *o*-quinones (Figure 1).
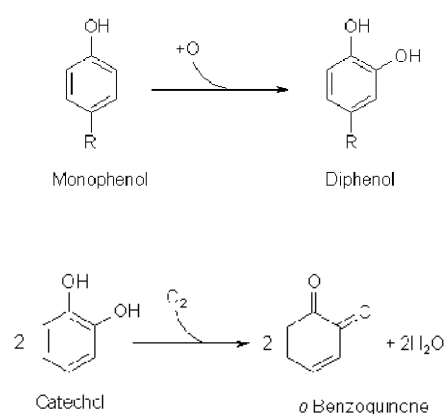
Figure 1. Enzymatic browning reactions catalyzed by polyphenol oxidase. Source: Marshall *et al.* (2000).

Water loss

Plant tissues are in equilibrium with an atmosphere at the same temperature and relative humidity. When tissues are exposed during cutting or peeling the water evaporation rate increases. The rate of water loss between intact and wounded plant surfaces varies according to the commodity (Watada and Qi, 1999).

2.2.3 Microbiological Changes

Microorganisms such as psychrothrophic or mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and molds have been found to actively grow in packaged fresh-cut fruits. Development of microbial populations is related to the increase in respiration rate and the release of nutrients from damaged tissue and broken cells, which can also provide a protective environment for growth of other types of microflora (Lamikanra, 2002).

Fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens capable of causing human illness. *Listeria monocytogenes*, *Salmonella* and *Escherichia coli* have been isolated from raw produce, which can become contaminated during production or harvest, postharvest handling, or distribution. The incidence of foodborne disease outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years (Mukherjee *et al*., 2006). The pathogens most frequently linked to produce-related outbreaks include bacteria (*Salmonella* sp., *E. coli*), viruses (Norwalk-like, hepatitis A) and parasites (Cryptosporidium, Cyclospora), with *Salmonella* sp. and *E. coli* O157:H7 being the leading causes of produce-related outbreaks in the USA. Fresh fruits and vegetables may therefore pose a food safety risk because they are consumed raw and are susceptible to be contaminated by sources of fecal material and soil on the farm (Mukherjee *et al*., 2004).

Although there have been a number of reports about microbiological contamination involving whole fresh produce, there is less information about microbial contamination of fresh-cut fruit (Abadias *et al*., 2008). Several national microbiological guidelines have been published for ready-to-eat food in countries such as: the EU (Graffham, 2006), UK (PHLS, 2000), HK (FEHD, 2001), AU and NZ (FSANZ, 2001) and TH (Department of Medical Sciences, 1993). Table 1 shows the

standards applied to the safety quality of fresh/fresh-cut fruit and ready-to-eat food in different countries.

Table 1. Safety quality standards for fresh/fresh-cut fruit and ready-to-eat food in different countries.

Microbiological criteria	EU (Fresh /Fresh-cut fruit)	UK, HK (Ready-to-eat)	AU, NZ (Ready-to-eat)	TH (Ready-to-eat)
Aerobic count	-	N/A	N/A	-
Salmonella spp.	Absence in 25 g	Absence in 25 g	Absence in 25 g	Absence in 25 g
Enterobacteriaceae	-	N/A	-	-
Escherichia coli (total)	$<10^4$ CFU/g	N/A	N/A	<10 (MPN)
E. coli O157 & other VTEC	-	Absence in 25 g	-	-
Listeria spp. (total)	-	N/A	-	-
L. monocytogenes	-	$<10^2$ CFU/g	$<10^2$ CFU/g	-
Staphylococcus aureus	-	$<10^4$ CFU/g	-	-
Clostridium perfringens	-	$<10^4$ CFU/g	$<10^4$ CFU/g	-
Campylobacter spp.	-	Absence in 25 g	Absence in 25 g	-
Bacillus spp.	-	$<10^5$ CFU/g	-	-
Vibrio cholerae	-	Absence in 25 g	-	-
V. parahaemolyticus	-	$<10^3$ CFU/g	$<10^4$ CFU/g	-
Yeast	-	-	-	$<10^4$ CFU/g
Mold	-	-	-	<500 CFU/g

Note: EU (Europe), UK (United of Kingdom), HK (Hong Kong), AU (Australia), NZ (New Zealand), TH (Thailand)

VTEC (Verocytotoxin producing *E. coli*), N/A (Not applicable)

2.3 Factors Affecting the Quality of Fresh-Cut Fruit

2.3.1 Raw Materials

Species and variety

Several studies in the literature report comparisons for the shelf-life of fresh-cut fruit from different cultivars and illustrate the importance of this variable on the selection of raw material for fresh-cut products. In a study on four cultivars of pear (Bartlet, Bosc, Anjou and Red Anjou), it was shown that Bartlett pears had better and

longer shelf-life than the others (Gorny *et al*., 2000). The other study, Gorny *et al*. (1999) examined the response of thirteen cultivars of peach and eight cultivars of nectarine to fresh-cut processing. It was found that the quality of slices based on visual appearance depending on cultivar, species and variety.

Raw material quality

Although fruit which may not be visually acceptable for fresh market is occasionally used for processing, it is generally recognized that raw product quality for fresh-cut should be high to insure good quality in the finished product. This was illustrated in a study comparing the quality of fresh-cut melon pieces made from melons of good quality and fruit grown in contact with the soil or with sunburn symptoms (Cantwell and Portela, 1998). Pulp removed beneath sunburned areas or where the melon was in contact with ground yielded softer pieces with poor overall quality scores.

Physiological maturity

 Generrally, climacteric fruits show a large increase in CO_2 and C_2H_4 production rates after harvesting coincident with ripening. Whereas non-climacteric fruits showed neither CO_2 nor C_2H_4 production rates change during ripening (Kader, 1992). The physiological maturity of fruits and vegetables impacts the wounding response, especially in climacteric fruits (Lamikanra, 2002; Kader, 2002). Gorny *et al*. (2000) studied the quality changes in fresh-cut pear as affected by maturity stage. Results showed that fruit maturity stage at cutting had a significant effect on shelf-life as indicated by both the rate and intensity of enzymatic browning.

2.3.2 Cutting Conditions

Shape of cutting

Fresh-cut fruits may be cut into a wide variety of shapes which influences the degree of damage and wound response. For example, Rivera-Lopez *et al*. (2005) compared the quality of cubes and slices prepared from papaya. The total soluble solids content, weight loss and overall quality index of slices were better than cubes because of the area damaged by the two cutting procedures.

Peeling method

The type of peeling method used may influence the degree of physiological response in the tissues. Lamikanra (2002) compared the effects of hand peeling, coarse and fine abrasion peeling on carrots. Fine abrasion peeling resulted in lower weight loss in packaged carrot slices compared to coarse abrasion and the carrots hand-peeled with a sharp blade exhibited lower water loss, respiration rate and microbial counts than sliced carrots made from either fine or coarse abrasion.

Sharp versus dull blades

Portela and Cantwell (2001) found that cutting cantaloupe melon pieces with a sharp borer resulted in longer shelf-life than cutting with a blunt borer. Slicing with a dull blade led to translucency, a common visual defect in commercial fresh-cut melon. Although the respiration and ethylene production rates were affected only slightly by the cutting treatment, blunt cut pieces had higher ethanol concentrations, off-odor scores and electrolyte leakage than sharp cut pieces.

2.3.3 Storage Temperature

Temperature is a critical factor affecting the quality of fresh-cut fruit. For an increase from 0 to 10 °C the respiration rate increases substantially, with the Q_{10} ranging from 3.4 to 8.3 among various fresh-cut products (Watada and Qi, 1999). Control of temperature is the most useful and important technique that is available to minimize the effect of wounding in fresh-cut fruit (Brecht, 1995). Low temperatures are needed to reduce respiration rates, retard microbial growth and deterioration processes such as softening and browning. In general, fresh-cut products should be stored at 0-5 °C to maintain quality. For chilling-sensitive fruits low temperatures retard the rate of deterioration of the fresh-cut products more than they induce chilling injury (Kader, 2002; Rivera-Lopez *et al.*, 2005).

2.3.4 Packing Systems

Modified atmosphere packaging (MAP) involves placing the product in a sealed package to achieve an atmosphere with the desired composition. The most important aspect of modified atmosphere packaging is to determine the composition

of the atmosphere and the type of materials that can be used to create the optimal environment. Critical conditions include atmospheric oxygen, carbon dioxide, ethylene and relative humidity. Shelf-life will be improved if all these conditions are chosen correctly, but incorrect conditions can lead to the opposite effect (Gorny, 1997).

Modified atmospheres can be achieved passively or actively. Passive MAP is achieved when fresh produce is hermetically sealed in a semi permeable container and the respiration process alters the gas composition. An active MAP could be achieved by flushing out the air within the package with a precise mixture of gases to obtain an initial atmosphere (Lamikanra, 2002).

One benefit of MAP is the maintenance of a high relative humidity around the fresh-cut product. Water condensation can be a disadvantage however, since it can promote the growth of spoilage microorganisms and fogging inside the package which can affect the visibility of the product by the consumer. Another benefit of MAP is that low oxygen or elevated carbon dioxide concentrations inside the package can slow browning reactions, reduce the rate of product respiration and reduce ethylene biosynthesis and their effects (Gorny, 1997).

CO_2 and O_2 concentrations in MAP packages change as a function of produce respiration rate, weight of sample, temperature and the properties of the film. Several models can combine these variables to generate an overall MAP model. As described by Exama et al. (1993), the transient O_2 level as a function of time can be expressed by ordinary differential equations (ODE)

$$\frac{dyiO_2}{dt} = \left(\frac{AP_{O_2}p}{VL}\right)(yeO_2 - yiO_2) - \left(\frac{WR_{O_2}}{V}\right)$$

where A = area of permeable film, P_{O_2} = O_2 permeability coefficient, p = pressure (1 atm), V = free volume of the package, L = film thickness, yeO_2 = external (atmospheric) O_2 concentration (21%), yiO_2 = internal O_2 concentration (%), W = weight of produce and R_{O_2} = O_2 consumption rate.

Al-Ati and Hotchkiss (2003) studied the effect of film permselectivity on the equilibrium gas composition of a model MAP produce, fresh-cut apple, using the model as described above. It was found that the calculated gas composition agreed with the observed values.

2.4 Quality Control of Fresh-Cut Fruits

Quality changes in fresh-cut fruit due to ethylene production, respiration rate, oxidative browning, loss of water and spoilage by microorganisms can be controlled to extend the quality of fresh-cut fruits using different strategies outlined below.

2.4.1 Chemical Methods

Acidulants

The optimum pH of polyphenol oxidase (PPO) has been reported to be from acid to neutral in most fruits and vegetables. The optimum activity is observed at pH 6.0-6.5 and minimum activity is minimal below pH 4.5. Chemicals that lower the product pH or acidulants can consequently be used for the control of enzymatic browning. Acidulants applied as pre-packaging dips are often used in combination with other treatments because reducing browning by only controlling the pH is difficult. Acidulants such as citric, malic and phosphoric acids are capable of lowering the pH of a system thus reducing polyphenol oxidase activity (Lamikanra, 2002; Marshall *et al*., 2000). Citric acid is widely used as an acidulant and is typically applied at levels ranging between 0.5 and 2 percent (w/v) for the prevention of browning in fruits and vegetables. Citric acid can be used in combination with other antibrowning agents such as ascorbic or erythorbic acids and their neutral salts, for the chelation of prooxidants and for the inactivation of polyphenol oxidase. Besides lowering the pH, citric acid acts by chelating the copper at the active site of the enzyme (Marshall *et al*., 2000).

Reducing agents

Reducing agents react with quinones by reducing them to phenols and act on the PPO by binding irreversibly with the copper of the enzyme. Reducing compounds are very effective in the control of browning (Lamikanra, 2002; Marshall *et al*., 2000).

One of the most widely used reducing agents is ascorbic acid. Ascorbic acid is a moderate reducing compound, acidic in nature, forms neutral salts with bases and is water-soluble. Erythorbic acid, which is the D-isomer of ascorbic acid but without the vitamin C activity, is cheaper than vitamin C and is believed to have the same antioxidant properties (Alzamora *et al.*, 2000).

Ascorbic acid delays PPO browning by reducing *o*-quinones back to phenolic compounds before they form brown pigments. However, ascorbic acid is consumed in the process, providing only temporary protection unless used at higher concentrations. Gorny *et al*. (1999) determined that 2% ascorbic acid with 1% calcium lactate reduced the browning of fresh-cut peaches initially but after 8 days storage at 0 °C the difference was minimal. Gil *et al*. (1998) determined that 2% ascorbic acid was effective in reducing the browning of fresh-cut Fuji apple slices but in combination with low oxygen atmospheres storage.

Sulphites are also reducing agents of enzymatic browning. These compounds include sulphur dioxide (SO_2) and several forms of inorganic sulfites that liberate SO_2. Gonzales *et al*. (2000) compared the efficacy of sulphites with other antibrowning agents. Sulphites are very effective compared with ascorbic acid. Although they are very effective, many regulatory agencies including the FDA have restricted their use due to potential adverse reactions in sensitive individuals (FDA, 2000).

Another effective reducing agent is cysteine but the amount of cysteine required for complete browning inhibition is often incompatible with product taste (Lamikanra, 2002). Thiol-containing compounds such as N-acetyl-L-cysteine and reduced glutathione are natural chemicals that react with quinones formed during the initial phase of enzymatic browning reactions. Oms-Oliu *et al*. (2006) used combinations of N-acetyl-L-cysteine, reduced glutathione, ascorbic acid and 4-hexylresorcinol and concluded that 0.75% of N-acetyl-L cysteine was effective to prevent browning of fresh-cut pears up to 28 days and 0.7% glutathione was effective up to 21 days. There was also an enhanced effect combining N-acetyl-L-cysteine with reduced glutathione. The combination of ascorbic acid and 4-hexyresorcinol was not effective.

Chelating agents

Chelating agents can prevent enzymatic browning through the formation of a complex between these inhibitors and copper through an unshared pair of electrons in their molecular structures. Some of the chelating agents used in fruits and vegetables are citric acid and ethylenediamine tetraacetic acid (EDTA) (Alzamora *et al.*, 2000). EDTA is used with other antibrowning chemicals in concentrations up to 500 ppm (Lamikanra, 2002). Some tests using EDTA as an inhibitor of peach PPO showed they were not totally effective (Marshall *et al.*, 2000).

Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one), a γ-pyrone derivative and a fungal metabolite produced by many species of *Aspergillus* spp. and *Penicillium* spp. is a good metal ion chelator (Marshall *et al.*, 2000). Son *et al.* (2001) used kojic acid and thirty six other antibrowning compounds to compare their ability to prevent browning of apple slices. Kojic acid, oxalic acid, oxalacetic acid, ascorbic acid, cysteine, glutathione, N-acetylcysteine and 4-hexyl resorcinol are the most effective antibrowning agents tested.

Calcium treatments

Calcium treatments are commonly used in the industry to improve the firmness of canned fruit and vegetable products and have been also reported to reduce browning. The effect of calcium on texture can be explained by different mechanisms: 1. Complexing of calcium ions with cell wall and middle lamella pectin; 2. Stabilization of the cell membrane by calcium ions and 3. Effect of calcium on cell turgor pressure (Luna-Guzman and Barret, 2000). The firming action of calcium at the same time contributes to reduced leakage of polyphenol oxidase and substrates at the exposed cut surfaces, thereby contributing to reduce browning (Lamikanra, 2002). Calcium chloride has been used as a firming agent, it may impart undesirable bitterness or flavor differences to the product. Calcium lactate has been considered as an alternative source of calcium to achieve the same effect (Luna-Guzman and Barret, 2000).

Sanitizers

Sanitizers are chemicals used to reduce microorganisms on the surfaces of whole and cut produce and processing equipment, including viruses, parasites, spoilage bacteria, molds and yeast, pathogenic species (Alzamora *et al*., 2000). Chlorine (Cl_2) and chlorinated compounds are effective sanitizers for surfaces. Chlorine from any source is commonly used at concentrations up to 200 ppm (free chlorine or concentration of hypochlorous acid) and at a pH below 8.0, with contact times of 1-2 minutes to sanitize whole fruits and vegetables and fresh-cut produce (Alzamora *et al*., 2000). Chlorination may not be desirable for all fresh-cut fruits. Washing or dipping after cutting may cause negative consequences, such flavor alteration or increased water activity (Lamikanra, 2002).

Chlorine dioxide (ClO_2) is widely used as a sanitizer. It has higher antimicrobial activity at neutral pH, is less affected by organic matter than other chlorinated sanitizers and is less corrosive (Olmez and Kretzschmar, 2009). It has been used at concentrations up to 200 ppm for washing whole fresh fruits and vegetables. However, the FDA currently does not permit the use of ClO_2 to sanitize fresh-cut fruit (Alzamora *et al*., 2000).

Sodium chlorite ($NaClO_2$) is an oxidizing agent, which is able to generate chlorine dioxide gas in an acidic environment. Lu *et al*. (2007) found that sodium chlorite inhibited the browning reaction in apple slices in addition to the control of microbial growth. Acidified sodium chlorite or ASC, the acidified form of sodium chlorite is a highly effective antimicrobial agent, which is produced by lowering the pH (2.5-3.2) of a solution of sodium chlorite with any GRAS (Generally Recognized as Safe) acid for example citric acid (Warf, 2001).

$$NaClO_2 + HA \longrightarrow HClO_2 \text{ (chlorous acid)} + NaA$$

The FDA has approved ASC use on raw fruits and vegetables in the range of 0.5-1.2 g/l followed by a potable water rinse. Allende *et al*. (2009) evaluated the efficacy of different sanitizing treatments on reducing *Escherichia coli* O157:H7 populations, aerobic mesophilic bacteria, yeast and molds on fresh-cut cilantro. ASC applied at a level of 1 g/l was the most effective treatment.

Hydrogen peroxide also has bactericidal activity through the formation of hydroxyl radicals. Ukubu *et al*. (2005) used hydrogen peroxide in combination with nisin, sodium lactate and citric acid to reduce the transfer of bacteria from whole melons surfaces to fresh-cut pieces, including *E. coli* 0157:H7 and *Listeria monocytogenes*.

Ozone has been used for the treatment of water and has been investigated for the decontamination of various types of foods. Kim *et al*. (2006) used cold ozonated water for washing fresh-cut lettuce inoculated with *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Staphylococcus aureus*. All pathogenic bacteria were decreased by 99% within 1 minute of treatment with 5 mg/l ozonated water and total bacterial populations were reduced by 3-4 log cfu/g, a result similar to that achieved with 100 ml/l chlorinated water.

Essential oils, gas phase antimicrobials

Plotto *et al*. (2006) studied the effect of an ethanol vapor treatment to extend the shelf-life of fresh-cut mango. A secondary objective of this work was the control of ripening. Ethanol vapor applied for 20 hours to whole mangoes prior to processing did not delay ripening but clearly reduced microbial contamination on the fruit surface resulting in lesser transfer to the flesh during processing.

Roller and Seedhar (2002) used carvacrol and cinnamic acid to inhibit microbial growth in fresh-cut kiwifruit stored in sealed jars at 4 °C. Carvacrol is a major component of the essential oil of oregano and thyme and cinnamic acid occurs in cinnamon, cloves, black pepper, coriander and turmeric. The treatment of fresh-cut kiwifruit with 5-15 mM carvacrol reduced total viable counts from 6 to 2 log cfu/g for 21 days, but undesirable color and changes to the odor may limit potential applications.

Wang and Buta (2003) used methyl jasmonate and other volatiles on fresh-cut kiwi slices placed in polystyrene trays at 10 °C. The volatile compounds were introduced inside the trays before covering with lids. Methyl jasmonate was effective in preserving the quality attributes of the slices for 3 weeks.

Vasantha *et al*. (2006) applied vanillin (hydroxy-3-methoxybenzaldehyde) solutions to fresh-cut apple slices inoculated with *Escherichia coli*, *Pseudomonas*

aeruginosa, *Enterobacter aerogenes* and *Salmonella enteric* and spoilage microorganisms including *Candida albicans*, *Lactobacillus casei*, *Penicillum expansum* and *Saccharomyces cerevisiae*. The concentrations of vanillin needed to inhibit the microorganisms varied between 6 and 18 mM, depending on species. Total aerobic microbial growth was inhibited by 37% in fresh-cut Empire apples and 66% in Crispin apples treated with a 12 mM vanillin solution.

Fisher and Phillips (2006) studied the effectiveness of lemon oils (*Citrus limon*), sweet orange (*Citrus sinensis*) and bergamot (*Citrus bergamia*) against a number of common foodborne pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157 and *Campylobacter jejuni*). All of the oils could inhibit the growth of common causes of food poisoning but bergamot was the most effective. However, this stands as a good example of a natural plant extract with excellent antimicrobial properties that would find limited applications in the preservation of fresh-cut fruits due to potential sensory impacts.

1-Methylcyclopropene

1-Methylcyclopropene (1-MCP) has been shown to block the action of ethylene, inhibiting ethylene responses such as ripening and softening. Therefore it could be useful for maintaining the quality of fresh-cut products (Lamikanra, 2002).

Linchun *et al*. (2007) reported that the application of 1-MCP treatments before cutting reduce the intensity of wound responses on kiwifruit stored at 2 °C for 10 days. Treatment with 1-MCP resulted in reduced respiration rate, ethylene production, lowered electrolyte leakage and delayed softening and color changes.

Calderon-Lopez *et al*. (2005) studied the effect of 1-MCP on whole fruit and fresh-cut apple slices prepared from five apple cultivars (Delicious, Empire, Idared, Law Rome and Mutsu). The treatment of apples at harvest with 1-MCP was shown to improve the shelf-life of fresh-cut slices.

2.4.2 Physical Treatments

Hot water treatment

Hot water treatment has been used in fruit and vegetables for several reasons including the control of fungal spores, insect infestation, ethylene synthesis, cell wall

degradation associated with hydrolytic enzymes, demethylation of pectin by pectin methylesterase and increased synthesis of heat shock proteins. Hot water treatment of whole cantaloupe melon at 50 ^{o}C prior to cutting reduced the rate of respiration and moisture loss during storage. The treatment also reduced total microbial count and prevented growth of lactic acid bacteria (Lamikanra *et al*., 2005a).

Packaging

Different types of film have been shown to provide benefits to fresh-cut products. Films with an adjustable "temperature switch" point have been developed in which the permeability changes rapidly once it is reached. Landec Company uses long-chain fatty alcohol based polymeric chains to achieve under/over temperature switch points influenced by the crystalline state of the acids, which result in changes to the gas barrier properties. The side chains melt to a gas permeable amorphous state at specified temperatures (Alzamora *et al*., 2000).

Films that are made from two dissimilar layers containing minute cuts have been developed in which the layers expand at different rates as the temperature rises or falls. The film at the cut edge retracts and curls upwards to enlarge holes thereby increasing permeability (Alzamora *et al*., 2000).

Films with incorporated antimicrobials, two-way humidity control to continually adjust the internal package relative humidity, carbon dioxide release, desirable aromas and time-temperature indicators have been considered for use with fresh-cut products, including for retail packages (IFPA, 2004).

Radiation

Early applications for irradiation on fruit and vegetables were aimed at insect and disease disinfestations or delay of ripening and sprouting. Recently, irradiation has been investigated for use in fresh-cut products although it might cause undesirable biochemical changes, such as enzymatic browning and flavor alteration (Lamikanra, 2002).

Irradiation may be used in combination with other preservation methods to improve the quality or safety of fresh-cut fruits. Fan *et al*. (2005) investigated the effect of low-dose ionizing radiation (gamma) and calcium ascorbate in fresh-cut

apple slices packed under modified atmosphere. The slices were treated with 7% calcium ascorbate and irradiated with 0.5 and 1.0 kGy followed by storage at 10 °C for up to 3 weeks. They conclude that the combination of calcium ascorbate and irradiation enhanced microbial safety while maintaining quality of fresh-cut apple slices. Despite the evident benefits of irradiation for food preservation, consumer acceptance and regulatory approval continue to hinder commercial applications.

2.4.3 Biopreservation

Biopreservation has been investigated as a means to control the growth of spoilage and pathogenic bacteria in fresh-cut fruit with or without modified-atmosphere packaging. Most of the work in this area has involved lactic acid bacteria (LAB). LAB can inhibit or eliminate the growth of many different microorganisms, including bacteria, yeast and fungi, through the production of organic acids, diacetyl, hydrogen peroxide, enzymes, defective phages, lytic agents and antimicrobial peptides or bacteriocins (Alzamora *et al*., 2000).

2.4.4 Others

Edible coatings

The application of a thin layer of protective material to the surface of the fruit with the objective of replacing the natural protective tissue could provide some benefits for shelf-life. Edible coating could reduce respiration, retard water loss, improve texture and retain volatile flavor compounds as well as reduce microbial growth. Coating formulations may include polysaccharides, proteins, resins, waxes or oils (Lamikanra, 2002). Another advantage of edible coatings in fresh-cut products is to serve as carriers of ingredients that perform a specific function, such as antimicrobials or antioxidants. An edible coating should also create a barrier that can retard the loss of desirable flavor volatiles and water vapor and restrict the exchange of carbon dioxide and oxygen (Baldwin *et al*., 1995).

Combined methods

A combination of treatment may be necessary when no single approach can provide the desired shelf-life. Gonzalez-Aguilar *et al*. (2000) applied chemical agents

and modified atmosphere packaging (MAP) in the processing of fresh-cut mangoes which resulted in a reduction of browning and deterioration at 10 °C. Solutions containing 4-hexylresorcinol (0.001 M) plus potassium sorbate (0.05 M), 4-hexylresorcinol, potassium sorbate and D-isoascorbic acid (0.5 M) reduced changes in color and microbial growth but did not affect the sensory properties of fresh-cut mangoes.

Mohammed and Wickham (2005) dipped pineapple slices in solutions containing 300 ppm ascorbic acid, 200 ppm 4-hexylresorcinol, 300 ppm ascorbic acid and 200 ppm 4-hexylresorcinol. Separate treatments with ascorbic acid or 4-hexylresorcinol in conjunction with MAP controlled browning and maintained quality for 2 days at 10 °C, but the combined treatment was more effective in browning inhibition and microbial spoilage was controlled over a longer storage period.

Gorny *et al*. (2002) found that a treatment with 2% ascorbic acid, 1% calcium lactate and 0.5% cysteine adjusted to pH 7.0 could inhibit loss of firmness and prevent browning in "Bartlett" pear slices, in combination with low O_2 and elevated CO_2 atmospheres, without producing objectionable off-flavors.

2.5 Mangosteen

2.5.1 General Information

Mangosteen (*Garcinia mangostana* L.) belongs to the family Guttiferae. The fruit is 4-7 cm across and is covered by a smooth and hard pericarp about 6-10 mm thick. The edible portion of the fruit (aril) is small in comparison with other tropical fruits due to the thickness of the pericarp. The edible aril, which makes up 20-30% of total fruit weight is a pearly white color, slightly translucent and consists of 4 or 8 segments with 1 or 2 large segments containing apomictic seeds. Mangosteen has a high percentage of sugars but a rather low mineral and vitamin content (Salunkhe and Kadam, 1995). The flesh is sweet, slightly acid and mild to distinctly acid in flavor and is acclaimed as exquisitely luscious and delicious (Morton, 1987).

2.5.2 Harvesting and Utilization

The harvesting index for mangosteen fruit is based on the extent and intensity of purple pericarp development. The timing of harvest depends on whether the fruit is

to be exported or supplied to local markets, exported fruit being picked at an earlier stage (Nakasone and Paull, 1998). Palapol *et al*. (2009) divided the harvest index of mangosteen into 7 stages as follows: stage 0 (yellowish white or yellowish white with light green), stage 1 (light greenish yellow with 5-50% scattered pink spots), stage 2 (light greenish yellow with 51-100% scattered pink spots), stage 3 (spots not as distinct as in stage 2 or reddish pink), stage 4 (red to reddish purple), stage 5 (dark purple) and stage 6 (purple black). Fruit is normally not harvested before the pericarp turns to a light greenish yellow, with distinct irregular pink spots (stage 2). Fruit with less color development have excessive latex exudation at the peduncle and have inferior flavor when ripened (Nakasone and Paull, 1998). The best flavor is achieved when the fruit is fresh and fully ripe (Macleon and Peieris, 1982).

Mangosteen is a highly regarded fresh fruit in Southeast Asia. It is mainly eaten fresh and in a partially frozen state, although some processed products are also available. The delicate flavor of the aril is believed to derive from hexyl acetate and cis-hex-3-enyl-acetate (Teylor *et al*., 1993). The degree to which flavor is retained depends on the nature of the process applied. Ripe fruit can be canned (Khantee, 1997), dried (Sophanodora *et al*., 2002) and frozen (Sripongpunkul, 1990; Sutthirak, 1996), but unripe fruit can be processed into a ready-to-eat format (Kitpipit, 2005; Manurakchinakorn *et al*., 2005; Ngarmsak, 2007). Some of these processes generate considerable byproducts for which some uses exist, although they are limited. For example, the pericarp is used to tan leather and to dye fabric black and the rind is an ingredient in traditional medicines.

2.5.3 Changes in Mangosteen During Storage

Respiration rate and ethylene production

Respiration is the process by which plants take up oxygen. The oxygen breaks down carbohydrates (stored starch or sugar) into carbon dioxide, water and energy (in the form of heat). The process is continuous and stops when reserves are exhausted. Ageing follows and the plant eventually dies and decays (FAO, 1989). Some work has been done on the respiration of ripe mangosteen fruit. Respiration increased 10-30 ml CO_2/(kg h) and ethylene production from 2-15 µl C_2H_4/(kg h) at 25 $^\circ$C (Ketsa and Koolplookee, 1993). It should be stressed here that the influence of variety

postharvest handling or stage of ripening on these parameters has not been established to date.

Mechanical and chilling injury

Careless handling of fresh produce causes internal bruising, which results in abnormal physiological damage or splitting and skin breaks, thus rapidly increasing water loss and the rate of normal physical breakdown. Skin breaks also provide sites for infection by spoilage microorganisms (FAO, 1989). Mangosteen fruit is very susceptible to mechanical injury. A drop of 10 cm can induce slight pericarp damage, indicated as hardening at the point of impact. Higher drops cause significantly greater damage, leading to downgrading of the fruit (Bunsiri *et al*., 2003)

Temperature plays a key role in the metabolism of fruits. Decreasing temperature lowers metabolism, thus prolonging shelf-life. Unfortunately, some tissues are sensitive to low temperature. Most tropical commodities are sensitive to chilling. The chilling injury physiological disorder induced by low temperature is a serious problem in postharvest handling of tropical commodities (Taiz and Zeiger, 1991). The first symptoms of chilling injury in mangosteen are the hardening and browning of the cortex. This occurs after about 6 weeks at 4 and 8 ^{o}C (Wang, 2000). Flesh segments were still acceptable, though with longer storage the segments become soft, browning is initiated and there is loss in original gloss. After 6 weeks at 4 and 8 ^{o}C, the segments develop a slight smell and flavor described as fermentative (Wang, 1982).

Quality changes

Mangosteen can be held 20 to 25 days in dry, warm and enclosed storage facilities. The outer skin toughens and the rind becomes rubbery after longer periods in storage. The rind eventually hardens and becomes difficult to open and the flesh turns dry.

Thomson (1996) reported that the shelf-life of whole mangosteen fruit, which was about 1 week at room temperature, could be extended up to 3 to 4 weeks at 4-12 ^{o}C and could be extended for up 5-7 weeks at 1.7-5 ^{o}C. Salunkhe and Kadam (1995)

found that the optimum storage condition for whole mangosteen fruit is between 4-6 ^{o}C with 85-90% RH to obtain a maximum shelf-life of 49 days.

During fruit development, percent total soluble solid (TSS) increase gradually from the early stage of fruit development to maturity. At 12 weeks old, the fruit has 16.14% TSS. Acidity tended to decrease during ripening and 12 week old fruit could contain 10.3 mg of acid equivalent per 100 ml of juice. In addition, TSS and acidity can vary throughout storage, although the range of differences tends to be small (Wang, 1982).

2.5.4 Medicinal Properties of Mangosteen

Antioxidant properties

The antioxidant activity of extracts and xanthones isolated from mangosteen has been shown using the following methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Yoshikawa *et al.*, 1994; Leong and Shui, 2002; Weecharangsan *et al.*, 2006; Chomnawang *et al.*, 2007; Haruenkit *et al.*, 2007), the ferric thiocyanate method (Yoshikawa *et al.*, 1994; Fan and Su, 1997) and the 2,20-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay (Leong and Shui, 2002; Haruenkit *et al.*, 2007). The data from the studies noted above confirm that mangosteen extracts have antioxidant activity that is primarily derived from xanthones (Pedraza-Chaverri *et al.*, 2008).

Antitumoral properties

Several studies have been designed to examine the anticancer activities of xanthones isolated from mangosteen-fruit pericarp. Hepatocellular carcinoma (Ho *et al.*, 2002), SKBR3 human breast cancer (Moongkarndi *et al.*, 2004) and human leukemia (Matsumoto *et al.*, 2003) cell lines have been used in this work. The results suggest that α-mangostin and its analogs were candidates for preventive and therapeutic applications for the control of cancer (Pedraza-Chaverri *et al.*, 2008).

Anti-inflammatory and antiallergy properties

There is some evidence that mangosteen has antiallergenic and anti-inflammatory properties. Supporting studies were carried out with RBL-2H3 cells

(Nakatani *et al*., 2002), C6 rat glioma cells (Nakatani *et al*., 2002, 2004; Yamakuni *et al*., 2006), rabbit thoracic aorta and guinea-pig trachea (Chairungsrilerd *et al*., 1996) and several *in vivo* models in rats (Nakatani *et al*., 2004). Data from this research indicates that xanthones isolated from mangosteen could be a novel target of anti-inflammatory and antiallergenic compounds (Pedraza-Chaverri *et al*., 2008).

Antibacterial, antifungal and antiviral properties

Several studies have demonstrated that mangosteen tissues contain antibacterial, antifungal and antiviral agents. Iinuma *et al*. (1996) studied the inhibitory effect of several xanthones isolated from mangosteen-fruit pericarp, against the growth of methicillin-resistant *S. aureus* (MRSA). α-mangostin exhibited high efficacy, with MIC values of 1.57-12.5 µg/ml. Suksamrarn *et al*. (2003) studied the antituberculosis potential of prenylated xanthones obtained from mangosteen-fruit pericarp. Among them α- and β-mangostins and garcinone B exhibited the most potent inhibitory effect against *Mycobacterium tuberculosis*, with an MIC of 6.25 µg/ml; whereas demethylcalabaxanthone and trapezifolixanthone had an MIC value of 12.5 µg/ml and γ-mangostin, garcinone D, mangostanin, mangostenone A and tovophyllin B had an MIC value of 25 µg/ml.

Chomnawang *et al*. (2005) evaluated the antibacterial activity of 19 medicinal plants from Thailand against *Staphylococcus epidermidis* and *Propionibacterium acnes*, pus-forming bacteria that could trigger an inflammation resulting in acne. Only 13 Thai medicinal plants were able to inhibit the growth of both bacteria. Among these, mangosteen exhibited the most potent inhibitory effect, with an MIC of 0.039 µg/ml against both of bacteria.

Gopalakrishnan *et al*. (1997) demonstrated the antifungal activity of several xanthones isolated from mangosteen-fruit pericarp and some α-mangostin-derivatives against three phytopathogenic fungi (*Fusarium oxysporum vasinfectum*, *Alternaria tenuis* and *Dreschlera oryzae*). α-mangostin, γ-mangostin, gartanin, garcinone D, BR-xanthone and euxanthone showed high inhibitory activity against all three species.

Chen *et al*. (1996) showed that an ethanolic extract of mangosteen effectively inhibited HIV-1 protease. Two xanthones were isolated from the ethanolic extract: α- and γ-mangostins, which exhibited an inhibitory concentration at 50% (IC_{50}) of 5.12 $\pm$

0.41 and 4.81 $\pm$ 0.32 µM, respectively. Pepstatin A (IC$_{50}$ = 76 $\pm$ 5.5 nM) was used as positive control.

In addition, Chanarat *et al*. (1997) found that polysaccharides obtained from mangosteen-fruit pericarp can stimulate the activity of polymorphonuclear phagocytic cells against *Salmonella enteritidis*. Hence the pericarp may contains compounds that can boost the immune response.

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CHAPTER 3

QUALITY CHANGES IN PACKAGED FRESH-CUT MANGOSTEEN

3.1 Abstract

Fresh-cut mangosteen was prepared, packed in PP trays sealed with OPP/LLDPE film and stored at 5 °C and 85% RH. The initial respiration rate of fresh-cut mangosteen was 10.7 ml CO_2/(kg h) and declined rapidly within 7 h, then gradually decreased and relatively stable until the end of storage. C_2H_4 and CO_2 production were high at the beginning and then gradually decreased. Atmospheric composition and ethylene inside package did not reach equilibrium because the transmission rate of the film may not fit to seal the trays. Flesh firmness decreased from 11.10 to 8.36 g/mm^2 as the storage time increased and correlated with the increasing in weight loss. The change of browning index (BI) was quite low, showing that low temperature storage plays an important role in the control of browning, due to inhibition of PPO enzyme activity. At the end of storage, the fresh-cut mangosteen became darker with lower L* and higher a* values. Acetaldehyde and ethanol contents increased slightly after cutting and then rose sharply, reaching 11.40 and 1,426.43 µl/kg fruit, respectively, at the end of storage. None of the microbial populations (aerobes, yeast, mold and *E. coli*) increased during storage and levels were deemed acceptable. Scores for sensory attributes including browning, off-odor, texture and overall visual quality increased during storage, thus the acceptance of fresh-cut mangosteen gradually decreased over time.

3.2 Introduction

Fresh-cut produce is a rapidly growing segment in the food service and retail markets. This new marketing trend is intended to meet consumer demand for both convenience and produce with "fresh-like" quality (Rocha and Morais, 2003). The development of new treatments to minimize the impact of processing operations and preserve the organoleptic characteristics and improvements in the distribution of refrigerated products are crucial to satisfying demand for these products. Unfortunately, there remains a lack of fundamental knowledge about the influence of

processing on the quality of many fresh-cut fruit commodities. Peeling, coring and slicing operation are critical because they may influence the shelf-life due to physiological stresses caused by physical damage or wounding. The respiration of fresh-cut tissues is greater than that of the intact fruit and ethylene production is known to be stimulated within a few minutes of processing. These responses increase biochemical reactions related to changes in color, flavor, texture and nutritional quality.

Cell disruption leads to the release and intermixing of enzymes and substrates that are responsible for these undesirable processes. Some of the released compounds may be used by native or exogenous microorganisms to grow on the product surface, thus increasing the perishability and reducing the microbiological shelf-life of the fresh-cut cut products. Furthermore, microorganism-induced browning or early changes in tissue appearance caused by pectinolytic breakdown can place further limits on the distribution and marketability of the product.

There is a general lack of information about quality changes in stored fresh-cut tropical fruits in general and about mangosteen in particular. The aims of this work were to measure some of the physical, chemical and microbiological changes that occur during storage of fresh-cut mangosteen packed in PP trays sealed with a sealing film. The knowledge derived from this work is meant to improve the preservation of fresh-cut mangosteen.

3.3 Materials and methods

Plant material

A southern cultivar of mangosteen fruit (*Garcinia mangostana*, Linn.) was grown in Chumphon province, Thailand by a commercial grower. Fruit with a mean weight of 120 ± 5 g were hand-harvested during May-June 2009 at stage 2 (light greenish yellow with 51-100% scattered pink spots) according to the scales described by Palapol *et al*. (2009). The crop was transported to the laboratory within 4 hours by truck without temperature control. The fruits were selected on the basis of firmness and surface color without damaged or diseased fruit. The firmness was set at about 4 Kg force with 1 cm diameter probe using fruit hardness tester (GY-1, Aliyiqi Instrument, China) and surface color of lightness (L*) 45-55, redness (a*) 10-20 and

yellowness (b*) 15-25 using Miniscan Hunter Colorimeter (Hunter Associates Laboratory, Inc., USA).

The fruit was processed into fresh-cut format in an isolated and cleaned minimal-processing room at 30 °C. The stalks and calyx ends were removed with a knife and the fruit were washed by hand under tap water. An incision was made around the periphery to remove the peel, the entire white arils were removed and immediately placed in cold water (10 °C). After 5 min, the arils were dried with a handheld blower and approximately 150 g of fresh-cut was packed in polypropylene (PP) trays (11.5×17.5×4.5 cm) which were sealed with OPP/LLDPE film with an OTR of 1,160 cm^3/(m^2 day). The samples were stored at 5 °C with 85% RH. Three replicates of two trays each were analyzed at the beginning of the experiment and after 3, 6, 9, 12 and 15 days of storage.

Respiration and ethylene production rate assessments

Fresh-cut mangosteen were weighed and placed in an open 0.75-L glass jar (three replicates) in a refrigerator at 5 °C. Every 1 h for the first 3 h and every 2 h later, a jar lid fitted with rubber septum was placed on the jar for 15 min. One ml of headspace gas was removed with a syringe for measurement of carbon dioxide (CO_2) and ethylene (C_2H_4) concentrations (Agar *et al*., 1999). The same glass jars were left open to air flow after sampling. The gas sample was injected into a gas chromatograph (GC) (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a thermal conductivity detector (TCD) for CO_2 under the oven and detector temperatures were 60 and 150 ^{o}C, respectively, with helium as the carrier gas. Ethylene (C_2H_4) production was measured with the same GC equipped with a flame ionization detector (FID). The oven, injector and detector temperatures were 40, 120 and 180 ^{o}C, respectively, with helium as the carrier gas. Measurements were repeated until CO_2 or C_2H_4 levels were stable. Respiration and ethylene production rate were expressed as ml CO_2/(kg h) and nl C_2H_4/(kg h), respectively.

In-package atmospheric gas analyses

Changes in the concentrations of O_2, CO_2 and C_2H_4 in the trays were measured at 3-day intervals by withdrawing air samples (1 ml) through a septum using a gas-

tight syringe. The concentrations were analyzed using a same gas chromatograph (GC) and condition as above. In-package gasses accumulations were expressed as $\%O_2$, CO_2 and ppm C_2H_4, respectively.

Physical analyses

Firmness was evaluated using a TA-XT2i texture analyzer (Stable Micro Systems, England) with a 25 kg load cell, equipped with a 2 mm diameter cylinder stainless steel probe (P/2). The measurements were taken as the maximum force value obtained during the penetration of probe into the fruit for 3 mm at 2.0 mm/s (Troncoso *et al*., 2009) and expressed as g/mm^2 force.

Weight loss was determined by weighing all samples using Metler balance, model AB204 (Mettler-Toledo Inc., USA) at initial storage and after storage. The difference between the values was used to calculate weight loss.

Browning index was estimated by extracting 5 g sample in 100 ml ethanol (67%) for 1 h and then filtered through Whatman No. 1 filter paper. The browning index in terms of absorbance at 420 nm (Spectrophotometer: UNICAM, Type Helios Alpha, England) was measured for the filtrate using 67% ethanol as blank (Supapvanich *et al*., 2011).

Fresh-cut color was measured using Miniscan Hunter Colorimeter (Hunter Associates Laboratory, Inc., USA) calibrated with a standard white tile with the following parameters: X = 79.6, Y = 84.4, Z = 89.9 with illuminant D65/10°, (light source used for the daylight), according to CIE L* (lightness), a* (green to red) and b* (blue to yellow) values. Numerical values of a* and b* were converted into chroma $[C = (a*^2+b*^2)^{1/2}]$ and hue angle ($H^o = tan^{-1} b*/a*$) (Francis, 1980).

Chemical analyses

Acetaldehyde and ethanol content determinations were based on the method described by Gonzalez-Aguilar *et al*. (2004). Five grams of tissue were weighed in 60 ml - amber colored bottles and placed in a 65 ^{o}C water-bath for 15 min. One ml of headspace gas was injected into a gas chromatograph (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a 60 m × 0.325 mm × 0.25 µm DB-WAX column (J&W Scientific, Folsom, California). The oven, injector and detector

temperatures were 60, 250 and 250 ^{o}C, respectively, with helium as the carrier gas. Retention times and standard curves of acetaldehyde and ethanol in water solutions were used for peak identification and quantification.

Microbiological analyses

Microbial determinations were carried out using standard methods (BAM, 2001). Twenty-five grams of samples were diluted in 225 ml of sterile buffered Butterfield's Phosphate (BPS) and homogenized for 2 min at normal speed in a Stomacher (Model 400 Circulator, Seward, Norfolk, England). Serial dilutions of the suspension were prepared in BPS and analyzed for total viable count (TVC), yeasts and molds and *E. coli* . Another 25 g of sample were diluted in 225 ml of buffered peptone water for the detection of *Salmonella* sp. TVC and yeast and mold populations were reported as cfu g^{-1} (colony forming units per gram of sample) whereas *E. coli* and *Salmonella* sp. were reported as the most probable number (MPN) and detected or not, respectively.

Sensory evaluation

Twenty semi-trained panelists were asked to rank for browning, off-odor, firmness and overall visual quality (OVQ) of treated fresh-cut mangosteen against reference samples using methods described by Gomez-Lopez *et al*. (2008) with some modifications. The 5-point ratings were assigned as follows: (5) not original, (4) slightly original, (3) moderately original, (2) very original and (1) extremely original. The reference samples were prepared from untreated fresh-cut fruit immediately after cutting as a good sample and from an untreated fresh-cut after 1 day at room temperature as a bad sample. Each treatment consisted of six fresh-cut fruit served on a white plastic plate within 2-3 h of holding time at room temperature (25°C) and the same samples were evaluated by each panelist within 1 h.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and mean differences estimated by Duncan's new multiple range test (DMRT) using SPSS

Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant.

3.4 Results and discussion

Respiration rate and ethylene production

Fresh-cut mangosteen showed a high initial respiration rate at 5 ^{o}C, 85% RH (10.7 ml CO_2/(kg h)), thereafter, it gradually decreased and remained relatively stable at 1 ml CO_2/(kg h) until the end of storage, 36 h (Figure 2). The high initial respiration rate is most likely due to the wound response induced by cutting. This is in agreement with findings with melon (Aguayo *et al*., 2004, 2007). A study on the respiration rate of intact and fresh-cut apple slices revealed comparatively higher CO_2 production rates in the fresh-cut slices immediately after cutting than in intact fruit, and the differences was maintained throughout the storage period (Mao *et al*., 2007). The effect was ascribed to the onset of wound responses in the cut tissues. A similar response has been reported for fresh-cut muskmelon (Luna-Guzman *et al*., 1999), honeydew (Saftner *et al*., 2003), apple slices (Calderon-Lopez *et al*., 2005) and watermelon (Saftner *et al*., 2007).

In this study, wound response-induced effects on the respiration rate of fresh-cut mangosteen were also detected immediately, as indicated by the increase in CO_2 production. The value of respiration rate in this study using stage 2 mangosteen stored at 5 ^{o}C was relatively low as compared to that report on fresh-cut stage 2 mangosteen stored at 10 ^{o}C (38.20×10^3 ml CO_2/(kg h)) (Kitpipit *et al*.,2005), probably due to various factors i.e. measurement system, ripening stage, storage temperature and degree of tissue wounding. Paull and Chen (1997) also showed that differences in the respiration rates of cut papaya depend on the ripening stage.

C_2H_4 production was relatively high at the beginning of the experiment and then decreased, much like CO_2 production. Synthesis was clearly higher than in intact mangosteen fruit, which was reported by Buntong (2004) as 0.26-0.51 ul/(kg h) at 13°C. This effect could be due to physiological responses to wounding incurred during cutting.
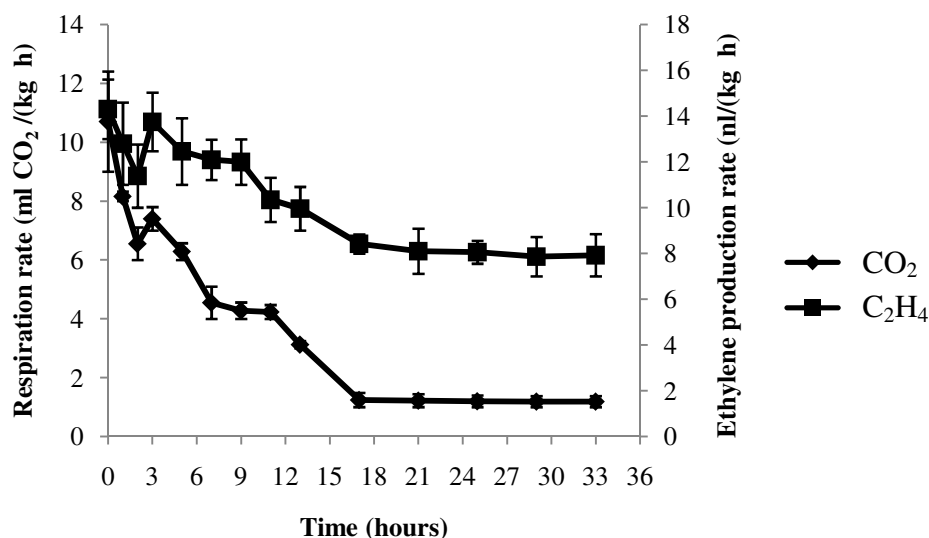
Figure 2. Respiration and ethylene production rate in fresh-cut mangosteen during storage.

It was clearly shown that processing of mangosteen into a fresh-cut product enhances both respiration rate and ethylene production. This is in agreement with the findings in other fresh-cut commodities such as 'Amarillo' melon (Aguayo *et al*., 2008) and fresh-cut apple (Mao *et al*., 2007). The higher respiration rate and ethylene production during storage indicated that peeling and storage at $5^{o}C$ induced important physiological responses in mangosteen tissues. It should be noted that removal of the peel is known to favor gas diffusion into interior tissues more rapidly and may lead to increased metabolic activity (Zagory, 1995).

In-package atmospheric changes

The change in gas composition inside the trays is shown in Figure 3. Concentrations of O_2 decreased to 15.33% by the third day in storage but subsequently increased until the trial was ended. A corresponding increase in CO_2 concentration to 5.25% after three days was followed by a progressive decline to day 15th. This result suggests that the respiration rate of fresh-cut mangosteen was highest over the first three days in storage and declined thereafter. The initial storage period of fresh-cut produce is characterized by intensive respiration due to the rapid utilization of substrates (primarily sugars, starches) released during peeling or cutting

(Kim *et al*, 1993a, Rocha *et al*., 1995). In this respect the behavior of fresh-cut mangosteen resembles that reported for fresh-cut tomato by Gil *et al*. (2002).
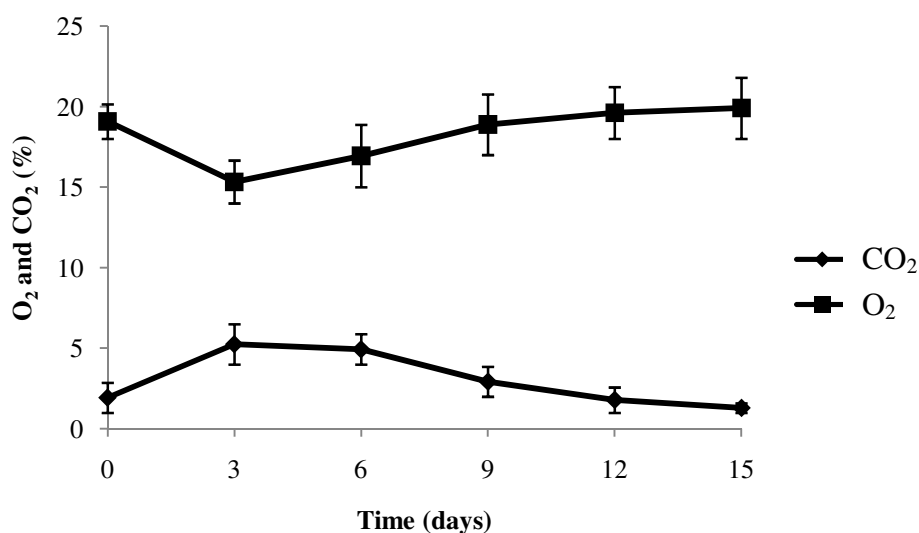
Figure 3. Changes in O_2 and CO_2 contents in the headspace of fresh-cut mangosteen during storage.

CO$_2$ and O$_2$ concentrations in the package of fresh-cut mangosteen at the end of the storage period were similar to those measured at the outset. This result suggests that the permeability of the film used to overlay the trays allowed the exchange of gases between the package and the atmosphere. This was evidently a poor permeability film that would limit gas exchange and may quickly lead to the deterioration of fresh-cut.

Ethylene accumulation started on the first day of storage (Figure 4). Concentrations increased rapidly to a maximum value of 6.99 ppm by the third day of storage, followed by a gradual decline to non-measurable levels by the ninth day. This behavior is again similar to that of results reported for fresh-cut tomato by Gil *et al*. (2002) who noted an immediate accumulation of ethylene attributable to the wound response, which was followed by a gradual decline after 7 days.
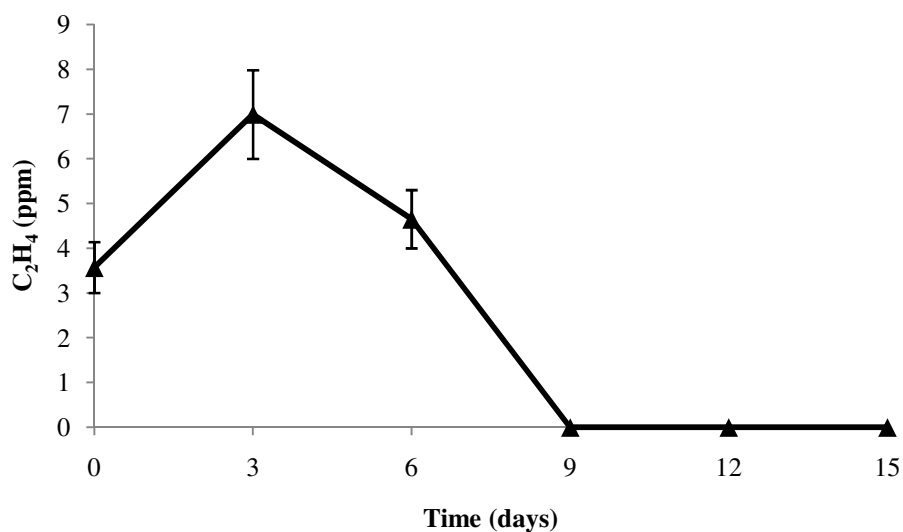
Figure 4. Changes in ethylene content in the headspace of fresh-cut
mangosteen during storage.

Ethylene production in stored plant tissues is known to be influenced by the composition of the atmosphere. For example, Solomos (1997) demonstrated that low CO_2 concentrations combined with elevated amounts of O_2 might act synergistically to inhibit ethylene production. Similar events likely influenced the production of ethylene in fresh-cut mangosteen.

Physical changes

Firmness and weight loss

The firmness and weight loss of fresh-cut mangosteen is shown in Figure 5. Firmness decreased rapidly over the first three days of storage, from 20.82 to 11.10 g/mm^2 (approximately 50% decline from the first day, followed by a more gradual reduction until the end of the storage period. Similar losses in texture have been reported in stored fresh-cut kiwifruit where measurements revealed a 32% reduction over the first three days. Rocha and Morais (2003) also noted a 50% reduction in the firmness of apple slices after 7 days of storage. Several factors may influence changes in the texture of fresh-cut produce, which is a fundamental problem for the extension of shelf-life and a hindrance to wider distribution. The action of endogeneous enzymes on the cell wall are believed to play a critical role as does

degradation due to the growth of microorganisms (Huxsoll and bolin, 1989; Rolle and Chism, 1987). In the present work the decline in texture correlated with the increase in weight loss (see the next section). Dehydration and/or the release of juice from cell sap can contribute to the softening that is observed in many minimally processed products (Karaibrahimoglu *et al*., 2004; Tian *et al*., 2004; Perera and Baldwin 2003).
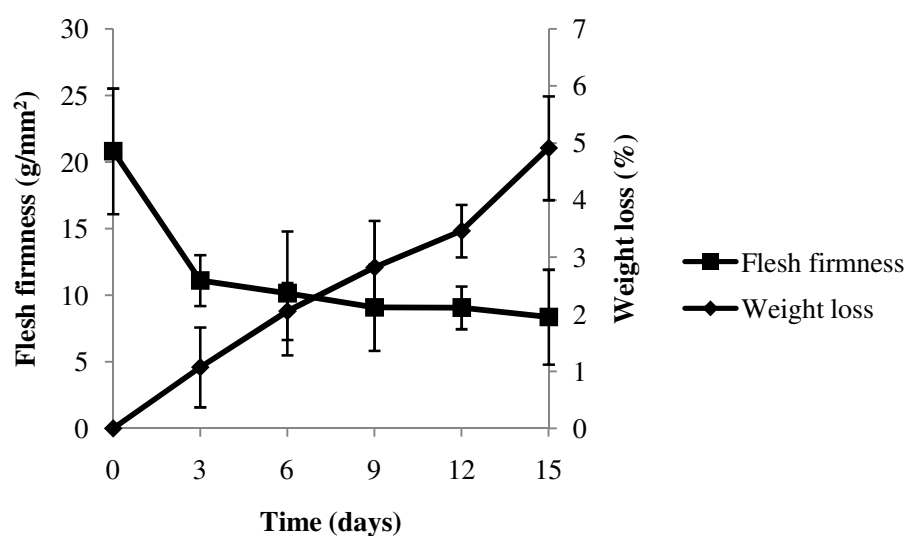
Figure 5. Flesh firmness and weight loss of fresh-cut mangosteen during storage.

Weight losses increased throughout the storage of fresh-cut mangosteen, ranging from 0% at the first sampling to 4.91% at the end of storage. There was no evidence of equilibrium during the storage period (Figure 5). Overall, weight losses in fresh-cut mangosteen were quite low and comparable with results achieved by Kim *et al*. (1993a) with several fresh-cut apple cultivars stored at 2 ^{o}C for 12 days. It is apparent that the atmospheres in the tray were saturated or nearly saturated with regard to water vapor, thus minimizing water losses (Rocha and Morais, 2003).

Browning index

Enzymatic browning assessed in term of a browning index (BI) as A_{420} values are given in Table 2. The change in BI values over the storage period was quite low. Browning after cutting was probably due to enzymatic browning reactions stimulated

by tissue damage with consequent enhanced contact between enzymes and substrates. It is possible that the low temperature (5°C) storage condition played an important role in restricting browning by reducing PPO activity.

Table 2. Browning index (A_{420}) of fresh-cut mangosteen during storage.

Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
0.0364$\pm$ 0.0011a*	0.0424$\pm$ 0.0015c	0.0419$\pm$ 0.0009c	0.0416$\pm$ 0.0009b	0.0424$\pm$ 0.0011c	0.0422$\pm$ 0.0009c

Note: * mean$\pm$SD from 3 replicates followed by the same letter within row are not significantly different ($p>0.05$).

Changes in color

Table 3 shows changes in the color of fresh-cut mangosteen stored in PP trays at 5 °C for 15 days.

Table 3. Color changes in fresh-cut mangosteen during storage.

Flesh color	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
L*	85.07$\pm$2.24d*	83.83$\pm$3.45c	81.68$\pm$3.57bc	80.81$\pm$4.59b	78.53$\pm$3.26a	75.26$\pm$5.05b
a*	0.24$\pm$0.77a	1.13$\pm$0.87b	1.29$\pm$1.23b	2.16$\pm$1.27c	2.60$\pm$1.36cd	2.68$\pm$1.45d
b*	14.51$\pm$2.23d	13.59$\pm$2.44c	13.06$\pm$2.13c	11.96$\pm$1.79 ab	11.16$\pm$2.77a	12.26$\pm$2.22b
C	14.53$\pm$2.24d	13.66$\pm$2.50c	13.17$\pm$2.21c	11.51$\pm$2.90a	12.20$\pm$1.90ab	12.60$\pm$2.40b
H$^{\circ}$	0.23$\pm$1.53a	1.41$\pm$0.49d	1.14$\pm$0.96b	1.35$\pm$0.09cd	1.31$\pm$0.51c	1.36$\pm$0.09c

Note: * mean$\pm$SD from 3 replicates followed by the same letter within row are not significantly different ($p>0.05$).

At the end of storage, the surfaces were darker (lower L*values and higher a* values), which could be attributed to the consumption of substrates by poly-phenoloxidase. A decrease in L* value and an increase in a* value are indicative of browning in some commodities (Petriella *et al*., 1985; Mastrocola and Lerici, 1991; Monsalve-Gonzalez *et al*., 1993). However Rocha and Morais (2001) reported that a change in the H$^{\circ}$ value may be a better indicator of the absence of oxidative browning

of flesh pulp in some fruit. In the present work the H^o value increased from day 0 to day 3rd, suggesting that most oxidative browning occurred over this time period. However the L* value continued to decrease throughout the storage period, which hints at other factors that influenced the color of the fruit. Increasing pigment concentration (Goupy *et al*., 1995) or slight browning attributed to loss of water (Rattanapanone *et al*., 2001) could be responsible for the effect. Gonzalez *et al*. (1993) reported that the lightness (L* value) of 'Red Delicious' apple rings decreased sharply during 48 h at 1 ^{o}C. Kim *et al*. (1993b) reported a similar result with color changes of 12 apple cultivars; all cultivars showed a rapid decrease in L* values and they assumed that the changes were due to enzymatic browning caused by tissue damage with consequent enhanced contact between enzymes and substrates.

Chroma (C), a measure of the intensity or purity of the hue, of fresh-cut mangosteen showed slightly decrease after three days of storage but remained relatively stable thereafter.

Chemical changes

Acetaldehyde and ethanol content in fresh-cut mangosteen increased slowly during 12 days storage at 5 ^{o}C (Figure 6) but increased dramatically at the end of the storage period. Acetaldehyde and ethanol contents are commonly used as indicators of anaerobic respiration. Concentrations of both compounds generally increase as O_2 concentrations declined in packaged fresh-cut produce. This relationship was not evident in the present work. Headspace O_2 concentrations remained high and it is unlikely that anaerobic respiration would be initiated under these conditions. However both acetaldehyde and ethanol contents are normally synthesized during fruit maturation (Paul, 1997). It is therefore plausible that the accumulation of acetaldehyde and ethanol contents in the tray were primarily associated with ripening processes in the mangosteen fruit.
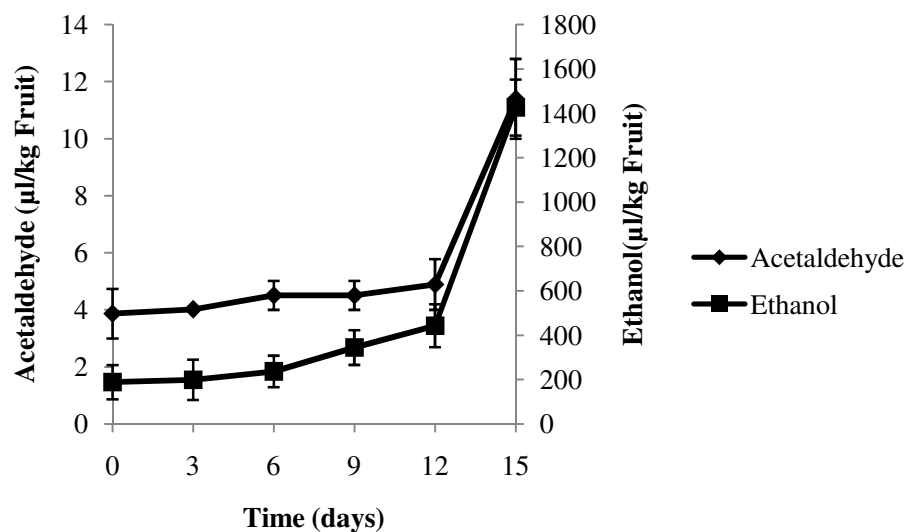
Figure 6. Acetaldehyde and ethanol contents in fresh-cut mangosteen during storage.

Microbiological changes

The results of microbiological analyses conducted on stored fresh-cut mangosteen are shown in Table 4.

Table 4. Microbial populations of fresh-cut mangosteen during storage.

Microorganisms	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
-TVC (CFU/g)	<10	10	10	10	20	20
-Yeast and mold (CFU/g)	<10	<10	<10	<10	<10	<10
-*E. coli* (MPN/g)	<3	<3	<3	<3	<3	<3
-*Salmonella* sp.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Note: N.D. (Not detected at dilution 10^{-1})

Populations of all of microorganism (total, yeast and mold, *E. coli*) changed little during storage and *Salmonella* was not detected. Cut or damaged surfaces provide availability of nutrients conditions therefore increase the numbers of microbes that can develop on most fresh-cut produce. Fresh-cut mangosteen is somewhat

unique among these products in that the membrane surrounding the pulp remains intact thereby limiting the release of potential nutrients. Moreover intrinsic factors such as pH affected on microbial growth (King and Bolin, 1989; Ahvenainen 1996), obviously acidic fruit such as mangosteen (pH 2-3) is not favorable food for general spoilage microorganisms. Besides extrinsic factors particularly the storage temperature was low and the CO_2 concentration was elevated, these factors would further limit microbial growth (Soliva-Fortuny and Martin-Belloso, 2003). Consequently it appears that fresh-cut mangosteen is relatively stable due to the interaction of several factors which restrict microbial growth.

Changes in sensory quality

The sensory scores for browning, off-odor, texture and overall visual quality (OVQ) of fresh-cut mangosteen during storage at 5°C increased with storage time (Table 5), which indicated the decrease in acceptability.

Table 5. Sensory scores of fresh-cut mangosteen during storage.

Sensory qualities	Day 0	Day 3	Day 7	Day 10	Day 15
-Browning	1.90+0.77a*	2.37+0.68b	3.21+0.79c	3.90+0.79d	4.58+0.61e
-Off-Odor	1.05+0.22 a	1.68+0.82 b	2.95+1.18 c	3.80+1.15d	4.53+0.70e
-Firmness	1.00+0.00 a	3.84+0.76 b	4.26+1.10 c	4.40+0.79cd	4.73+0.76d
-Overall visual quality	1.25+0.60 a	3.19+0.76 b	3.57+1.12 c	3.98+1.50cd	4.09+1.38d

Note: * mean+SD from 20 panelists followed by the same letter within row are not significantly different ($p>0.05$).

Sensory scores for browning increased sharply immediately after cutting and progressively afterward. This finding correlated well with the browning index discussed previously. The panelists found that the odor declined with prolonged storage. Initially, fresh-cut mangosteen has a fresh fruit aroma. Owing to changes during storage, scores increased from 1.05-4.53 due to the development of off-odors. This could be related to the increasing accumulation of acetaldehyde and ethanol documented in the previous section.

Similarly, the increase in scores for firmness was in accordance with decreasing weight and flesh firmness reported previously. The wide range in intensity scores recorded over the course of the storage period suggests that firmness is a critical sensory quality parameter for fresh-cut mangosteen.

The OVQ score correlated well with other sensory attributes. Results fro Table 5 demonstrated that the acceptance of fresh-cut mangosteen decreased with storage time. Comments received from the panelists suggested that products stored for <7 days most closely resembled fresh mangosteen fruit.

3.5 Conclusions

According to the results of this study, fresh-cut mangosteen evidently suffered from undesirable changes during storage. The packaging system used for the experiments may not provide sufficient extension of shelf-life to increase the marketability of the product. Hence further means to reduce undesirable changes need to be investigated. In addition, the film used in this study may not act as a good barrier to gas exchange, resulting in undesirable alterations in product quality. Other films may prove more effective for the preservation of fresh-cut mangosteen. Finally, further research needs to address changes in texture and the appearance of odors/ flavors which may be associated with the accumulation of ethanol and acetaldehyde in the fresh-cut.

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CHAPTER 4

EFFECT OF 1-MCP AND ASC ON THE QUALITY OF PACKAGED
FRESH-CUT MANGOSTEEN

4.1 Abstract

Fresh-cut mangosteen is a potentially marketable product but enzymatic and microbial activities could lead to deterioration of quality during storage. Therefore, the effect of 1-MCP and ASC on the quality of stored fresh-cut mangosteen was investigated. Mangosteen fruit were treated with 0, 20, 40 or 80 ppm 1-MCP for 12 h at 28 ± 2 ^{o}C before preparation into fresh-cut product packed in PP trays sealed with OPP/LLDPE film. After storage at 5 ^{o}C for 12 days, fresh-cut mangosteen without 1-MCP treatment showed rapid softening and continuous weight loss. Ethylene production and respiration rate were lower in 1-MCP treated fruits and both softening and weight losses were delayed. Therefore, the treatment could provide better quality in packaged fresh-cut mangosteen. The 1-MCP (40 ppm) treated fruits were cut and dipped in tap water (control), 500 or 1,000 ppm acidified sodium chlorite (ASC) for 1 min before packing in PP trays sealed with OPP/LLDPE film for storage at 5 ^{o}C for 12 days. The results indicated that ASC application did not significantly reduce initial microbial populations. However, ASC could control browning as revealed by significantly lower browning index (BI) values throughout the storage period.

4.2 Introduction

In recent years, there has been a considerable increase in demand for high quality fruits and vegetables, coupled with a requirement for convenience and safety. Consumers are indicating a strong preference for fresh fruits and vegetables over their processed counterparts (Bolin and Huxsoll, 1989). Furthermore, changes in consumer lifestyles have led to a desire for ready-to-eat or ready-to-use products and therefore, interest in "minimally" or "lightly" processed products. Despite their popularity, the production of minimal processed fruit is limited because of rapid deterioration and senescence. The plant tissues incur damage during processing, leading within minutes

to increased rates of respiration and ethylene production (Brecht 1995) due to the onset of "wound responses". Injury stresses caused by minimal processing result in mechanical plant cell rupture and cellular de-compartmentalization, leading to delocalization and intermixing of enzymes and substrates. Microbial proliferation and desiccation are accelerated by the removal of the protective peel. Quality is reduced because of associated processes that lead to degradation of tissues and the shelf-life of these products is limited.

Although the shelf-life of fresh-cut vegetables and salads is generally adequate, marketing of fresh-cut fruit has been limited to 4 to 7 days. The industry would much prefer a 21days marketing window (Ahvenainen, 1996). One major challenge is to develop techniques that will effectively extend the shelf-life of these products while ensuring product quality and safety.

The gaseous plant hormone, ethylene, induces synthesis of enzymes that lead to fruit ripening, senescence and degradation. Therefore, one strategy to retard deterioration and senescence of minimally processed fruit would be to remove this hormone and/or block its effects. A number of technologies have been used to reduce ethylene synthesis or action including antisense gene technology (Hamilton *et al*., 1990) and application of compounds such as silver in the form of thiosulfate (STS) and diazocyclopentadiene (DACP) (Blankenship and Sisler 1993; Sisler and Serek 1997). However, few of these compounds have been fully successful or their use has been limited to nonfood products. Recently, a new organic molecule, 1-methylcyclopropene (1-MCP), has been discovered that can block the ethylene receptor for extending periods (Blankenship and Dole, 2003). If 1-MCP is effective in delaying the ripening of partially ripe (climacteric or post-climacteric) fruits, it would be useful in extending the shelf-life of fresh-cut fruits.

Furthermore, washing of fresh-cut produce is an important step in reducing microbial populations. Chlorine (sodium hypochlorite) has been widely used in produce washes in order to inactivate microorganisms and ensure quality and safety (Li *et al*., 2001; Wei *et al*., 1985). However, recent studies have shown that chlorine lacks efficacy for pathogen reduction (Zhang and Farber, 1996; Beuchat *et al*., 1998); the formation of chlorine by-products is also deleterious to human health (Richardson

et al., 1998). Thus, there is much interest in developing a safer and more effective antimicrobial alternative to chlorine.

Acidified sodium chlorite (ASC) has recently been approved by the FDA for spray or dip application on various food products, including fresh and fresh-cut produce (FDA, 2001). Studies have shown that ASC has strong antimicrobial effects against various human pathogens inoculated onto cantaloupes and asparagus (Park and Beuchat, 1999). ASC was also found to inhibit browning in fresh-cut fruit. Lu *et al*. (2007) reported that ASC was not only an effective sanitizer for inhibiting microbial growth but is also a browning control agent on fresh-cut apple slices.

The objectives of this study were to evaluate the effects of 1-MCP on the physiological, chemical, microbiological and sensory quality of packaged fresh-cut mangosteen, and to study the efficacy of ASC for reducing microbial populations and preventing color changes due to browning.

4.3 Materials and Methods

Plant material

A southern cultivar of mangosteen fruit (*Garcinia mangostana*, Linn.) was grown in Songkhla province, Thailand by a commercial grower. Fruit with a mean weight of 120±5 g were hand-harvested during July-August 2009 at stage 2 (light greenish yellow with 51-100% scattered pink spots) according to the scales described by Palapol *et al*. (2009). The crop was transported to the laboratory within 1 hour by truck without temperature control. Fruit were selected on the basis of firmness and surface color. The firmness was set at about 4 Kg force with 1 cm diameter probe using a fruit hardness tester (GY-1, Aliyiqi Instrument, China) and surface color of lightness (L*) 45-55, redness (a*) 10-20 and yellowness (b*) 15-25 using Miniscan Hunter Colorimeter (Hunter Associates Laboratory, Inc., USA).

Effect of 1-MCP on the quality of packaged fresh-cut mangosteen

After grading the fruit was placed in polystyrene containers (45 l) with 50 ml glass beakers containing sufficient 1-MCP (AnsiP®, Lytone Enterprise, Inc., Taiwan) to obtain final concentrations of 0, 20, 40 or 80 ppm. After 12 h the treated fruit was processed into fresh-cut form in an isolated and cleaned minimal-processing room at

30 °C. The fresh-cut mangosteen were washed under tap water and placed in cold water (10 °C) for 5 min, then drained and dried by handheld blower. Approximately 150 g of fruit was packed in polypropylene (PP) trays (11.5×17.5×4.5 cm) which were sealed with OPP/LLDPE film (OTR of 1,160 $cm^3/(m^2$ day)). The trays were stored at 5 °C under 85% RH in an incubator. Three replicates with two trays per replicate were analyzed after 0, 3, 6, 9 and 12 days of storage.

Measurement of gas concentrations in the package headspace

Changes in the concentrations of O_2, CO_2 and C_2H_4 in the trays were measured at 3-day intervals by withdrawing air samples (1 ml) through a septum using a gas-tight syringe. Gas concentrations were measured with a gas chromatograph (GC) (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a thermal conductivity detector (TCD). The oven and detector temperatures were 60 and 150 ^{o}C, respectively, with helium as the carrier gas. Ethylene (C_2H_4) was measured with a same GC but equipped with a flame ionization detector (FID). The oven, injector and detector temperatures were 40, 120 and 180 ^{o}C, respectively, with helium as the carrier gas. In-package gasses accumulations were expressed as %O_2, CO_2 and ppm C_2H_4, respectively.

Physical analyses

Firmness was evaluated on a TA-XT2i texture analyzer (Stable Micro Systems, England) with a 25 kg load cell, equipped with a 2 mm diameter cylinder stainless steel probe (P/2). Firmness measurements were taken as the maximum force value obtained during the test to penetrate the fruit 3 mm at 2.0 mm/s (Troncoso *et al*., 2009). Data were expressed as g/mm^2 force.

Weight loss was determined by weighing all samples with a balance (model AB204, Mettler-Toledo Inc., USA) at the beginning and end of the storage period. The difference between the values was used to calculate weight loss.

Chemical analyses

Acetaldehyde and ethanol content determinations were based on the method described by Gonzalez-Aguilar *et al*. (2004). Five grams of tissue were placed in amber colored bottles with a 60 ml capacity and kept in a 65 ^{o}C water-bath for 15 min. One ml of headspace sample was injected into a gas chromatograph (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a 60 m × 0.325 mm × 0.25 μm

DB-WAX column (J&W Scientific, Folsom, California). The oven, injector and detector temperatures were 60, 250 and 250 ^{o}C, respectively, with helium as the carrier gas. Retention times and standard curves of acetaldehyde and ethanol in water solutions were used for peak identification and quantification.

Sensory evaluation

Twenty semi-trained panelists were asked to rank the samples for browning, off-odor, firmness, wateriness and overall visual quality (OVQ) against reference samples using methods described by Gomez-Lopez *et al*. (2008) with some modifications. The 5-point ratings were assigned as follows: (5) not original, (4) slightly original, (3) moderately original, (2) very original and (1) extremely original. The reference samples were prepared from fruit cut immediately before the evaluations (good sample) and from processed fruit held for 1 day at room temperature (bad sample). Each sample consisted of six fresh-cut fruit served on a white plastic plate after 2-3 h at the room temperature (25 ^{o}C) and the same samples were evaluated by each panelist within 1 h.

Effect of ASC on the quality of packaged fresh-cut mangosteen

Mangosteen fruit was treated with 40 ppm 1-MCP for 12 h at 28$\pm$2 ^{o}C. The treated fruit was processed and washed with 0, 500 or 1000 ppm ASC solutions at 10 °C for 1 min., packed in PP trays and sealed with OPP/LLDPE film. Samples were subjected to analyses for physical (color) and microbiological (psychrotrophic microorganisms, total viable count (TVC), yeasts, moulds, *E. coli* and *Salmonella* sp.) quality after 0, 3, 6, 9 and 12 days of storage at 5 °C under 85% RH .

Physical analyses

Browning index was estimated by extracting a 5 g sample in 100 ml of 67% ethanol for 1 h. The extract was filtered through Whatman No. 1 filter paper and the browning index in terms of absorbance at 420 nm (Spectrophotometer: UNICAM, Type Helios Alpha, England) was measured for the filtrate using 67% ethanol as a blank (Supapvanich *et al*., 2011).

Fruit color was measured using a colorimeter (Miniscan 45/0-L, Hunter Associates Laboratory, Inc., USA) calibrated with a standard white tile with the following parameters: X = 79.6, Y = 84.4, Z = 89.9 with illuminant D/65/10°, (light

source used for the daylight), according to CIE L* (lightness), a* (green to red) and b* (blue to yellow) values. Numerical values of a* and b* were converted into chroma [C = (a*^{2}+b*^{2})$^{1/2}$] and hue angle (H^o = tan^{-1} b*/a*) (Francis, 1980).

Microbiological analyses

Microbial determinations were carried out using standard methodologies (BAM, 2001). Twenty-five grams of sample were diluted into 225 ml of sterile buffered Butterfield's Phosphate (BPS) and homogenized for 2 min at normal speed using a Stomacher (Model 400 Circulator, Seward, Norfolk, England). Serial dilutions of the suspension were prepared in BPS and analyzed for Psychrophile at 4 ^{o}C, total viable count (TVC), yeasts and molds and *E. coli*. Additional 25 g/samples were diluted with 225 ml buffered peptone water for the detection of *Salmonella* sp. The Psychrophile, TVC, yeast and mold populations were reported as CFU/g (colony forming units per gram of sample) whereas *E. coli* and *Salmonella* sp. are reported as the most probable number (MPN) and detected or not, respectively.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and mean differences were estimated by Duncan's new multiple range test (DMRT) using SPSS Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant.

4.4 Results and discussion

Effect of 1-MCP on gas concentrations in the package headspace

Figure 7 shows the O$_2$, CO$_2$ and C$_2$H$_4$ levels measured in the package headspace during storage. A rapid O$_2$ decrease and CO$_2$ increase occurred during the first three days as a consequence of fresh-cut mangosteen tissue respiration,. Afterwards, O$_2$ levels increased and CO$_2$ levels decreased gradually during the remainder of the storage period.
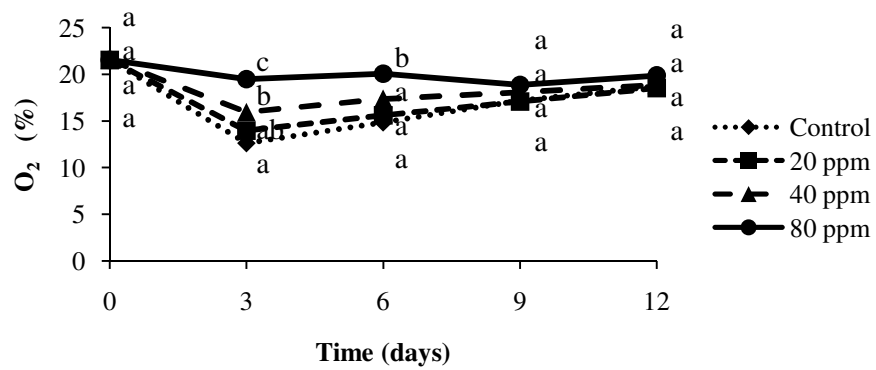
(A)
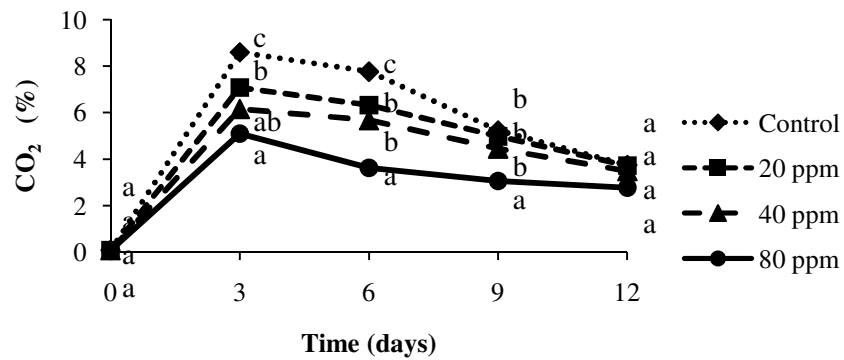
(B)
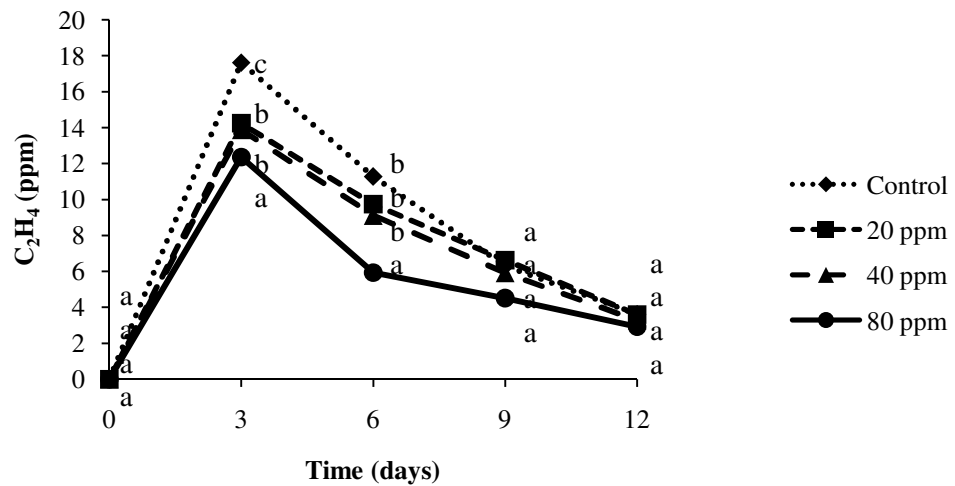
(C)

Figure 7. Concentrations of gases in the headspace of 1- MCP treated fresh-cut mangosteen during storage. A) O_2, B) CO_2 and C) C_2H_4. The same letter within storage day means no significant difference ($p>0.05$).

These results indicated that the respiration rate of fresh-cut mangosteen reached the maximum after three days. Subsequently, the slower respiration rate of the tissues combined with transmission through the film led to reduced CO_2 accumulation. As a consequence CO_2 accumulation was reduced and O_2 concentrations remained comparatively higher after the first three days of storage.

O_2 levels were higher and CO_2 levels were lower in the package headspace of 1-MCP-treated fresh-cut mangosteen than in controls. Accumulation of CO_2 was slower and that of O_2 was faster in fresh-cut mangosteen prepared from fruit treated with 80 ppm 1-MCP than with 40 ppm, 20 ppm or the control. Therefore, 1-MCP had a concentration dependant effect on respiration, in terms of O_2 depletion and CO_2 production inside the package. Similar results were obtained by Buda and Joyce (2003), who showed that 1-MCP decreased the respiration rate of fresh-cut pineapple.

Ethylene production in both control and 1-MCP-treated fresh-cut mangosteen increased rapidly and reached maximum values on day 3 (Figure 7) which were approximately 12-18 fold higher than at day 0. Ethylene production was slower in product made from 1-MCP-treated fruit than controls throughout the storage period.

Treatment with 80 ppm 1-MCP led to lower ethylene accumulation than treatment with 40 ppm, 20 ppm and controls, further evidence that ethylene production was concentration dependant. However, ethylene accumulation declined after 3 days of storage in all treatments. Two factors likely account for this observation: 1-MCP suppressed ethylene production and the permeability of the molecule through the film used to seal the trays.

These results demonstrated that 1-MCP suppressed maximum ethylene production, similar to reports in apple (Pre-Aymard *et al*., 2003), kiwifruit (Mao *et al*., 2007), pineapple slices (Buta and Joyce, 2003) and plum (Khan and Singh, 2007 and 2009; Manganaris *et al*., 2008). However, Vilas-Boas and Kader (2006) did not observe any influence of 1-MCP treatment on ethylene production in fresh-cut banana, although the respiration rate decreased. The reduction in ethylene production in 1-MCP-treated fruits may be due to 1-MCP interfering with the autocatalytic production of ethylene, as ethylene binding sites can be irreversibly blocked by 1-MCP (Sisler *et al*., 1996). Chrriboga *et al*. (2007) reported that at a physiological

level, 1-MCP can inhibit the metabolism of 1-aminocyclopropane 1 carboxylic acid (ACC), the substrate of ethylene production process.

Wounding from cutting generally results in higher respiration rates and ethylene production (Brecht, 1995; Rosen and Kader, 1989; Watada *et al*., 1996). This could be due to both the increased surface area exposed to the atmosphere after cutting, which allows oxygen to diffuse into the interior cells more rapidly, and to increased metabolic activity in injured cells (Zagory, 1995). With the application of 1-MCP, fresh-cut mangosteen generally had a lower respiration rate and ethylene production than untreated controls. Hence the present study confirmed that application of 1-MCP can provide advantages in term of reduced ethylene production and respiration rates in packaged fresh-cut fruit.

Effects of 1-MCP on physical properties

   Firmness

Firmness was retained better in product made from fruit treated with 1-MCP throughout the entire storage period (Figure 8). Firmness decreased after cutting irrespective of treatment but remained significantly higher if fruit was treated with 1-MCP before processing ($p \leq 0.05$).
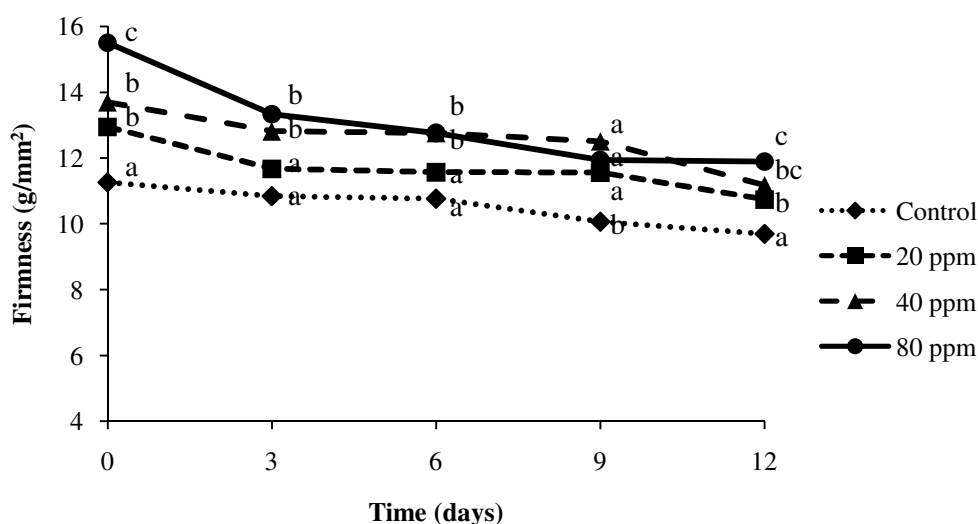
Figure 8. Firmness of 1-MCP treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p > 0.05$).

Giovanonni (2001) and Lelievere *et al*. (1997) reported that the ripening of climacteric fruits was regulated by ethylene which triggers the physical and chemical changes related to fruit softening, color, sugar and organic acid changes. The main consequence of these changes is a reduced shelf-life, which is not longer than several days, and softening of tissue is a major problem (Perera *et al*., 2003). The atmosphere inside the package with the O_2 concentration decreasing, the concentration of CO_2 and C_2H_4 increasing in the first three days of storage, may lead to physical disorders in the tissue that affect firmness (Arias *et al*., 2009). Hence the retention of firmness in1-MCP fruit is likely associated with the suppression of CO_2 and C_2H_4 production. The effectiveness of 1-MCP treatment for maintaining firmness of fresh-cut mangosteen was concentration dependant, and the highest concentration provided the best results.

Weight losses

Weight losess increased over the whole storage period for all treatments although losses were lower by application of 1-MCP before processing (Figure 9). At the end of storage, weight losses reached 5.08%. 4.51%, 4.49% and 3.56% in the control, 20 ppm, 40 ppm and 80 ppm 1-MCP treatments, respectively. There were no significant differences between the 40 and 80 ppm treatments (*p>0.05*) but weight losses were significantly lower than in the control and 20 ppm 1-MCP-treated (*p≤0.05*) fruit. These results showed that treatment with 1-MCP reduced weight losses in fresh-cut mangosteen and that treatment with 40 ppm would provide the same benefits as the 80 ppm treatment.

Ethylene may have played a role in reduced weight losses since accumulation in 1-MCP-treated fruit was lower than in controls. Ripening leading to flesh softening is induced by ethylene, as demonstrated in treatments that led to higher ethylene concentrations (Figure 7). I should be noted that liquid accumulation was evident in samples where softening was substantial. Overall, treatments that resulted in higher ethylene content showed higher weight losses. These findings are in agreement with those of Luo *et al*. (2009) who studied the effect of 1-MCP on weight losses in 'Qingnai' plum.
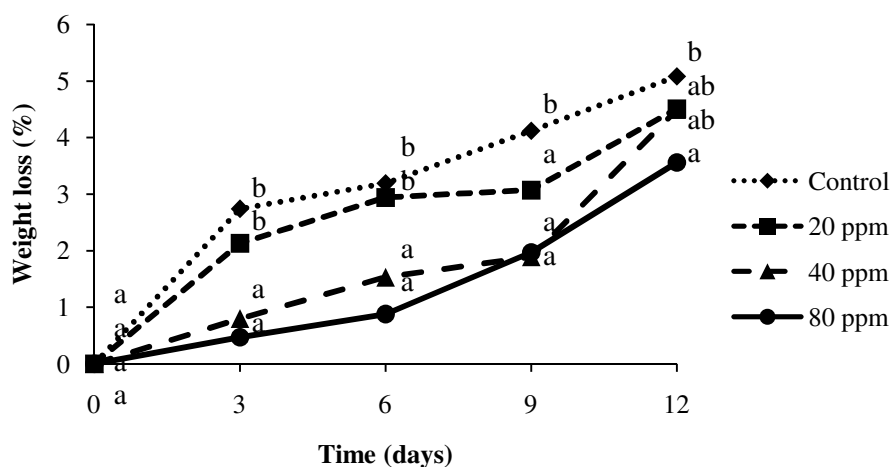
Figure 9. Weight losses of 1-MCP treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p>0.05$).

Effects of 1-MCP on chemical properties

Acetaldehyde and ethanol accumulation in packaged fresh-cut mangosteen were affected by 1-MCP and storage time (Figure 10). Both acetaldehyde and ethanol concentrations were significantly lower than in controls ($p≤0.05$). Treatment of the fruit with 40 and 80 ppm 1-MCP reduced acetaldehyde and ethanol accumulation compared with treatment with 20 ppm 1-MCP and controls ($p≤0.05$). This indicated that fermentation products that influence aroma, such as acetaldehyde and ethanol, could be inhibited by 1-MCP treatment depending on concentration. Accumulation of these products is influenced by the ripening process or ethylene accumulation, as discussed in the previous section which documents lesser ethylene production in 1-MCP-treated fruit. It can be concluded that production of volatile fermentation products that influence aroma was an ethylene-dependent process. Abdi *et al.*, 1998 also concluded that volatile products could be derived from ethylene-dependent processes in fruit. A similar response has also been reported for fresh-cut apple (Fan *et al.*, 2001; Fan and Mattheis, 1999), who found that 1-MCP inhibited total alcohol and total ester formation in 'Fuji' and 'Gala' apples.
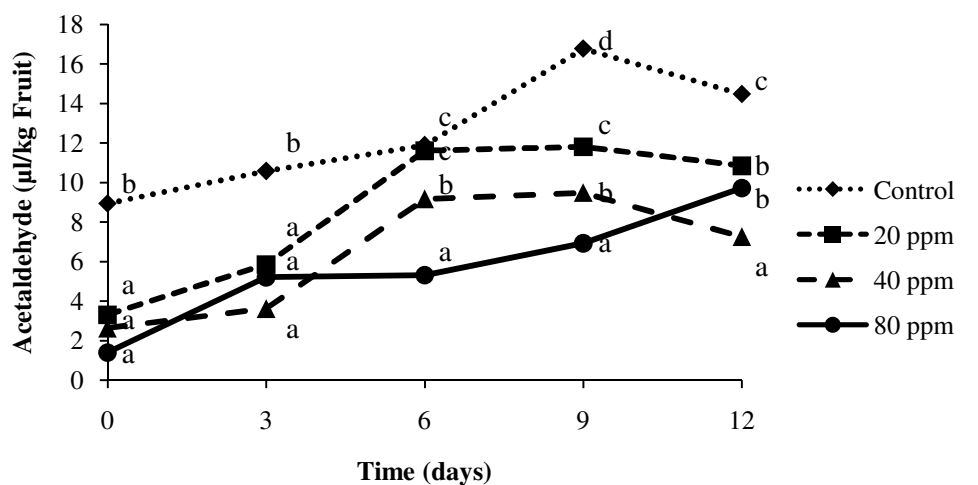
(A)
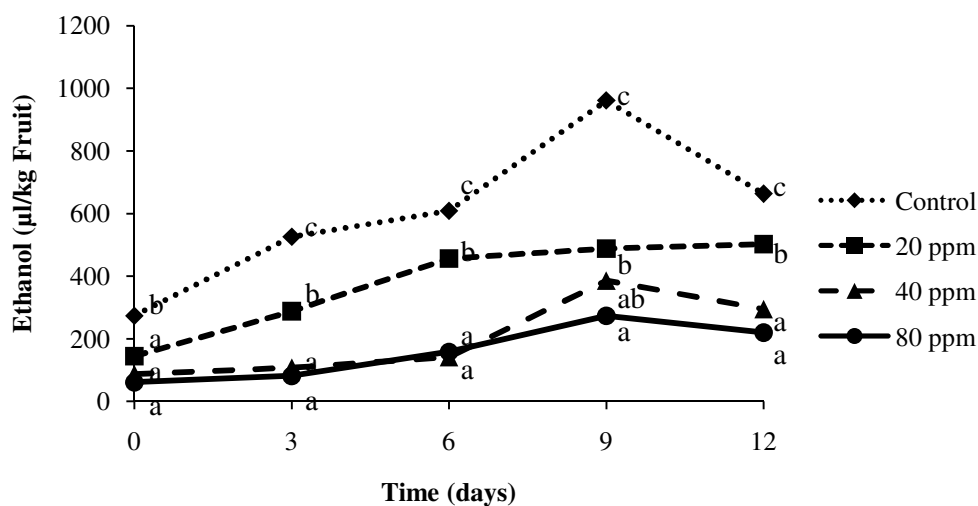
(B)

Figure 10. Changes in acetaldehyde (A) and ethanol (B) concentrations in 1-MCP treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p > 0.05$).

Effects of 1-MCP on microbiological quality

Treatment with 1-MCP before cutting did not affect microbial populations on packaged fresh-cut mangosteen (data not shown). No differences were detected between controls, 20 ppm, 40 ppm and 80 ppm 1-MCP-treated fresh-cut mangosteen throughout the duration of the experiment. Buda and Joyce (2003) also found that

treatment with 1-MCP did not show any beneficial effects in terms of microbial growth inhibition.

Effects of 1-MCP on sensory quality

Table 6 summarizes statistical contrasts for the five attributes for 1-MCP-treated and control of fresh-cut mangosteen and significant differences induced by the treatments ($p \leq 0.05$). Intensity scores for all treatments continuously increased during the storage period. Control samples suffered from poor firmness and wateriness, while 1-MCP-treated samples were subject to browning. No significant differences were found between treatments for off-odor and overall visual quality ($p > 0.05$).

Browning

Browning intensity scores increased after cutting throughout the storage period. The highest browning scores (range of 3.60-4.25 at the end of storage) were recorded for fruit treated with 20 ppm 1-MCP before processing. Browning color was more easily detected in fresh-cut mangosteen made from fruit treated with 1-MCP than in controls.

It should be noted that 1-MCP treated fruit were less ripe than controls. Latex exudation during cutting would be greater in unripe fruit thereby enhancing the risk of flesh contamination and browning at the surface. This risk would be comparatively lower in control fruit in which the ripening process continues unabated. It is therefore likely that browning in fresh-cut mangosteen made from fruit treated with 1-MCP is the consequence of an indirect effect.

Firmness

The increase in firmness scores and the corresponding decrease in fresh-cut fruit weight during storage were reported in a previous section. Firmness scores were directly affected by 1-MCP. No differences were found between treatments immediately after cutting but variances appeared during storage. Fresh-cut mangosteen prepared from 40 and 80 ppm 1-MCP treated fruit were judged to be different from the 20 ppm 1-MCP and control treatments. Overall, the best firmness scores were recorded with the 40 ppm 1-MCP-treatment.

Table 6. Sensory scores of 1-MCP treated fresh-cut mangosteen during storage.

		Storage time (days)				
		0	**3**	**6**	**9**	**12**
Browning						
	Control	2.81±1.06a*	2.94±1.26b	2.93±1.07a	2.65±1.00a	4.06±1.06ns
	20 ppm	3.60±.10b	3.56±0.92c	3.71±1.14b	3.53±0.94b	4.25±0.77ns
	40 ppm	2.60±0.99a	2.17±0.92a	3.50±0.94b	3.47±0.80b	3.94±0.93ns
	80 ppm	2.65±1.09a	2.83±0.92b	3.00±0.96a	3.24±1.03b	3.88±1.09ns
Off-Odor						
	Control	1.40±0.60ns	2.5±1.10ns	2.57±1.16ab	2.94±1.20ns	3.25±1.07ns
	20 ppm	1.90±1.02ns	2.56±0.92ns	3.00±0.88c	2.88±1.17ns	3.00±1.03ns
	40 ppm	1.80±0.89ns	2.06±0.80ns	2.86±1.41b	2.88±0.86ns	3.31±0.95ns
	80 ppm	1.70±1.08ns	2.22±0.88ns	2.29±1.07a	2.82±1.13ns	2.94±0.93ns
Firmness						
	Control	2.2±0.83ns	2.72±0.96b	3.43±0.76d	3.65±0.86d	4.44±0.73b
	20 ppm	2.0±0.73ns	2.67±1.03b	3.14±0.77c	3.41±1.00c	3.63±0.89a
	40 ppm	1.8±0.89ns	1.94±0.80a	2.64±1.22b	2.76±0.75a	3.31±0.95a
	80 ppm	2.2±0.83ns	2.11±0.76a	2.00±0.68a	2.94±1.03b	3.69±0.87a
Wateriness						
	Control	2.05±0.89b	2.61±0.98b	3.00±0.88c	3.47±1.12ns	4.25±0.77b
	20 ppm	2.15±0.93b	2.44±0.98b	2.57±1.02b	3.12±0.99ns	3.75±0.93a
	40 ppm	1.55±0.60a	1.67±1.14a	2.71±1.20b	3.06±0.83ns	3.56±1.21a
	80 ppm	1.60±0.60a	1.83±0.86a	2.00±0.78a	3.00±0.79ns	3.56±1.03a
Overall visual quality						
	Control	2.05±0.94c	2.56±0.81c	2.93±1.23b	2.91±1.88b	3.94±1.00c
	20 ppm	2.48±1.04b	2.61±1.05c	2.90±1.72b	2.94±1.54b	3.60±1.26b
	40 ppm	1.80±0.76a	1.81±0.65a	2.68±1.95b	2.88±1.44b	3.57±1.03b
	80 ppm	2.05±1.05c	2.03±0.47b	2.36±1.64**a**	2.65±1.57a	3.35±0.82a

Note: * mean±SD from 20 panelists followed by the same letter within column for each attribute are not significantly different (p>0.05).

Firmness retention in 1-MCP treated fruit is likely associated with reduced weight losses. Suppression of C_2H_4 production and the resulting lower respiration rate therefore appear to be intimately associated with the retention of firmness in stored fresh-cut mangosteen.

Off-odor

Sensory analysis indicated that the off-odor attribute of fresh-cut mangosteen increased with prolonged storage. Initially, the produce was scored as "extremely original" with a fresh fruit aroma, but by the end of storage most panelists scored it as "moderately original". No differences were found between the control, 20, 40 and 80 ppm 1-MCP-treated samples. Hence treatment of the fruit with 1-MCP did not affect the off-odor attribute of fresh-cut mangosteen.

Overall, higher acetaldehyde and ethanol concentrations were associated with low residual O_2 in the package. Anaerobic respiration may be responsible for this finding but volatile accumulation could also be related to the ripening process or to microbial spoilage. However microbial populations were very low throughout the storage period (Table 2) and both O_2 and CO_2 concentrations in the package were not characteristic of produce undergoing anaerobic respiration. Paull (1997) reported that ethanol and acetaldehyde normally accumulate during maturation or ripening of pineapple. It is possible that accumulating ethanol and acetaldehyde in the present work were derived from the normal ripening process of mangosteen. Consequently, their presence in packaged fresh-cut mangosteen may not correlate with the sensory off-odor attribute measured in this study.

Wateriness

Wateriness intensity scores were consistent with the degree of weight loss, as mentioned in the previous section. Weight losses in all treatments increased over the entire storage period. A similar pattern was observed in this attribute when the samples were evaluated by the panelists. The wateriness scores recorded for control samples increased more rapidly than in 1-MCP-treated samples. This means that wateriness of packaged fresh-cut mangosteen was reduced by the 1-MCP treatment, particularly at concentrations of 40 and 80 ppm.

Overall visual quality (OVQ)

The OVQ correlated well with firmness and wateriness attributes, but not with browning and off-odor attributes. Table 3 demonstrates that the OVQ scores from all treatments increased with storage time in parallel with firmness and wateriness scores. 1-MCP was shown to influence firmness and weight losses (see sections 3.2.1 and 3.2.2) of fresh-cut mangosteen and clearly influenced the OVQ scores.

Off-odor scores did not correlate with OVQ, likely because of the lack of microbial growth and relatively aerobic conditions maintained in the packages. The browning attribute was also poorly correlated with OVQ. Browning was significantly affected by the 1-MCP treatment. 1-MCP-treated fruit was more prone to browning during storage but scores for the OVQ attribute were seemingly unaffected. It should be stressed that the panelists were all Thai and were therefore familiar with mangosteen which readily browns after peeling. Consequently, it is probable that slight browning did not influence their overall assessment of the samples.

Effect of ASC on browning index and color

Browning index

Enzymatic browning in term of browning index (BI) measured as A420 values is shown in Figure 11. The BI index for control samples was significantly higher than in ASC-treated samples throughout the storage period. The lowest BI values were measured in 500 and 1,000 ppm ASC-treated samples although differences between the treatments were not significantly different (*p>0.05*).
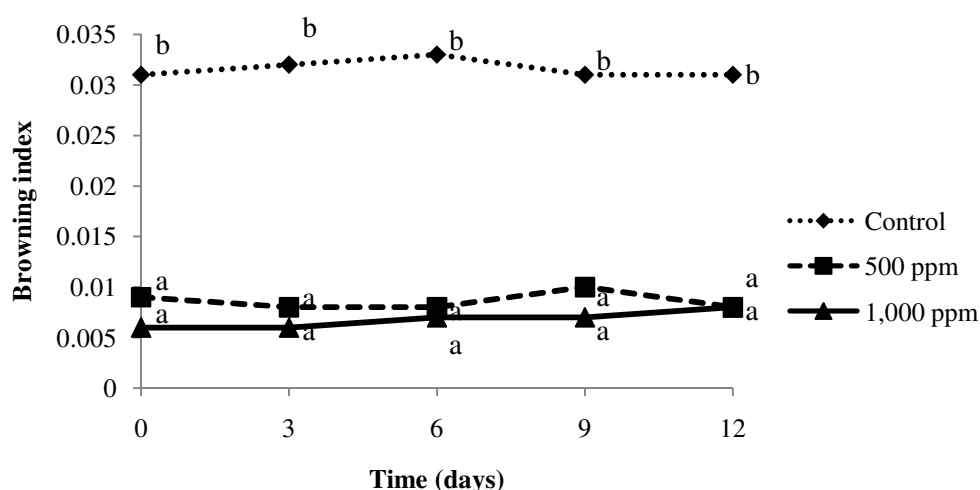
Figure 11. Changes in the browning index of ASC treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference (*p>*0.05).

This result is consistent with observations reported by Lu *et al.* (2007) who also found that ASC retarded the browning reaction in fresh-cut apple. He *et al.* (2008) reported that the anti-browning property of ASC is due to two effects derived from the sodium chlorite ion, the direct inactivation of polyphenol oxidase and the oxidative degradation of phenolic substances.

Color

Figure 12 shows the decrease in L* values in stored packaged fresh-cut mangosteen. Lightness decreased rapidly in all treatments over the first 6 days of storage but remained comparatively stable thereafter. No significant differences were found between the treatments at each day of storage ($p>0.05$).

This result suggested that decreasing L* values were not the consequence of oxidation reactions, a finding is consistent with results obtained by Rocha and Moris (2003) who reported that decreasing of L* values might be caused by either oxidative browning or increasing pigment condensation, with the latter more likely in fresh-cut fruit. Water losses could result in localized concentration of some fruit pigments leading to darkening and a decrease in L* values. In contrast, Lu *et al.* (2007) reported that ASC does not lead to decreases in L* values of stored fresh-cut apple slices, which hints that oxidative browning reactions are more likely responsible for browning in the latter.

Chroma values, an indicator of the intensity of hue, were relatively stable throughout the storage period. Hue angle (H^o), an indicator of browning, increased over the first 3 days but remained stable thereafter suggesting that most of the visible browning of stored fresh-cut mangosteen would occur during the first 3 days of storage.
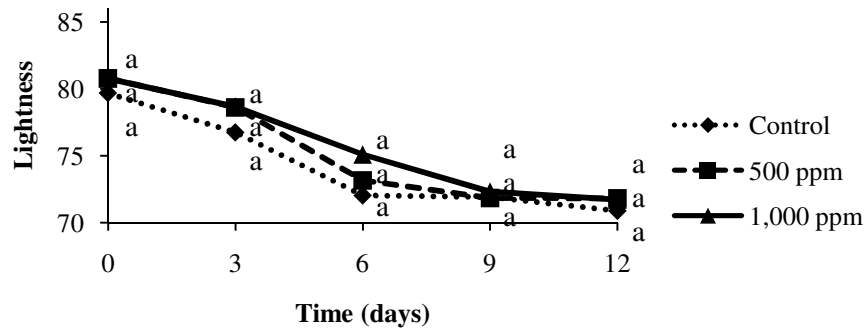
(A)
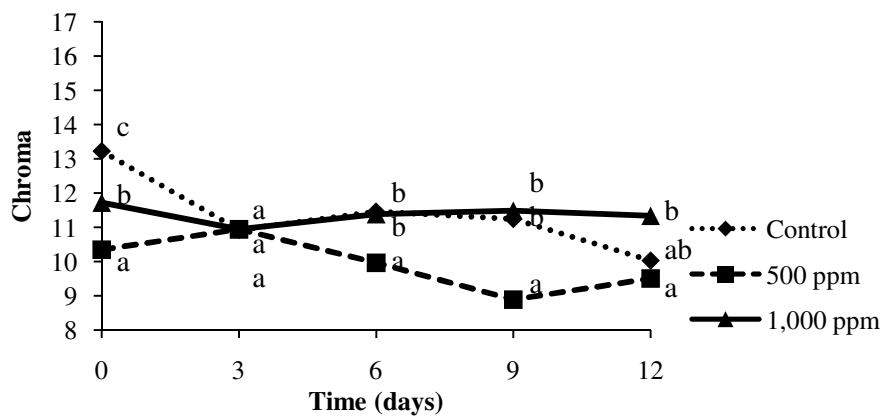
(B)
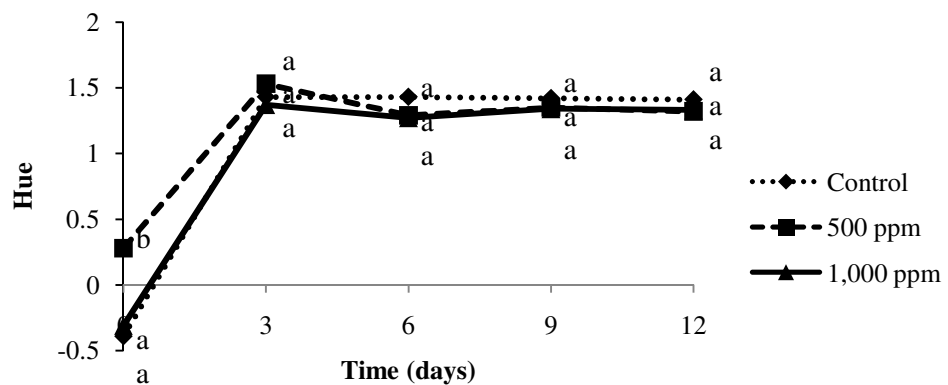
(C)

Figure 12. Changes in color of ASC treated fresh-cut mangosteen during storage A) lightness, B) chroma and C) hue values. The same letter within storage day means no significant difference ($p > 0.05$).

Effect of ASC on microbiological quality

ASC is widely reported to be an inhibitor of microbial growth. Ruiz-Cruz *et al.* (2007) reported that 250 and 500 ppm could reduce bacterial counts on fresh-cut carrots by 1.5 and 2.5 log CFU/g. Kim *et al.* (2007) and Allende *et al.* (2009) reported that 100 and 1,000 ppm can reduce *E. coli* on fresh-cut cilantro by 1.6 and 3.0 log CFU/g, respectively. In the present study the level of contamination with microorganisms at the outset was very low and there was no evidence of growth during storage, even in controls (Table 7). Low initial microbial populations, storage at refrigeration temperature (5 $^{\circ}$C) and the relatively high acid content of mangosteen fruit likely all contributed to the microbiological stability of the product. Hence it was not possible to determine if ASC can influence the development of microorganisms in fresh-cut mangosteen.

Table 7. Microbial populations of all treatments (control, 500 and 1,000 ppm ASC solution).

Microorganism	Storage time (days)				
	0	3	6	9	12
Psychrophile (CFU/g)	<10	<10	<10	<10	<10
TVC (CFU/g)	<10	<10	<10	<10	<10
Yeast&Mold (CFU/g)	<10	<10	<10	<10	<10
E. coli (MPN/g)	<3	<3	<3	<3	<3
Salmonella sp.	N.D.	N.D.	N.D.	N.D.	N.D.

Note: N.D. (Not detected)

4.5 Conclusions

Treatment of fruit with 1-MCP before processing influenced the physical, chemical and sensory qualities of fresh-cut mangosteen. Deterioration during storage was mainly due to softening and weight losses. These events were delayed by treatment with 1-MCP which suppressed ethylene production. Fresh-cut mangosteen made from fruit treated with 1-MCP-treated retained reasonable quality during storage. Treatment with 20 ppm did not provide evident advantages and treatment with 80 ppm yielded un-ripened fruit which was difficult to process and sensitive to

browning caused by contamination of the surface by latex. In contrast, fruit treatment with 40 ppm 1-MCP provided some clear advantages in terms of quality retention and this concentration can be recommended for fresh-cut mangosteen processing. Washing fresh-cut mangosteen with an ASC solution significantly reduced browning intensity. An ASC concentration of 500 ppm is a practical alternative for the control of browning during storage. However the value of the treatment for the control of microorganisms could not be assessed from this work. Further studies are needed to determine whether ASC can provide such activity when higher levels of bacteria are present on fresh-cut mangosteen. It would also be useful to examine the effect of the treatment against food-borne pathogens deliberately inoculated onto fresh-cut mangosteen.

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CHAPTER 5

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACTS PREPARED FROM MANGOSTEEN PARTS AND SOME ESSENTIAL OILS

5.1 Abstract

The antioxidant and antimicrobial activities of the extracts from pericarp, leaf and bark of mangosteen (*Garcinia mangostana* L.) and cinnamon and citrus essentials oils were investigated. The antioxidant activities (IC_{50}) of pericarp, leaf and bark extracts, which were measured using the DPPH method, were 5.94, 9.44 and 6.46 µg/ml, respectively. Both cinnamon and citrus essential oil showed no antioxidant activities with the DPPH method. A broth dilution method was employed to measure antimicrobial activity against some Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella* sp.). The minimum inhibitory concentrations (MIC) of pericarp, leaf and bark extracts against Gram-positive bacteria ranged from 0.025 to 0.78 mg/ml. Minimum bactericidal concentrations (MBC) ranged between 0.05-0.39 mg/ml. MIC and MBC values of cinnamon oil against *S. aureus*, *E. coli* and *Salmonella* sp. were 3.13 and 6.25 mg/ml, respectively. Citrus oil showed antibacterial effecst against *S. aureus* only with MIC and MBC values of 6.25 and 12.50 mg/ml, respectively.

5.2 Introduction

Plant extracts have long been added to foods to improve their flavor and organoleptic properties. Extracts from herbs and spices have well known antimicrobial and antioxidation potential. The active principles extracted from plants are generally referred to as natural compounds, and are primarily secondary metabolites. These metabolites have demonstrated biological activities and have received particular attention as potential natural agents for food preservation. Nowadays consumers demand more natural and fresh-like foods with fewer synthetic additives but increased safety and shelf-life (Negi *et al*., 2008). Plants and plant extracts have consequently considered as potential ingredients for replacing synthetic

antimicrobial and antioxidant agents (Mayachiew and Devahastin, 2008).

Mangosteen (*Garcinia mangostana* L.) is one of the most famous fruits grown in Thailand. Previous studies have shown that extracts prepared from various plant parts contain a variety of secondary metabolites such as prenylated and oxygenated xanthones. Xanthones or xanthen-9H-ones are secondary metabolites found in some higher plants including mangosteen (Peres *et al.*, 2000). Xanthones can be isolated from the pericarp, whole fruit, bark and leaf of mangosteen. Several studies have shown that xanthones from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral activities (Suksamrarn *et al.*, 2006; Pedraza-Chaverri *et al.*, 2008).

Preparations with biological activity that can be extracted from plants by distillation are frequently referred to as essential oils. Essential oils from aromatic plants, spices and herbs have been used historically in the pharmaceutical, food and perfume industries because of their antibacterial, culinary and fragrant properties (Salehi *et al.*, 2005). In addition, essential oils have been used for preventing food spoilage and deterioration and also for extending the shelf-life of food since ancient times. Cinnamon and citrus essential oils have been shown have antimicrobial activity and could serve as a source of antimicrobial agents against foodborne microorganisms. Several references on their antimicrobial efficiency are available in literature reviews, such as cinnamon (Lopez *et al.*, 2005; Tzortzakis, 2009) and citrus (O'Bryan *et al.*, 2008; Fisher and Phillips, 2006)

Because of increasing consumer demand for healthy food, food processors have a need to reduce the use of synthetic chemicals in food products. Common culinary plants could provide a source of natural alternatives. Although mangosteen and essential oils from cinnamon and citrus contain potent antimicrobial and antioxidative activities, such extracts have not been sufficiently tested. While plant extracts are generally considered to be safe (GRAS) their uses are often limited by organoleptic criteria. For this reason, it will be necessary to determine the minimum concentration needed to inhibit the growth of foodborne microorganisms without affecting the sensory quality of the food. The objectives of the present study were to determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and antioxidant activity of mangosteen pericarp, leaf and bark

extracts and cinnamon and citrus essential oil against Gram-positive and Gram-negative bacteria.

5.3 Materials and methods

Plant material and extraction method

The whole pericarp (outer and inner peels) of fruit at ripening stage 3, leaf and bark of mangosteen were harvested from Songkhla province in April, 2009. The samples were first cleaned to remove any residual compost and washed thoroughly to remove impurities. After washing, the samples were chopped into small pieces (0.5 × 1.0 cm^2) and dried overnight in a tray dryer at 45 ^{o}C. Then chopped samples were ground with a grinder to make powder (around 18 meshes).

All ground samples were placed in 70 ^{o}C distilled water for 15 min at the ratio of sample powder:water of 1:4. The mixtures were boiled 4 times or until no content of tannin was found by dropping a 2% gelatin solution in the mixtures (Weecharangsan *et al*., 2006). The mixtures were filtered and the residues were dried at 40-45 °C in an oven. The dried powder was macerated at room temperature for 7 days with 50% ethanol. In order to know the exact weight, the crude extracts were filtered and evaporated to obtain the dried crude extracts. Final extracts were stored in a desiccator containing dry silica gel.

Essential oils

Cinnamon and citrus essential oils (0.05%, food grade) were purchased from Flavor-Focus (Bangkok, Thailand).

Microbial cultures

Escherichia coli DMST 15537, *Salmonella* sp. DMST 4464, *Listeria monocytogenes* DMST 17303 and *Staphylococcus aureus* DMST 6512 were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The microorganisms were maintained in TSA at 5 ^{o}C. Microbial stock cultures were grown in MHB (*E. coli*, *S. aureus* and *Salmonella* spp.) and TSB (*L. monocytogenes*) at 37$\pm$2 ^{o}C in a shaker incubator for 3.5 h at 110 rpm (cells in early stationary phase).

The bacterial suspension was subsequently adjusted to 10^6 CFU/ml using MHB and TSB.

Antioxidant activity assay

The scavenging of DPPH free radicals was used for measuring the antioxidant activity of the extracts according to the method of Weecharangsan *et al.* (2006) with slightly modifications. Briefly, stock solutions of the crude extracts were prepared by dissolving 0.1 g of dry extracts in 50 ml 50% ethanolic solution. The stock solution was diluted with 50% ethanolic solution to obtain sample solutions at the concentrations of 1, 10, 50 and 100 µg/ml. The sample solutions were thoroughly mixed with freshly prepared 0.05% DPPH ethanolic solutions at the ratio of 1:1 and kept for 30 min in the dark at room temperature. The amount of the reaction mixture was determined by UV-VIS spectrophotometer at 517 nm. Neutralisation of DPPH radical was calculated using the equation: DPPH inhibition (%) = $100 \times (A_0 - A_S)/A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound) and A_S is the absorbance of the tested sample.

The antioxidant activity of the crude extract was expressed as IC_{50}, defined as the concentration of the crude extract required to inhibit DPPH radicals by 50%, using the linear regression analysis. Ascorbic acid was used as a standard antioxidant.

Antimicrobial activity assay

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed by a broth dilution technique, using 96-well microtitre plates according to Kuete *et al.* (2008) and Saker *et al.* (2007). Bacterial inocula contained approximately 1.0×10^5 cells in a final volume of 100 µl/well.

The crude extracts were dissolved in 10% dimethylsulfoxide (DMSO) in sterile MHB to obtain a stock concentration of 12.50 mg/ml. Serial two-fold dilutions of each sample were made with MHB to yield volumes of 100 µl/well with final concentrations ranging from 0.05-12.50 mg/ml. MHB was used as a negative control while ciprofloxacin and vancomycin were used as positive controls (0.5-2.0 µg/ml). The microplates were incubated for 24 h at 37 ^{o}C.

The MIC of samples was detected following the addition of 10 µl resazurin (0.75%). Viable bacteria can reduce the blue dye to a pink color. Microbial growth was determined by observing changes of color in the wells. MIC was defined as the lowest sample concentration that had prevented this change. The minimum bactericidal concentration (MBC) was determined by subcultivation of 50 µl of each blue well in plates containing Mueller-Hinton agar followed by incubation for 24 h at 37 ^{o}C. The lowest concentration with no visible growth was defined as the MBC.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and mean differences estimated by Duncan's new multiple range test (DMRT) using SPSS Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant.

5.4 Results and discussion

Antioxidant activity

G. mangostana pericarp extracts had the strongest activity (IC_{50} = 5.94 µg/ml) while bark and leaf extracts showed moderate activities (IC_{50} = 6.46 and 9.44 µg/ml, respectively). Cinnamon oil showed no activity and the citrus oil did not reach 50% of DPPH-neutralisation at the highest concentration applied (Table 8).

Table 8 shows that the mangosteen pericarp extract had the highest antioxidant activity among these extracts when compared to L-ascorbic acid. The IC_{50} value for pericarp extracts measured in the present work was lower than that reported by Weecharangsan *et al*. (2006). For the present study raw materials were derived from fruit at maturity stage 3 while Weecharangsan *et al*. (2006) used fruit at stage 5 or 6 maturity. Hence disparities in maturity may have led to differences in experimental outcomes. Okonogi *et al*. (2007) reported that the antioxidant activity (IC_{50}) of mangosteen pericarp extract was 0.023 µg/ml, which is considerably lower than the IC_{50} found in this experiment (5.94 µg/ml). However these authors used a different extraction method, which provides further evidence that the antioxidant activity of plant extracts is highly influenced by the approach used in their preparation, as reported by Liu *et al*. (2008).

Table 8. Antioxidant activity (IC$_{50}$, µg/ml) of *G. mangostana* pericarp, leaf and bark extracts and some essential oils.

Extracts	IC$_{50}$
Mangosteen	
-Pericarp	5.94$\pm$0.14b*
-Leaf	9.44$\pm$0.39d
-Bark	6.46$\pm$0.36c
Cinnamon oil	no activity
Citrus oil	no activity
L-Ascorbic acid	4.30$\pm$0.14a

Note: * mean$\pm$SD from 3 replicates followed by the same letter within column are not significantly different ($p > 0.05$).

Extracts prepared from mangosteen pericarp, leaf and bark had different IC$_{50}$ values. Pericarp extract exhibited the lowest IC$_{50}$, followed by bark and leaf extracts with IC$_{50}$ values of 5.94, 6.46 and 9.44 µg/ml, respectively. Zadernowski *et al*. (2009) established that the rind (inner peel) of mangosteen has a higher phenolic content than the pericarp (outer peel) or aril. Tachakittirungrod *et al*. (2007) reported that the active compounds in higher plant are located in different parts with different contents. Hence differences derived from variable phenolic composition in the pericarp, leaf and bark were likely responsible for differences in IC$_{50}$ values indicative of differences in antioxidant activity. Maisuthisakul *et al*. (2008), Mayachiew and Devahastin (2008) and Liu *et al*. (2008) reported similar correlations between phenolic content and antioxidant activity in plant extracts from various sources. Moreover, Mayachiew and Devahastin (2008) reported that differences in the chemical composition of extracts can influence antioxidant activity. It is likely that mangosteen pericarp extracts contain more or different profiles of phenolic compounds than bark or leaf extracts.

Table 8 shows that no activity was detected in either cinnamon or citrus essential oils by the DPPH method. This result agrees with the findings of Poloteo *et al*. (2006) who reported that cinnamon essential oil had minimal activity. Eyob *et al*.

(2008) suggested that the activity of essential oil from korarima was very low, for the reason that the OH group in the aromatic rings of phenolic compound was replaced by other functional groups as a result of their hydrogen donating ability. Similar substitutions may be responsible for the lack of activity in cinnamon and citrus essential oils.

Antimicrobial activity

The MIC and MBC of the extracts ranged from 0.05 to 6.25 mg/ml against the 4 microorganisms (Table 9). This experiment confirmed the strong antibacterial activity of mangosteen extracts against Gram-positive bacteria and the lack of activity against Gram-negative bacteria. The lowest MIC value (0.025-0.05 mg/ml) was observed with pericarp and bark extracts against *L. monocytogenes* and *S. aureus.* Cinnamon oil was moderately active against *E. coli*, *Salmonella* sp. and *S. aureus* (MIC 3.13 mg/ml, MBC 6.25 mg/ml) while citrus oil was active only against *S. aureus* (MIC 6.25 mg/ml, MBC 12.5 mg/ml).

Table 9. Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of *G. mangostana* pericarp, leaf and bark extracts and some essential oils.

Bacteria	MIC/ MBC	Mangosteen extracts (mg/ml)			Cinna-mon (mg/ml)	Citrus (mg/ml)	Cipro-floxacin (μg/ml)	Vanco-mycin (μg/ml)
		Pericarp	Leaf	Bark				
L. monocytogenes DMST 17303	MIC	0.05c*	0.78b	0.025a	>12.5	>12.5	0.78b	0.78b
	MBC	0.10b	1.56d	0.05a	-	-	0.78c	0.78c
S. aureus DMST 6512	MIC	0.025a	0.20c	0.05b	3.13e	6.25f	<.05	0.78d
	MBC	0.05a	0.39c	0.10b	6.25e	12.50f	-	0.78d
E. coli DMST 15537	MIC	>3.13	>3.13	>3.13	3.13	>12.5	<.05	N.D.
	MBC	-	-	-	6.25	-	-	N.D.
Salmonella sp. DMST 4464	MIC	>3.13	>3.13	>3.13	3.13	>12.5	<.05	N.D.
	MBC	-	-	-	6.25	-	-	N.D.

Note: N.D. (Not Detected)

* mean±SD from 3 replicates followed by the same letter within row are not significantly different ($p > 0.05$).

Xanthones are biologically active compounds found in all parts of the mangosteen plant. More than 20 xanthones from mangosteen have been characterized. Both of α- and β-mangostin are mainly found in the fruit (Furukawa *et al.*, 1996; Chen *et al.*, 2008), especially in the pericarp. Xanthones can inhibit several microorganisms. Iinuma *et al.* (1996) and Sakagami *et al.* (2005) reported that the MIC value of α-mangostin against *S. aureus* was lower than β-mangostin (6.25 and >100 µg/ml, respectively). It is likely that xanthones such as α-mangostin were responsible for the activity of extracts prepared in the present work. The higher activity of pericarp and bark extracts against *L. monocytogenes* and *S. aureus* may have been the consequence of higher xanthone content than leaf extracts.

Canillac and Mourey (2001) reported that if the MBC/MIC ratio is less than or equal to 4, bacteria can be considered susceptible to an antimicrobial; on the other hand, if this ratio is greater than 4, they can be considered tolerant. The MBC/MIC ratios of all extracts against Gram-positive bacteria tested in the present work were less than 4, and they can be considered sensitive to the extracts. Differences in the cell membranes of Gram-positive and Gram-negative bacteria are responsible for differences in susceptibility to phenolics (Negi *et al.*, 2008). The Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier (Scherrer and Gerhardt, 1971). The resistance of Gram-negative bacteria towards antibacterial substances may be due to outer phospholipidic membranes carrying the structural lipopolysaccharide components, which makes it impermeable to lipophilic solutes and porins which constitute a selective barrier to hydrophilic solutes (Nikaido and Vaara, 1985).

The effects of cinnamon and citrus essential oils against the 4 microorganisms were different than mangosteen extracts. The essential oils had higher MIC or MBC values than the extracts. Citrus oil could inhibit only Gram-positive bacteria, particularly *S. aureus*. In contrast the cinnamon oil inhibited all of the Gram-negative bacteria.

The main components of citrus oil are limonene, linalool and citral. Among these components, limonene is the most abundant, followed by linalool and citral. Fisher and Phillips (2006) reported that limonene had the lowest effect against microorganisms. Inhibition was due primarily to linalool rather than citral or

limonene. Gram-negative bacteria were tolerant to citrus oil because of the lipopolysaccharide present in the outer membrane which provides protection against different agents (Mahboubi and Haghi, 2008; Oussalah *et al.* 2007).

Cinnamaldehyde and eugenol were found to be the main chemical constituents of cinnamon essential oil. Oussalah *et al.* (2007) reported that cinnamaldehyde was found mainly in the bark or branch of the cinnamon tree while eugenol was found primarily in the leaf. Eugenol has lower activity against microorganisms than cinnamaldehyde. Hussain *et al.* (2008) reported that differences in essential oil composition could result in different biological activity. Lopez *et al.* (2005) and Inouye *et al.* (2001) showed that cinnamon with higher eugenol content has higher activity against Gram-negative than Gram-positive bacteria. In contrast, cinnamon with higher cinnamaldehyde content showed higher activity against Gram-positive than Gram-negative bacteria. In the present study, cinnamon inhibited Gram-positive and Gram-negative bacteria, in agreement with the results of Oussalah *et al.* (2007). It is therefore probable that the cinnamon oils used in both of these works contained high concentrations of both of eugenol and cinnamaldehyde.

Differences in the susceptibilities of the test microorganisms to essential oils suggest that the ability of oil constituents to penetrate the cell wall and cell membrane structures affect their activity. The ability of essential oil constituents to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for their lethality (Cox *et al.*, 2000). Nedorostova *et al.* (2009) reported that the leakage of intracellular metabolites due to damage to cell membranes is the main mechanism or mode of action underlying their antimicrobial activity. However other mechanisms may contribute to their effect, including interactions with intracellular structures or specific proteins after penetration into the cells.

The MBC/MIC ratio of citrus oil against *S. aureus* and the MBC/MIC ratio of cinnamon oil against *E. coli*, *Salmonella* sp. and *S. aureus* were less than 4 according to Table 9. Therefore, *S. aureus* can be considered sensitive to both essential oils while *E. coli* and *Salmonella* sp. are sensitive only to cinnamon essential oil.

5.5 Conclusions

This work showed that mangosteen pericarp extracts may have potential as preservative agents due to their intrinsic antibacterial and antioxidant activities and provided important baseline information for the use of pericarp for this purpose in some types of food. *In vivo* experiments should be conducted to further assess this potential. More specifically, the extracts may be useful for the preservation of desirable quality and the control of microorganisms in fresh-cut mangosteen.

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CHAPTER 6

INHIBITION OF FOODBORNE MICROORGANISMS BY AQUEOUS AND METHANOLIC EXTRACTS OF MANGOSTEEN LEAF, BARK AND FRUIT PERICARP

6.1 Abstract

Antimicrobial activity was examined in mangosteen (*Garcinia mangostana* L.) bark, leaf and fruit pericarp extracts prepared in methanol and in hot water following optimization using response surface methodology. None of the extracts could inhibit growth of the fungi *Botrytis cinerea* and *Penicllium expansum,* the yeast *Saccharomyces cerevisiae* or the Gram-negative bacteria *Escherichia coli* and *Salmonella* Typhimurium. In contrast, both methanolic and aqueous extracts prepared from bark, leaf or fruit pericarp exhibited strong bacteriostatic and bactericidal effects against *Listeria monocytogenes* and *Staphylococcus aureus.* Further examination of activity against *Listeria* species including *L. monocytogenes, L. grayi, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* and *S. aureus* revealed that this activity was greatly enhanced by lowering test pH from pH 7 to pH 4. The strength of inhibition was lower in aqueous extracts and chemical analysis indicated lesser concentrations of tartaric acid esters and flavonols. Measurement of propidium iodide uptake and ATP leakage indicated that all the extracts induced damage to the cell membrane of Gram-positive bacteria. This research showed that mangosteen fruit pericarp is a source of antimicrobials that could be of value for the control of undesirable Gram-positive bacteria in the food or food processing environment.

6.2 Introduction

Aqueous and alcoholic extracts of many plant species contain bioactive phytochemicals with antimicrobial activity. One such plant is the mangosteen tree (*Garcinia mangostana* L.*)* which produces a widely consumed fruit in southeast Asian countries. Mangosteen has a long history of medicinal use, primarily for the control of microbial infections. Chomnawang *et al.* (2005) evaluated the antibacterial activity of 19 medicinal plants from Thailand against *Staphylococcus epidermidis* and

Propionibacterium acnes, pus-forming bacteria that trigger inflammation resulting in acne symptoms. Mangosteen exhibited the most potent antimicrobial effects with minimum inhibitory concentrations (MIC) of 0.039 µg/ml against both species and minimal bactericidal concentrations (MBC) of 0.039 and 0.156 µg/ml against *P. acnes* and *S. epidermidis*, respectively. Voravuthikunchai and Kitpipit (2005) examined the activity of ethanolic extracts obtained from 10 traditional Thai medicinal plants against a *Staphylococcus aureus* strain resistant to multiple antibiotics (MRSA). Mangosteen extracts were among the most potent preparations tested (MIC = 0.05 µg/ml). Chomnawang *et al*. (2009) also tested extracts from Thai medicinal plants against MRSA and mangosteen extract again exhibited the strongest activity against MRSA (MIC and MBC = 1.95 and 3.91 µg/ml, respectively). These findings indicate that *Garcinia mangostana* tissues contain phytochemicals with potent antimicrobial activity.

The nature of compounds responsible for antimicrobial activity in plant tissues from all species including mangosteen is often unclear. Extracts prepared in alcohols or by steam distillation invariably contain high concentrations of phenolic compounds. Zadernowski *et al*. (2009) reported that the pericarp, rind and aril of the mangosteen fruit contain complex mixtures of free or bound phenolic acids, primarily protocatecuic acid, hydroxybenzoic acid and their derivatives. Various parts of the fruit or tree also contain important classes of secondary phenolic metabolites, notably prenylated and oxygenated xanthones and xanthen-9H-ones (Peres *et al*., 2000). Xanthones have been isolated from the mangosteen fruit pericarp, flesh, tree bark and leaf. One well characterized xanthone extracted from the species, α-mangostin, has been examined in some detail (Sundaram *et al.*, 1983). The α-mangostin exhibits strong activity against *S. aureus*, *P. aeruginosa*, *Salmonella* Typhimurium and *Bacillus subtilis*, and moderate activity against *Proteus* sp., *Klebsiella* sp. and *Escherichia coli*, with MIC measurements ranging between 12.5 and 50 µg/ml. Inhibition of MRSA has also been reported (Sakagami *et al.*, 2005). Mahabusarakam *et al.* (1986) showed that mangostins and other xanthones from mangosteen including gartanin, γ-mangostin, 1-isomangostin and 3-isomangostin inhibit *S. aureus*, including penicillin-resistant strains. Phongpaichit *et al*. (1994) and Iinuma *et al*. (1996) showed that MRSA is also very sensitive to the antimicrobial effects of α-mangostin. Other

prenylated xanthones from the pericarp of mangosteen fruit including garcinone B, demethylcalabaxanthone, trapezifolixanthone, garcinone D, mangostanin, mangostenone A and tovophyllin B also exhibit antimicrobial activity (Suksamrarn *et al.*, 2003). It is likely that the antimicrobial activity reported in crude mangosteen tissue extracts is due to the combined action of mixtures of compounds co-extracted from the plant tissues by solvents commonly used for the purpose.

Antimicrobials from natural sources have been widely investigated to fill a nascent demand for safe food products free of synthetic preservatives. While there is a considerable body of scientific literature on the antimicrobial properties of mangosteen against medically relevant microorganisms, there is comparatively little information about effects against microorganisms of significance in food preservation or food safety. Recently, Palakawong *et al*. (2010) showed that crude ethanolic extracts prepared from bark, leaf and fruit pericarp strongly inhibit Gram-positive bacteria but that Gram-negative species were comparatively less sensitive. The investigation was carried out in a defined microbiological medium at a single pH and temperature. Antimicrobial activity in phenolic compounds is known to be influenced by pH. For example, Cerrutti and Alzamora (1996) and Delaquis *et al*. (2005) showed that the antimicrobial activity of vanillin and vanillic acid is enhanced at pH values ≤ 7. Many common and highly perishable foods such as fresh red meat, fermented dairy products or fresh-cut fruits have pH values well below 7.0. Hence investigations of antimicrobial activity in mangosteen extracts against foodborne microorganisms with a view to their application in foods should include consideration of activity over a range of pH values. Furthermore, the application of natural antimicrobials in food preservation or safety enhancement is hampered by high costs in comparison to conventional preservatives. The latter is influenced by the cost of extraction and the need for purification to meet regulatory guidelines for solvent residues. The availability of less expensive, solvent free extracts could reduce production costs and stimulate applications for natural antimicrobials. One approach with the potential to lower production costs is hot water extraction (Sin *et al.*, 2006). The required equipment is relatively simple and the lack of a solvent eliminates the need for disposal of toxic processing waste and post-extraction solvent removal.

We report here on the antimicrobial activity of phenolic fractions extracted from the bark, leaf and fruit pericarp of mangosteen in methanol and hot water. Optimization techniques were used to maximize extraction of phenolic compounds with hot water. Antimicrobial effects were examined against Gram-positive and Gram-negative bacteria and fungi in laboratory media adjusted to pH values between 4.0 and 7.0. Attempts were made to ascribe antimicrobial activity to specific classes of phenolic compounds extracted by each method.

6.3 Materials and methods

Plant materials

Mangosteen bark, leaf and fruit pericarp collected from an orchard in Songkhla province, Thailand in April, 2009 were rinsed with water to remove soil. The tissues were cut into pieces (0.5-1.0 cm^2), dried overnight in a tray dryer at $45\ ^{\circ}$C and ground to yield material of approximately 18 mesh.

Preparation of extracts in methanol

Ground materials were extracted six times with 80% methanol as describes by Zadernowski *et al* (2009). The recovered supernatants were passed through filter paper (Whatman #4) to remove remaining bulk impurities before lyophilization at $30\ ^{\circ}$C. Dried extracts were stored in the dark at $4\ ^{\circ}$C until used.

Optimization of phenolic extraction in hot water

Extraction of phenolics from all tissues with hot water was optimized using response surface methodology as described by Haaland (1989). A 2 factor 5 level central composite experimental design was used consisting of thirteen runs including the independent variables temperature and water to solid ratio (Table 10). Extraction time was fixed at 30 min to minimize thermal degradation of phenolic compounds during extraction.

Table 10. Central composite experimental design with three variables for the extraction of phenolics from mangosteen bark, leaf and fruit pericarp.

Run	Temperature (^{o}C)	Water/solid ratio
1	31.2 (-1.41)	1:60 (0)
2	40 (-1)	1:40 (-1)
3	40 (-1)	1:80 (+1)
4	60 (0)	1:31.2 (-1.41)
5	60 (0)	1:88.2 (+1.41)
6	60 (0)	1:60 (0)
7	60 (0)	1:60 (0)
8	60 (0)	1:60 (0)
9	60 (0)	1:60 (0)
10	80 (+1)	1:40 (-1)
11	80 (+1)	1:80 (+1)
12	88.2 (+1.41)	1:60 (0)
13	88.2 (+1.41)	1:88.2 (+1.41)

Chemical analyses

The total phenolic content of extracts prepared for optimization studies was estimated using the method of Singleton and Rossi (1965). Results were expressed as gallic acid equivalents (GAE) in mg GAE/g on a dry weight basis. Where needed, the relative concentrations of the major classes of phenolics in the extracts were measured according to methods described in Mazza *et al.* (1999) as modified by Oomah *et al.* (2010) for total phenolics, tartaric esters and flavonols and Rakic *et al.* (2007) for tannins. Briefly, 100 µL samples or standards and 150 µL 0.1% HCl in 80% ethanol were added to the wells of ultraviolet flat-bottomed microtiter plates (Greiner Bio-One Inc., Frickenhausen, Germany). The solutions were mixed and absorbances were read at 280, 320, 360 and 520 nm with a spectrophotometer (Specramax Plus 384, Molecular Devices Corp, Sunnyvale, CA, USA). The absorbance (*A*) at 280 nm was used to estimate total phenolic content, *A*320 nm was used to estimate tartaric esters

and $A360$ nm to estimate flavonols. Gallic acid, caffeic acid, quercetin and cyanidin-3-glucoside in 80% aqueous ethanol were used as standards for total phenolics, tartaric esters, flavonols and anthocyanins respectively. All standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA) except cyanidin-3-glucoside from Extrasynthese (Genay Cedex, France).

Microorganisms and cultural conditions

Listeria monocytogenes, L. grayi, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri, Staphylococcus aureus, Salmonella Typhimurium, Escherichia coli, Botrytis cinerea (2 isolates), Penicillium expansum (2 isolates) and Saccharomyces cerevisiae were from the culture collection at the Pacific Agri-Food Research Centre, Summerland, BC, Canada. Stock bacterial and yeast cultures were maintained on plates at 4 ^{o}C on Trypticase Soy Agar (BBL, Cockeysville, Maryland, USA) supplemented with 5 g/l yeast extract (TSAYE). Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to tubes of Trypticase Soy Broth supplemented with 5 g/l yeast (TSBYE) followed by incubation without agitation at 30 ^{o}C for 24 h. Inocula for experiments were prepared by dilution with fresh TSBYE to achieve optical densities corresponding to 10^7 CFU/ml. Stock fungal cultures were maintained at 4 ^{o}C on Potato Dextrose Agar (PDA). Active fungal cultures were prepared on PDA agar incubated for 10 days at room temperature.

Microbial inhibition assays

Screening of extracts and measurements of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations against bacteria and yeast were accomplished using a broth dilution technique performed in 96-well microtitre plates according to Delaquis et al. (2002). Stock solutions of each extract in water were adjusted to pH 4, 5, 6 and 7 with 5 N HCl or 10 N NaOH. Suitably diluted aliquots (100 µL) were transferred to the wells of microtiter plates containing 100 µL TSBYE + 0.30% (w/v) agar adjusted to the desired pH to achieve final extract concentrations ranging from 0.02-2.50 mg/ml for methanol extracts and 0.08-10.00 mg/ml for hot water extracts. Inocula containing approximately 1.0×10^4 CFU/ml were then added to

each well. The plates were incubated at 30 ^{o}C for 24 h with the lids on and the wells were examined for the presence of growth. Minimum inhibitory concentration (MIC) was defined as the lowest concentration that prevented visible growth. Aliquots (10 µL) from wells without growth were transferred to TSAYE which was incubated for 24 h at 30 ^{o}C to permit recovery of viable bacterial cells. Minimum bactericidal concentration (MBC) was defined as the lowest concentration for which there was no evidence of growth after recovery. Three independent replicates performed with inocula prepared from fresh cultures were carried out for each MIC/MBC determination. Screening of extracts for antifungal effects was accomplished by the poisoned agar method. Extracts were added to cooled (50 ^{o}C) PDA at the desired concentration before dispensing into Petri plates. Mycelial plugs (approximately 2 mm) removed from active cultures with a sterile cork borer were added to the centre of the PDA plates. The plates were observed for evidence of radial growth after 5 days incubation at room temperature.

Assessment of membrane damage by propidium iodide uptake

Propidium iodide uptake was performed by a modification of methods described by Niven and Mulholland (1998). *L. monocytogenes* and *S. aureus* cells were obtained from overnight cultures (27 ml) prepared as described above. The cells were harvested by centrifugation at 6000 x g for 10 min and the resulting pellets were suspended in sterile phosphate buffer solution (PBS) adjusted to pH 7.4. The procedure was repeated twice and 10 ml of the final suspensions were added to glass test tubes containing 5 ml of extract (5 g/ml) and 5 ml of twice concentrated PBS. After one hour of incubation at 30 ^{o}C 4 ml of the treated cell suspensions were mixed with 4 ml of a propidium iodide solution (100 µM) in a fresh test tube which was held in the dark for 10 min at room temperature. The treated cell suspensions were then spun for 10 min at 6,000 x g, the pellets were suspended in fresh PBS and spun again at the same speed. Aliquots (200 µL) were transferred to a 96 well black color microtiter plates (Greiner bio-one, Fluotrac 200, Frickenhausen, Germany) and fluorescence was measured in a spectrophotometer (Gemini EM, molecular Device Corp. Sunnyvale, CA, USA) at the following settings: λ_{ex} = 490 nm, λ_{em} = 610 nm, cutoff = 590 nm. Cetyl trimethylammonium bromide (CTAB, 1 mM) and vanillic acid

(5 mg/ml) were used as positive and negative control, respectively. The experiments were performed three times with each species.

Assessment of membrane damage by ATP leakage

L. monocytogenes and *S. aureus* cells were obtained from overnight cultures prepared as described above. The cells were harvested by centrifugation at 6000 x g for 10 min and washed twice with phosphate buffer solution (PBS) adjusted to pH 7.4. Samples (10 ml) of the final cell suspensions were transferred to glass test tubes containing 5 ml of extract (5 g/ml) and 5 ml of twice concentrated PBS. After one hour incubation at 30 ^{o}C 4 ml were filtered by passage through 0.45 µm membranes. Relative ATP concentrations in the filtrates were measured using the NovaLum system from Charm Sciences Inc. (Massachusetts, USA). Leakage of ATP was deduced from comparisons with positive and negative controls. Experiments were performed three times with each species.

Statistical analyses

MIC and MBC data and results from chemical analyses were subjected to analysis of variance (ANOVA) using SPSS Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant. Data from optimization experiments were analyzed using the PROC GLM procedure of SAS (SAS Institute, Cary, NC, USA). Plots were fitted with Sigma Plot (Systat Software, San Jose, CA, USA) using the 3-D plot interpolation function for missing data.

6.4 Results and discussion

Extraction of phenolic fractions from mangosteen bark, leaf and fruit pericarp

Response surface methodology was used to optimize the extraction of phenolic compounds from mangosteen bark, leaf and fruit pericarp with water. Plots showing the influence of the independent variable temperature and solid to water ratio on the recovery of phenolics from each tissue are shown in Figure 13. Extraction from pericarp and bark was influenced by both parameters and yields increased linearly with temperature and solid to water ratio. Higher yields were obtained from bark (maximum 65.94±0.76 mg GA/g) than pericarp (45.33±0.37 mg GA/g), particularly at

higher extraction temperatures (Table 11). In contrast, the highest yields were obtained from leaf (106.21±0.63 mg GA/g) but extraction rate was not affected by temperature. Hence the major phenolic fraction in leaf appears to be soluble in water at temperatures ≤40 ^{o}C. Alternatively, it is possible that thermal degradation occurs at temperatures >40 ^{o}C as some phenolic compounds are heat-labile (Cacace and Mazza, 2002). Extracts were also obtained in methanol using a procedure reported to maximize recovery of phenolic compounds from mangosteen tissues (Zadernowski *et al.*, 2009). The procedure yielded bark, leaf and pericarp extracts containing 70.18±6.51, 37.68±2.98 and 110.24±9.12 mg GA/g total phenolics, respectively. Hence the relative ranking and yields obtained by extraction with methanol were similar to those achieved with water under optimum conditions.
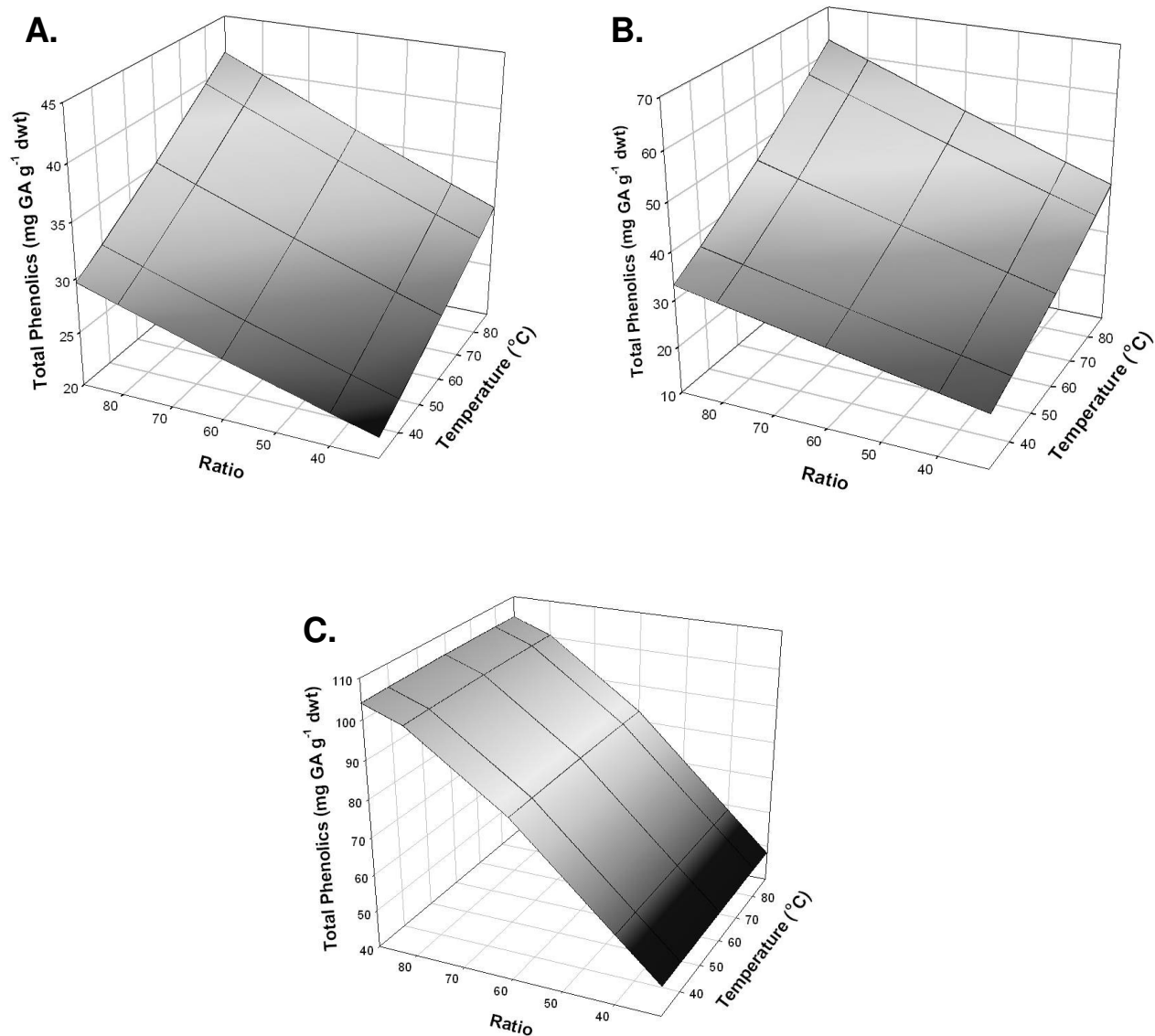
Figure 13. Response surface plots for the effect of water to solid ratio and temperature on total phenolics extracted from mangosteen A) pericarp, B) bark and C) leaf.

Table 11. Total phenolic concentration in aqueous extracts of mangosteen bark, leaf and fruit pericarp following extraction at various temperatures and water to solid ratios.

		Total phenolics (mg GA/g dwt)		
Temp.	Water/solid ratio	Bark	Leaf	Pericarp
31.2	60	33.65±0.80	83.20±0.13	26.80±0.07
40	40	22.32±0.21	59.51±0.15	26.32±0.25
40	80	33.45±0.52	99.11±0.24	29.93±0.25
60	31.2	32.58±0.17	46.75±0.24	26.01±0.14
60	88.2	40.98±0.83	105.26±0.22	33.80±0.04
60	60	40.95±0.58	83.93±0.63	28.44±0.22
60	60	40.70±0.39	83.95±0.49	29.96±0.37
60	60	41.91±0.19	84.82±0.45	29.29±0.70
60	60	40.74±0.59	85.01±0.25	30.12±0.19
80	40	43.20±0.23	58.46±0.36	30.01±0.24
80	80	60.42±0.25	97.37±0.55	35.23±0.17
88.2	60	55.54±0.37	83.01±0.13	36.33±0.31
88.2	88.2	65.94±0.76	106.21±0.63	45.33±0.37

Screening of antimicrobial activity in mangosteen bark, leaf and fruit pericarp extracts

Six extracts obtained in water (1: 60 ^{o}C 1:60 solid to water ratio for bark; 2: 80 ^{o}C: 1:80 bark; 3: 40 ^{o}C: 1:60 leaf; 4: 40 ^{o}C: 1:80 leaf; 5: 60 ^{o}C: 1:60 pericarp; 6: 80 ^{o}C: 1:80 pericarp) and the three methanolic extracts (7: MeOH bark; 8: MeOH leaf; 9: MeOH pericarp) were selected for screening. Antimicrobial effects against four fungal isolates, one yeast, two Gram-positive and two Gram-negative bacteria were examined at extract concentrations of 0.25 mg/ml in media adjusted to pH 5 and 7. Results shown in Table 12 revealed that none of the extracts could prevent the growth of the fungi, yeast or Gram-negative bacteria under these conditions. Inhibition was

restricted to the Gram-positive bacteria *L. monocytogenes* and *S. aureus*, notably at pH 5. Antifungal activity in mangosteen extracts has been reported previously although this was restricted to highly purified xanthone fractions from fruit pericarp (Gopalakrishnan *et al.,* 1997). The lack of activity against Gram-negative bacteria is in agreement with the findings of Priya *et al*. (2010) and Palakawong *et al*. (2010) and the common observation that essential oils and phenolic compounds are usually less active against Gram-negative than Gram-positive bacteria (Zaika, 1988; Shan *et al*., 2007; Soeksmanto *et al.* 2010). The sensitivity of Gram-positive bacteria has been attributed to greater ease of permeation into the cell due to differences in cell membrane architecture and constituents, notably the outer peptidoglycan layer which is a comparatively less effective permeability barrier (Scherrer and Gerhardt, 1971; Negi *et al*., 2008). In contrast, the outer phospholipidic membrane of Gram-negative bacteria is made up of structural lipopolysaccharide components which can make it impermeable to lipophilic solutes and porins that restrict the passage of hydrophilic solutes (Nikaido and Vaara, 1985).

Table 12. Screening of antimicrobial activity in aqueous and methanolic extracts of mangosteen bark, leaf and pericarp.

Extracts *	E. coli				Salmonella				L. monocytogenes				S. aureus				S. cerevisiae				B. cinerea 1		B. cinerea 2		P. expansum 1		P. expansum 2	
	CT	pH7	CT	pH5	CT	pH7	CT	pH5	CT	pH7	CT	pH5	CT	pH7	CT	pH5	CT	pH7	CT	pH5	CT	pH5	CT	pH5	CT	pH5	CT	pH5
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+

Note: CT (Control); +: clear evidence of growth; ±: weak growth ; -: no growth;

*1: 60 °C:1:60 bark; 2: 80 °C:1:80 bark; 3: 40 °C:1:60 leaves; 4: 60 °C:1:80 leaves; 5: 60 °C:1:60 pericarp; 6: 80 °C:1:80 pericarp; 7: MeOH bark; 8: MeOH leaves; 9: MeOH pericarp

Leaf extracts displayed little antimicrobial activity despite having the highest total phenolic content. Some investigators report correlation between the quantity of phenolic compounds extracted and antimicrobial activity in various plant tissues (Gursoy and Tepe, 2009; Alberto *et al*., 2006; Esekhiagbe *et al*., 2009; Kahkonen *et al*., 1999). There was no evidence of such a relationship in the present work, in agreement with the findings of Sengul *et al*. (2009) for extract prepared from the aerial parts of *Crocus sativus,*. Oliveira *et al*. (2008) studied the total phenolic content and antimicrobial activities in aqueous extracts prepared from the green husks of walnut (*Juglans regia* L.) from five different cultivars. Here total phenolic content ranging from 32.61 mg GA/g to 74.08 mg GA/g did not correlate with activity against *Bacillus subtilis,* a result the authors attributed to variability in the chemical

composition of extracts prepared from the various cultivars. Differences in composition were likely responsible for the lack of a relationship between phenolic content and antimicrobial activity in the mangosteen extracts examined in the present work.

Inhibition of Gram-positive bacteria by mangosteen extracts

The antimicrobial activity of pericarp and bark extracts prepared in water (60 °C, 1:60 ratio) and methanol against the Gram-positive bacteria *L. monocytogenes* and *S. aureus* was examined in greater detail at pH 4, 5, 6 and 7. Results presented in Table 13 show that MIC and MBC measurements varied according to source and solvent used for extraction, but that all extracts exhibited bacteriostatic activity at all test concentrations. Overall, pericarp extract prepared in methanol had the highest activity, followed by methanolic bark extract. MIC and MBC measurements were significantly affected by medium pH ($p > 0.05$) and the lowest measurements were achieved at pH 4. Aqueous pericarp and bark extracts were comparatively less effective although here the influence of medium pH was more pronounced. Aqueous bark extract, for example, had little activity against most *Listeria* species at medium pH>5. The effect of pH on the activity of plant extracts and individual phenolic compounds has been reported previously. Wen *et al.* (2003) showed that the antilisterial activity of caffeic acid increases with decreasing pH. This effect was ascribed to the influence of medium pH on the state of dissociation of the molecule which has a pKa of 4.45. The antimicrobial activity of weak organic acids increases at pH values approaching pKa because the proportion of undissociated molecules is highest, a condition that favors diffusion of the molecule through the negatively charged bacterial cell membrane (Cuchural *et al.*, 1988; Delaquis *et al.*, 2005).

Table 13. Minimum inhibitory concentrations (MIC, mg/ml) of mangosteen bark and pericarp extracts from aqueous and methanolic extractions.

Test microorganism	pH	Bark extract		Pericarp extract	
		Water	Methanol	Water	Methanol
S. aureus	4	2.08±0.72x c*	0.02±0.00x a	0.42±0.18x b	0.05±0.02x a
	5	6.67±2.89y d	0.31±0.00y b	4.17±1.44y c	0.08±0.00y a
	6	6.67±2.89y c	0.42±0.18y b	4.17±1.44y c	0.15±0.02z a
	7	8.00±2.89y d	0.42±0.18y b	4.17±1.44y c	0.11±0.04z a
L.monocytogenes	4	0.84±0.36x b	0.05±0.02x a	0.16±0.00w b	<0.02- -
	5	8.33±2.89y d	0.26±0.09y c	1.25±0.00x b	0.07±0.02x a
	6	10.00±0.00y c	0.26±0.09y a	2.50±0.00y b	0.16±0.01y a
	7	10.00±0.00y d	0.52±0.18z b	5.00±0.02z c	0.19±0.04y a
L.grayi	4	3.33±1.44x c	0.03±0.01x a	0.63±0.00x b	0.03±0.01x a
	5	10.00±0.00y d	0.63±0.00y b	2.50±0.00y c	0.08±0.00y a
	6	10.00±0.00y d	0.63±0.00y b	3.33±1.44y c	0.15±0.02z a
	7	10.00±0.00y d	0.84±0.36y b	3.33±1.44y c	0.10±0.02z a
L. innocua	4	0.84±0.36x b	0.11±0.05x a	0.26±0.09x a	<0.02- -
	5	10.00±0.00y d	0.26±0.09xy b	2.50±0.00y c	0.14±0.05x a
	6	10.00±0.00y c	0.52+0.18y a	6.67±2.89z b	0.52±0.18y a
	7	10.00±0.00y c	0.52±0.18y a	8.33±2.89z b	0.45±0.16y a
L. seeligeri	4	0.52±0.18x c	0.07±0.02x a	0.16±0.00x b	<0.02- -
	5	10.00±0.00y d	1.04±0.36y b	3.33±1.44y c	0.21±0.09x a
	6	10.00±0.00y d	1.04±0.36y b	3.33±1.44y c	0.29±0.04x a
	7	10.00±0.00y d	1.25±0.00y b	6.67±2.89z c	0.63±0.32y a
L.ivanovii	4	0.42±0.18x a	0.13±0.05x a	1.67±0.72x b	<0.02- -
	5	1.67±0.72y c	0.52±0.18y b	4.17±1.44y d	0.08±0.00x a
	6	3.75+2.17z c	0.52±0.18y b	6.67±2.89yz d	0.16±0.13x a
	7	4.00±1.44z c	0.63±0.00y b	8.33±2.89z d	0.13±0.05x a
L. welshimeri	4	0.84±0.36x b	0.02±0.00x a	0.63±0.00w b	<0.02- -
	5	10.00±0.00y d	0.26±0.09y b	1.25±0.00x c	0.12±0.04x a
	6	10.00±0.00y d	0.31±0.00y b	2.50±0.00y c	0.16±0.00x a
	7	10.00±0.00y d	0.31±0.00y b	5.00±0.02z c	0.21±0.09x a

Note: *mean±SD from 3 replicates followed by the same letter (x, y, z) in the same column within each microorganism are not significant difference ($p>0.05$), the same letter (a, b, c) in the same row within each pH are not significant difference ($p>0.05$).

Table 14. Minimum bactericidal concentrations (MBC, mg/ml) of mangosteen bark and pericarp extracts from aqueous and methanolic extractions.

Test microorganism	pH	Bark extract		Pericarp extract	
		Water	Methanol	Water	Methanol
S. aureus	4	8.33±2.89x c*	0.52±0.18x ab	3.33±1.44x b	0.19±0.03x a
	5	10.00±0.00x c	1.67±0.72y a	8.33±2.89y c	2.50±0.00y b
	6	10.00±0.00x c	1.67±0.72y a	8.33±2.89y c	2.50±0.00y b
	7	10.00±0.00x c	1.67±0.72y a	8.33±2.89y c	2.50±0.00y b
L.monocytogenes	4	>10.00- -	0.63±0.00x a	>10.00- -	0.43±0.07x a
	5	>10.00- -	1.67±0.72y a	>10.00- -	2.09±0.36y b
	6	>10.00- -	1.67±0.72y a	>10.00- -	2.50±0.00z b
	7	>10.00- -	2.5±0.00z a	>10.00- -	2.50±0.00z a
L.grayi	4	10.00±0.00x c	0.16±0.00x a	3.33±1.44x b	0.24±0.08x a
	5	10.00±0.00x b	1.67±0.72y a	8.33±2.89y b	1.46±0.36x a
	6	>10.00- -	2.50±0.00z a	10.00±0.00y b	2.29±0.36y a
	7	>10.00- -	2.50±0.00z a	10.00±0.00y b	2.29±0.36y a
L. innocua	4	>10.00- -	0.63±0.00x b	>10.00- -	0.17±0.06x a
	5	>10.00- -	1.25±0.00x a	>10.00- -	2.50±0.00y b
	6	>10.00- -	1.25±0.00x a	>10.00- -	2.50±0.00y b
	7	>10.00- -	2.08±0.72y a	>10.00- -	2.50±0.00y a
L. seeligeri	4	>10.00- -	0.31±0.00x b	>10.00- -	0.17±0.02x a
	5	>10.00- -	2.50±0.00y b	>10.00- -	1.81±0.73y a
	6	>10.00- -	2.50±0.00y b	>10.00- -	2.09±0.36y a
	7	>10.00- -	2.50±0.00y a	>10.00- -	2.50±0.00y a
L.ivanovii	4	>10.00- -	0.52±0.18x b	>10.00- -	0.16±0.00x a
	5	>10.00- -	1.25±0.00y b	>10.00- -	0.66±0.26x a
	6	>10.00- -	1.67±0.72y b	>10.00- -	2.50±0.00y a
	7	>10.00- -	2.50±0.00z a	>10.00- -	2.15±0.60y a
L. welshimeri	4	>10.00- -	0.26±0.09x a	>10.00- -	0.31±0.00x a
	5	>10.00- -	1.25±0.00y a	>10.00- -	2.09±0.35y b
	6	>10.00- -	1.67±0.72y a	>10.00- -	2.50±0.00z b
	7	>10.00- -	1.67±0.72y a	>10.00- -	2.50±0.00z b

Note: *mean±SD from 3 replicates followed by the same letter (x, y, z) in the same column within each microorganism are not significant difference ($p>0.05$), the same letter (a, b, c) in the same row within each pH are not significant difference ($p>0.05$).

Methanolic mangosteen pericarp and bark extracts evidently contain potent inhibitors of Gram-positive bacteria. Vaquero *et al.* (2007) studied the antimicrobial properties of polyphenols extracted from wine including the flavonoids rutin, catechin

and quercetin and the non-flavonoid gallic, vanillic, protocatechuic and caffeic acids against *L. monocytogenes*. Caffeic acid exhibited the strongest activity and 0.5 mg/ml was reported to completely inhibit growth, although the effect of pH was not considered. In the present work MIC measurements well below this value were obtained with pericarp extract prepared in methanol, particularly at low pH. Aqueous extracts were comparatively less effective and both MIC and MBC values were significantly higher under all experimental conditions ($p>0.05$). Weaker antimicrobial activity in plant extracts obtained with water compared with other solvents including alcohols or non-polar solvents has been reported previously. For example, Bassam *et al*. (2004) showed that methanol or ethanol extracts of *Syzyium aromaticum*, *Cinnamomum cassi*, *Salvia officinalis*, *Thymus vulgaris* and *Rosmarinus officinalis* were more effective against a range of microorganisms than those prepared in hot water. Baram *et al*. (2010) studied the antimicrobial activity of ethanol, methanol, water and acetone extracts of *Tulipa sintenisii* (Baker). All exhibited antimicrobial effects against *S. aureus*, *E. coli* and *Pseudomonas syringae* but acetone extracts had the highest activity.

Variability in potency is usually ascribed to differences in the relative amount and composition of phenolic compounds extracted with specific solvents. Some plant tissues yield fractions of comparatively simple composition and the source of antimicrobial activity can be deduced with some certainty. In contrast, mangosteen tissues contain heterogeneous mixtures of molecules belonging to several major groups of phenolic compounds (Zadernowski *et al.*, 2009). Chemical analysis was therefore carried out with a view to identify differences in the major classes of phenolic compounds recovered with the two solvents. Table 15 shows the concentrations of total phenolics, tartaric esters, flavonols and tannins in the water and methanol extracts. Anthocyanins were not detected in the samples. The method used for these analyses can only provide estimates of tannin concentration due to interference by non-phenolic compounds during absorbance measurements. It was therefore not possible to calculate accurate percentages of each class of phenolics. Nevertheless the results indicated that extraction of pericarp with methanol yielded the highest relative concentrations of tartaric esters and flavonols, followed by bark extract. These classes of phenolics were also present in aqueous extract prepared from

pericarp, although in comparatively lower concentrations and aqueous bark extract contained the lowest amounts. Hence the relative activity of mangosteen extracts against Gram-positive bacteria was strongest in extracts with high tartaric ester and flavonol contents. Interestingly, tartaric acid and tartaric acid derivatives are found in significant amounts in mangosteen pericarp (Zadernowski *et al.*, 2009). The antimicrobial activity of tartaric acid and common phenolic acids with pKa values ≤5 is known to increase with decreasing pH (Wen *et al.,* 2003). The flavonol group also includes well known antimicrobial compounds however, including xanthones which have been shown to impart activity to mangosteen pericarp extracts. Hence activity against Gram-positive bacteria reported in the present work was likely due to the combined action of several phenolic compounds.

Table 15. Major classes of phenolics in mangosteen bark and pericarp extracts from aqueous and methanolic extraction.

Extracts	Total Phenolics mg GA/g dwt	Tartaric esters mg CA/g dwt	Flavonols mg QU/g dwt	Tannins (estimate) mg GA/g dwt
Bark-water extract	67.95±2.36b	4.70±0.27b	4.01±0.25b	44.86±1.87d
Pericarp-water extract	35.87±0.37a	3.51±0.06a	2.19±0.05a	29.69±0.45a
Bark-methanol extract	69.11±0.88b	16.80±0.70c	7.63±0.16c	38.26±1.87b
Pericarp-methanol extract	67.41±4.36b	49.52±3.44d	22.37±1.27d	35.08±2.22c

Note: GA (Gallic acid), CA (Caffeic acid), QU (Quercetin)

*mean±SD from 3 replicates followed by the same letter in the same column are not significant difference (*p*>0.05).

Mode of action of mangosteen extracts

Phenolic compounds are believed to induce lesions in cell membranes that initiate a series of events that can lead to cell death (Palombo and Semple, 2001; Shah *et al.*, 2004). The effects of mangosteen extracts on membrane integrity were verified using two independent methods based on the uptake of propidium iodide (PI) or leakage of ATP by cells with compromised membranes. The phenolic vanillic acid which has no antimicrobial activity against Gram-positive bacteria at neutral pH (Delaquis *et al.*, 2005) was used as a negative control and cetyl trimethylammonium served as a positive control for the measurements. Table 16 reveals that uptake of PI by *L. monocytogenes* and *S. aureus* cells was dramatically enhanced by exposure to pericarp and bark methanol extracts. Relative PI uptake was higher than in control samples treated with the powerful cell membrane disruptor CTAB (Niven and Mulholland, 1998). PI uptake by cells exposed to aqueous extracts was comparatively lower, although differences with controls indicate that some membrane damage occurred. Leakage of ATP by cells treated with the extracts is illustrated by measurements summarized in Table 17. Extracellular ATP concentrations were clearly higher in treated samples and methanol extracts again induced increases in excess of those obtained with the positive control. These observations provided strong evidence that mangosteen extracts caused injury to the Gram-positive cell membrane and that the extent of damage was greatest in methanol extracts.

Table 16. Propidium iodide uptake (in fluorescence units) by *L. monocytogenes* and *S. aureus* cell suspensions treated with mangosteen extracts.

Treated substrate	*L. monocytogenes*	*S. aureus*
Negative control	18.11±3.34a*	12.24±2.68a
CTAB	194.28±21.87e	163.79±23.61f
Vanillic acid	24.26±3.45b	12.98±1.66b
Bark-water extract	33.52±4.62c	54.72±3.09c
Pericarp-water extract	50.42±5.38d	59.78±3.74d
Bark-methanol extract	270.53±17.56f	134.60±6.42e
Pericarp-methanol extract	312.02±15.53g	231.21±14.92g

Note: * mean±SD from 3 replicates followed by the same letter within column are not significantly different (*p*>0.05).

Table 17. Relative ATP concentrations (derived from fluorescence measurements) in *L. monocytogenes* and *S. aureus* cell suspensions treated with mangosteen extracts.

Treated substrate	*L. monocytogenes*	*S. aureus*
Negative control	40.91±1.34a*	8.61±0.26a
CTAB	1,086.02±7.63e	124.32±3.01c
Vanillic acid	61.18±1.52b	27.39±0.66b
Bark-water extract	554.99±20.59d	920.11±31.46e
Pericarp-water extract	73.00±1.37c	178.94±5.12d
Bark-methanol extract	2,175.36±44.60f	1,610.35±84.08f
Pericarp-methanol extract	5,082.02±45.16g	3,214.63±81.28g

Note: * mean±SD from 3 replicates followed by the same letter within column are not significantly different (*p*>0.05).

6.5 Conclusions

Mangosteen bark, leaf and pericarp extracts recovered in methanol had consistently greater antimicrobial activity than those prepared with water. Activity was highest in extracts recovered from the pericarp and this waste material could represent a potential source of antimicrobials for the control of Gram-positive bacteria. Strong effects against *Listeria* species were noteworthy given the need for improved strategies for the control of *L. monocytogenes* in food or in the food processing environment. Chemical analysis indicated that the reduced activity of aqueous extract was due to poor extraction of tartaric acid esters and flavonols. The aqueous extraction process used in this work was performed at atmospheric pressure at temperatures <100 ℃. Recent research indicates that the efficiency and selectivity of phenolic extraction in water can be improved by subcritical fluid extraction. It may be possible to exploit these new technologies for the production of mangosteen pericarp extracts with the desired composition and antimicrobial activity.

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CHAPTER 7

EFFECT OF MANGOSTEEN PERICARP EXTRACT ON THE PHYSICAL, CHEMICAL AND MICROBIOLOGICAL QUALITIES OF FRESH-CUT MANGOSTEEN

7.1 Abstract:

The effect of a mangosteen pericarp extract on the physical and chemical properties of fresh-cut mangosteen stored in PP trays at 5 $^{\circ}$C with 85% RH was investigated. Mangosteen pericarp extract did not affect headspace gas composition (O_2 and CO_2), fruit firmness, weight loss, acetaldehyde or ethanol contents, but the lightness and hue values of the tissue were altered. Fresh-cut mangosteen dipped in 0.25 g/l of the pericarp extract retained lightness and hue values better than the control. The extract could not inhibit *S. aureus* or *E. coli* inoculated onto fresh-cut mangosteen.

7.2 Introduction

The tissues of fruits and vegetables are rich in bioactive phytochemicals, particularly the phenolic group which includes compounds with antioxidant and antimicrobial activities (Ajila *et al*., 2010). Thus, the microbiological quality and antioxidant value of fresh-cut fruits could be improved by using phytochemicals derived from the fruit itself as processing aids.

The microbiological quality of fresh-cut fruits is affected by unit operations during processing and notably by the cutting process, which can cause contamination by bacteria, fungi and yeast (Raybaudi-Massilia *et al*., 2009). Microbiological safety and quality have always been important to consumers and continue to be a basic requirement of any modern food system. Consequently chemicals have been used to control microbial decay in fresh-cut produce. However, most of these compounds do not satisfy the concepts of "natural" and "healthy" that consumers prefer and that the food industry therefore needs to provide (Marriott, 2010).

Fruits yield between 25% and 30% of non-edible products (Ajila *et al*., 2007; Ajila *et al*., 2010). The by-products of most fruits are made up of skins and

seeds of different shapes and sizes that normally have no further use and are commonly discarded (Ajila *et al*., 2007). This could represent an important environmental problem if it is not addressed by the industry (Ajila *et al*., 2010). It should be noted that fresh-cut processing generates considerable amounts of waste and the possibility of creating alternative processes to give added value to this material must be considered.

The application of bioactive extracts from processing waste as alternatives to chemical preservatives could help to achieve consumer demand for fresh-cut fruit free of synthetic chemicals. The most abundant by-product of minimal processing of fresh-cut mangosteen is the pericarp which is reported to contain high amounts of phenolic compounds with antioxidant and antimicrobial properties (Palakawong *et al*., 2010). These compounds could be of use in fresh-cut mangosteen processing and preservation. There has been little research on the preservation of fresh-cut fruits with antimicrobials from plant sources. The following describes the effects of extracts recovered from mangosteen pericarp on the storage quality and microbiology of the fresh-cut fruit.

7.3 Materials and methods

Preparation of the extract

Mangosteen fruit collected from an orchard in Songkhla province, Thailand in April, 2009 were rinsed with water to remove dust and other impurities. The pericarps were removed and cut into pieces (0.5-1.0 cm^2), dried overnight in a tray dryer at 45 °C and ground to yield material of approximately 18 mesh. Ground materials were extracted six times with 80% methanol as describes by Zadernowski *et al*. (2009). The recovered supernatant was passed through filter paper (Whatman #4) to remove remaining bulk impurities before lyophilization at 30 °C. Dried extract was dissolved in water (0.25 g/l) at room temperature and filtered through Whatman #4 filter paper prior to use.

Mangosteen processing

A southern cultivar of mangosteen fruit (*Garcinia mangostana*, L) was grown in an orchard in Songkhla province, Thailand by a commercial grower. Fruit with a

mean weight of 120+5 g were hand-harvested during July-August 2011 at stage 3 (reddish pink) according to the scales described by Palapol *et al*. (2009). The crop was packed and transported to the laboratory by a truck without temperature control taking 30 min from the orchard, (approximately 25 km). The fruit stalks and calyx ends were removed with a sharp knife and the trimmed fruit were cleaned by hand under running tap water. A lateral incision was made around the fruit to remove the white arils which were immediately placed in cold water, 10 ^{o}C (control) or a solution containing 0.25 g/l mangosteen extract for 1 min. The fresh-cut mangosteen were then dried, packed in PP trays (11.5×17.5×4.5 cm) and sealed with OPP/LLDPE film with OTR of 1,160 cm^3/(m day) and placed in an incubator at 5 ^{o}C with 85% RH. Samples were removed after 0, 3, 6, 9 and 12 days to measure headspace gas concentrations, physical and chemical properties.

Headspace gas analysis

O$_2$, CO$_2$ and C$_2$H$_4$ concentrations were measured by withdrawing air samples (1 ml) through a septum using a gas-tight syringe. The samples were injected into a gas chromatograph (GC) (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a thermal conductivity detector (TCD). The oven and detector temperatures were 60 and 150 ^{o}C, respectively, with helium as the carrier gas. The C$_2$H$_4$ production was measured with the same GC but equipped with a flame ionization detector (FID) on 1 ml gas sample. The oven, injector and detector temperatures were 40, 120 and 180 ℃, respectively, with helium as the carrier gas. Gas concentrations were expressed as %O$_2$, %CO$_2$ and ppm C$_2$H$_4$.

Measurement of physical properties

Firmness was evaluated using a TA-XT2i texture analyzer (Stable Micro Systems, England) with a 25 kg load cell, equipped with a 2 mm diameter cylinder stainless steel probe (P/2). The measurements were taken as the maximum force value obtained during the penetration of probe into the fruit for 3 mm at 2.0 mm/s (Troncoso *et al*., 2009) and expressed as g/mm^2 force.

Weight loss was determined by weighing all samples with a Metler balance, model AB204 (Mettler-Toledo Inc., USA) at the beginning and end of the storage period. The difference between the values was used to calculate weight loss.

Fresh-Cut color was measured with a colorimeter (Miniscan 45/0-L, Hunter Associates Laboratory, Inc., USA) calibrated with a standard white tile with the following parameters: X = 79.6, Y = 84.4, Z = 89.9 with illuminant D/65/10°, (light source used for the daylight), according to CIE L* (lightness), a* (green to red) and b* (blue to yellow) values. Numerical values of a* and b* were converted into chroma $[C = (a*^2+b*^2)^{1/2}]$ and hue angle $[H^o = tan^{-1} b*/a*]$ (Francis, 1980).

Chemical analyses

Acetaldehyde and ethanol content were measured using the method of Gonzalez-Aguilar *et al*. (2004). In brief, tissue (5 g) was placed in amber colored bottles with 60 ml capacity and placed in a 65 ℃ water-bath for 15 min. Headspace samples of 1 ml were injected into a gas chromatograph (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a 60 m × 0.325 mm × 0.25 µm DB-WAX column (J&W Scientific, Folsom, California). The oven, injector and detector temperatures were 60, 250 and 250 ℃, respectively, with helium as the carrier gas. Retention times and standard curves of acetaldehyde and ethanol in water solutions were used for peak identification and quantification.

Sensory evaluation

A paired difference test was used to assess differences in color, off-odor, firmness, wateriness and overall visual quality. A 30 member panel without training evaluated paired of untreated (control) and treated samples as described by Meilgaard *et al*. (1999). Briefly, treatment and control samples from each day of storage were evaluated by the panelists in a testing room. 'Difference' or 'no difference' was recorded on a paper ballot. Chi-square test was used to analyses the data.

Microorganisms and inoculation

Escherichia coli DMST 15537 and *Staphylococcus aureus* DMST 6512 were inoculated onto fresh-cut mangosteen to evaluate potential antimicrobial effects of the extracts. *S. aureus* and *E. coli* were cultured for 24 h at 37 ^{o}C in 100 ml tryptic soy broth amended with 5 g/l yeast extract. Inocula for experiments were prepared by centrifugation of cultures at 3,000 rpm for 15 min. Where required, inoculation was performed by exposing fresh-cut mangosteen to a cell suspension of *E. coli* and *S. aureus* mixed in a plastic bag. The contents were gently tossed and rolled for 1 min to distribute the inoculum evenly over the fruit surfaces. This procedure yielded initial populations of approximately 10^5 CFU/g confirmed by plating on Eosin methylene blue (EMB) agar and Baird–Parker (BP) agar for *E. coli* and *S. aureus*, respectively. Treated fruit were dipped in the pericarp solution 1 min, placed in PP trays and sealed with OPP/LLDPE film. Control fruit were packed without further treatment. All the samples were placed in an incubator at 5 ^{o}C. Samples were removed for enumeration of each species after 0, 3, 6, 9 and 12 days in storage by plating on EMB agar and BP agar as mentioned above at 37 ^{o}C and mean populations at each sampling time were calculated in triplicate.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and mean differences estimated by Duncan's new multiple range test (DMRT) using SPSS Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant.

7.4 Results and discussion

Changes in headspace gas composition

The concentrations of gases in the headspace above fresh-cut mangosteen were monitored over 12 days of storage. Carbon dioxide and oxygen content were not significantly different ($p > 0.05$) (Figure 14). Hence the mangosteen pericarp extract did not affect the respiration rate of the samples. Martin-Diana *et al*., (2008) studied the effect of green tea extract on respiration of fresh-cut lettuce. Significantly ($p \leq 0.05$) lower oxygen and higher carbon dioxide levels were observed in samples

treated with high concentrations of the extract (0.5 and 1.0 g/l) compared to those treated with the lowest concentration (0.25 g/l). Some phenolics extracted from plants can induce stress at high concentrations (Surjadinata and Cisneros-Zevallos, 2003). Only one concentration (0.25 g/l) of extract was examined in the present work and it appears that this was not sufficient to induce stress-associated increases in respiration. This may be an advantage in fresh-cut mangosteen since high respiration rates are associated with declines in product quality during storage. A similar conclusion was reached by Xu *et al.* (2009) who reported that lesser respiration and ethylene production rates in 'Redglobe' grapes treated with grapefruit seed extract would provide advantages during storage.
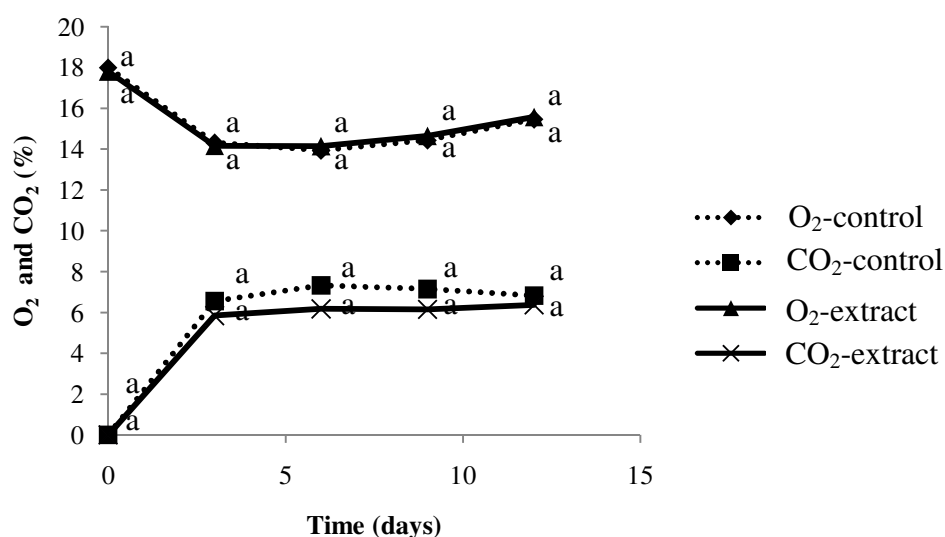
Figure 14. Changes in O_2 and CO_2 concentrations in the headspace of mangosteen pericarp extract treated fresh-cut mangosteen during storage. The same letter within storage day in each gas means no significant difference ($p > 0.05$).

Changes in physical properties

    Firmness

       Changes in firmness were monitored throughout the 12-day storage period (Figure 15). No significant differences ($p > 0.05$) in firmness were observed between the samples in each day of storage. The pericarp mangosteen extract did not affect

firmness of fresh-cut mangosteen, consistent with previous report for pears (Xiao *et al*., 2010). In those report, the combined effects of chitosan coating containing 0.03% rosemary extracts on the quality of fresh-cut pears were investigated. There were no significant variations between the control and rosemary sample.
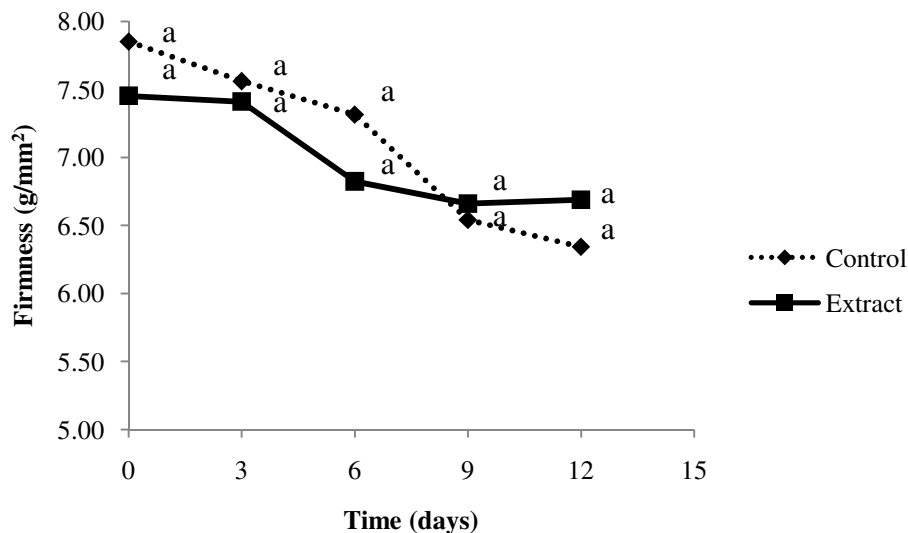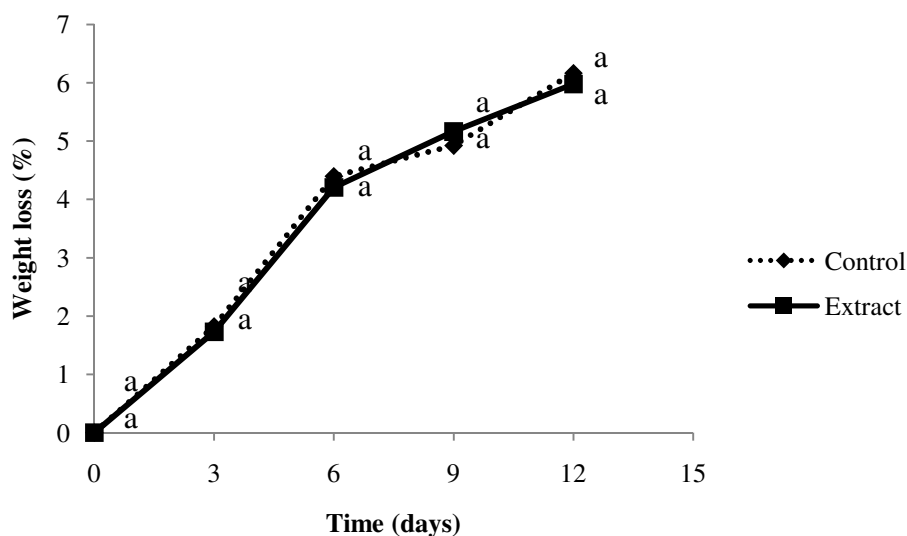

Figure 15.   Flesh firmness of mangosteen pericarp extract treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p>0.05$).

Weight losses

Treatment with the mangosteen pericarp extract did affect weight losses of fresh-cut mangosteen during storage (Figure 16). These findings agree with those reported by Xiao *et al*. (2010) following studies on fresh-cut pear treated rosemary extract. No significant differences were found between control and rosemary sample.
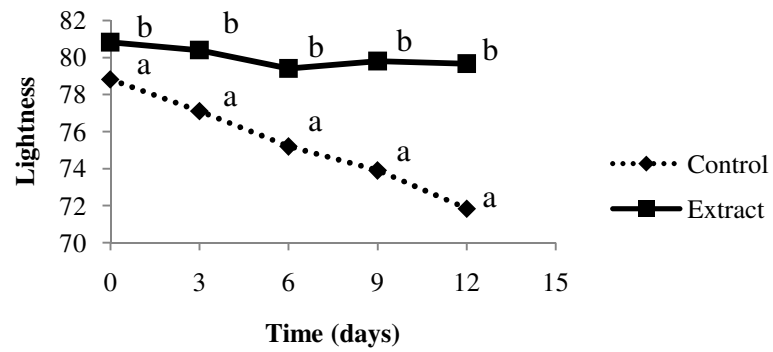
Figure 16.   Weight losses of mangosteen pericarp extract treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p > 0.05$).

Changes in color

Color was monitored in all the samples during the entire storage period. Lightness values (L*) decreased during storage, a change which normally correlates with the appearance of browning (Martin-Diana *et al.*, 2008). Prior treatment with the mangosteen pericarp extract significantly reduced losses in lightness ($p \leq 0.05$) (Figure 17A). Contrary outcomes were reported by Martin-Diana *et al.* (2008) with a green tea extract which decreased lightness values in stored fresh-cut lettuce. In the latter work, higher browning levels in the lettuce were induced by higher green tea extract concentrations. Browning can be caused by the direct oxidation of pigments or by the activation of specific enzyme pathways in plant tissues such as polyphenol oxidase, catechol oxidase leading to the formation of melanins and benzoquinone, resulting in a brown color. It is unclear which mechanism was responsible for browning in stored fresh-cut lettuce and mangosteen in the two studies. However the results reported here point to different mechanisms given variance in response to treatment with plant extracts. Palakawong *et al.* (2010) studied the antioxidant activity of mangosteen pericarp extracts. Activity (IC_{50}) evaluated using the DPPH method was 5.94 μg/ml compared with 4.30 μg/ml for ascorbic acid. It appears that

antioxidant activity in the mangosteen extract was responsible for the improved retention of lightness in the stored fresh-cut fruit. Green-tea extracts are also well known to contain phenolics with strong antioxidant activity but this property evidently does not to provide control over mechanisms responsible for browning in fresh-cut lettuce.
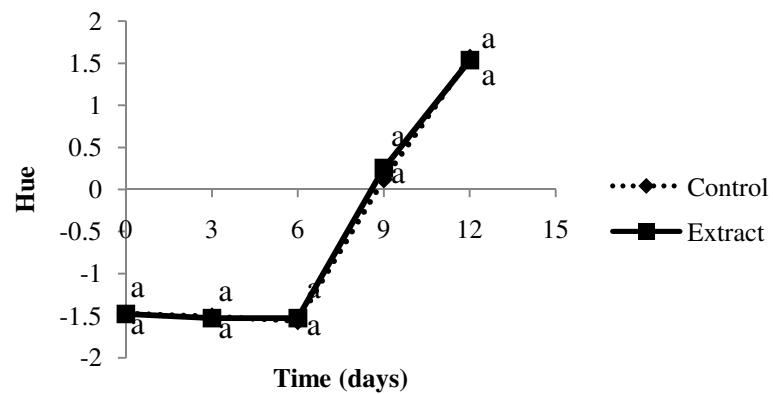
Several substances have been used in the food industry to prevent browning. Ascorbic acid and derivatives are well known to retard this process. The results of the present study suggest that mangosteen pericarp extract could be used for this purpose in fresh-cut processing of mangosteen fruit.

A)
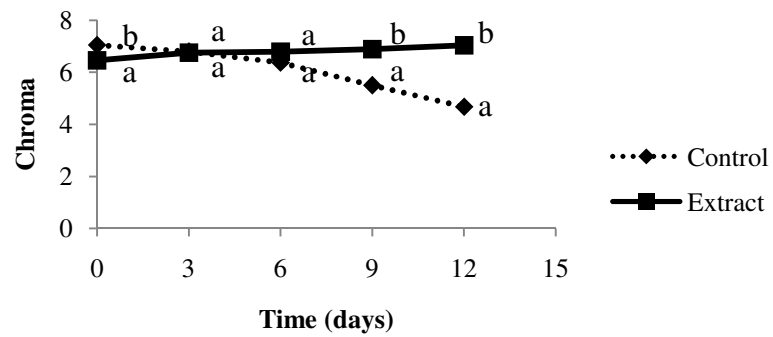
B)

C)

Figure 17. Color changes of mangosteen pericarp extract treated fresh-cut mangosteen during storage A) lightness, B) chroma and C) hue values. The same letter within storage day means no significant difference ($p > 0.05$).

The other parameters related to color are the chroma and hue value. A significant decrease in chroma value indicative of browning was observed in fresh-cut mangosteen not treated with the pericarp extract (Figure 17B). This value confirmed that the extract can decrease browning in the stored fresh-cut fruit. The extract did not show an effect on hue value and no difference ($p>0.05$) was found between the control and treatment (Figure 17C).

Chemical changes

Treatment with the mangosteen pericarp extract did not affect the accumulation of acetaldehyde or ethanol in the tissues of stored fresh-cut mangosteen (Figure 18 and 19).
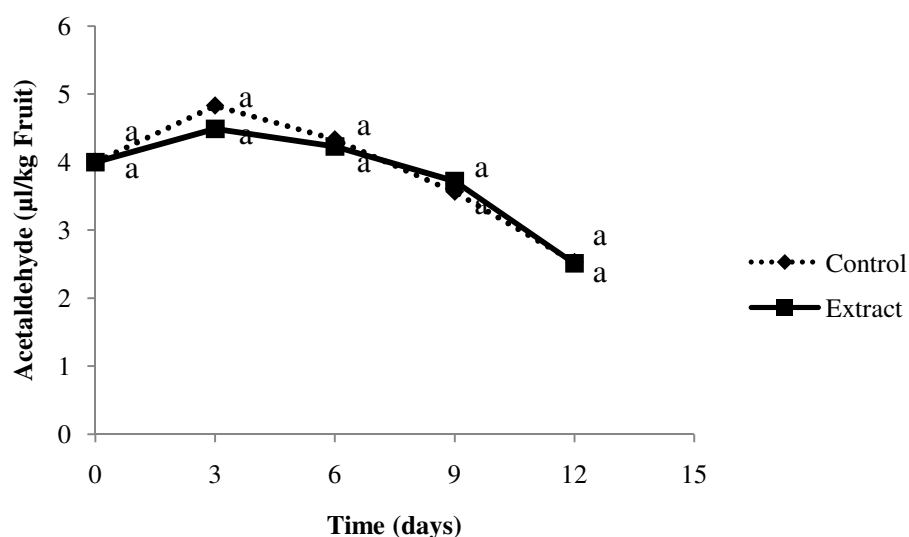

Figure 18. Acetaldehyde content of mangosteen pericarp extract treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p>0.05$).
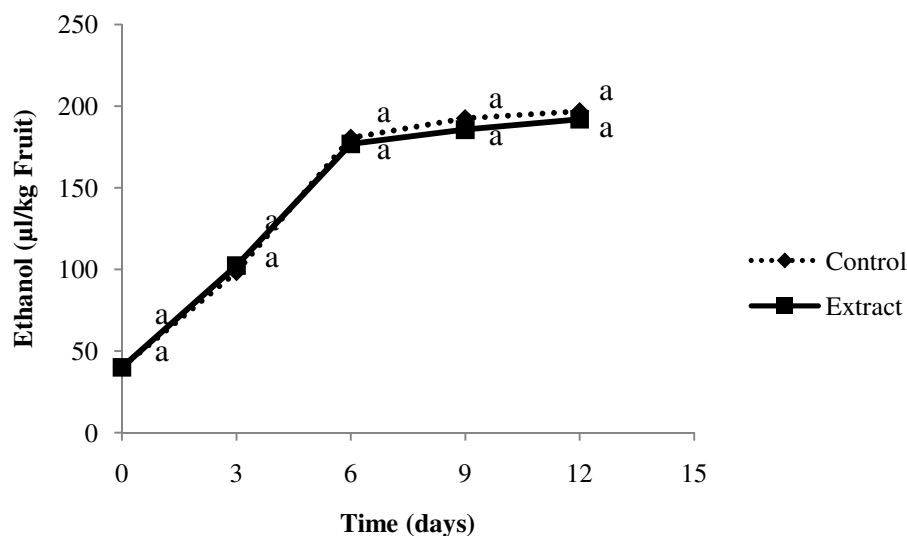
Figure 19. Ethanol content of mangosteen pericarp extract treated fresh-cut mangosteen during storage. The same letter within storage day means no significant difference ($p>0.05$).

Sensory analysis

Color, off-odor, firmness, wateriness and overall visual quality of the samples were evaluated by a sensory panel. The panelists did not detect treatment induced differences in any of the parameters. Hence mangosteen pericarp extracts did not affect the sensory properties of fresh-cut mangosteen. The effect of a green tea extract on the sensory properties of fresh-cut lettuce was investigated by Martin-Diana *et al.* (2008). The extract had a significant effect on the appearance of the lettuce, particularly at the end of the storage period. The extract concentration used to treat the fruit in the present study was comparatively small, which hints that the dosage applied in preservation of fresh-cut produce has to be maintained at low levels to avoid changes to sensory quality.

Effect of mangosteen pericarp extracts on microorganisms in stored fresh-cut fruit

Neither *E. coli* nor *S. aureus* grew on fresh-cut mangosteen stored at 5 °C and *S. aureus* populations declined slightly over time. There was no convincing evidence that the extracts could exert antimicrobial effects against *E. coli* or *S. aureus* (Figure 20). The lack of effect against *E. coli* was not unexpected given widespread reports

that plant extracts, including those prepared from mangosteen (present work), have weak activity against Gram-negative bacteria. Gram-positive bacteria including *S. aureu* are reported to be more sensitive (present work) but it is clear that the concentrations applied here were not sufficiently high to induce antimicrobial activity that could lead to losses in viability. Furthermore, the solubility of plant extracts in water can be highly variable as different solvents used for extraction can lead to changes in the composition that affect solubility. The pericarp extract used in this work was obtained by methanol extraction. Hence it is possible that reduced antimicrobial activity on fresh-cut mangosteen was related to restricted solubility.
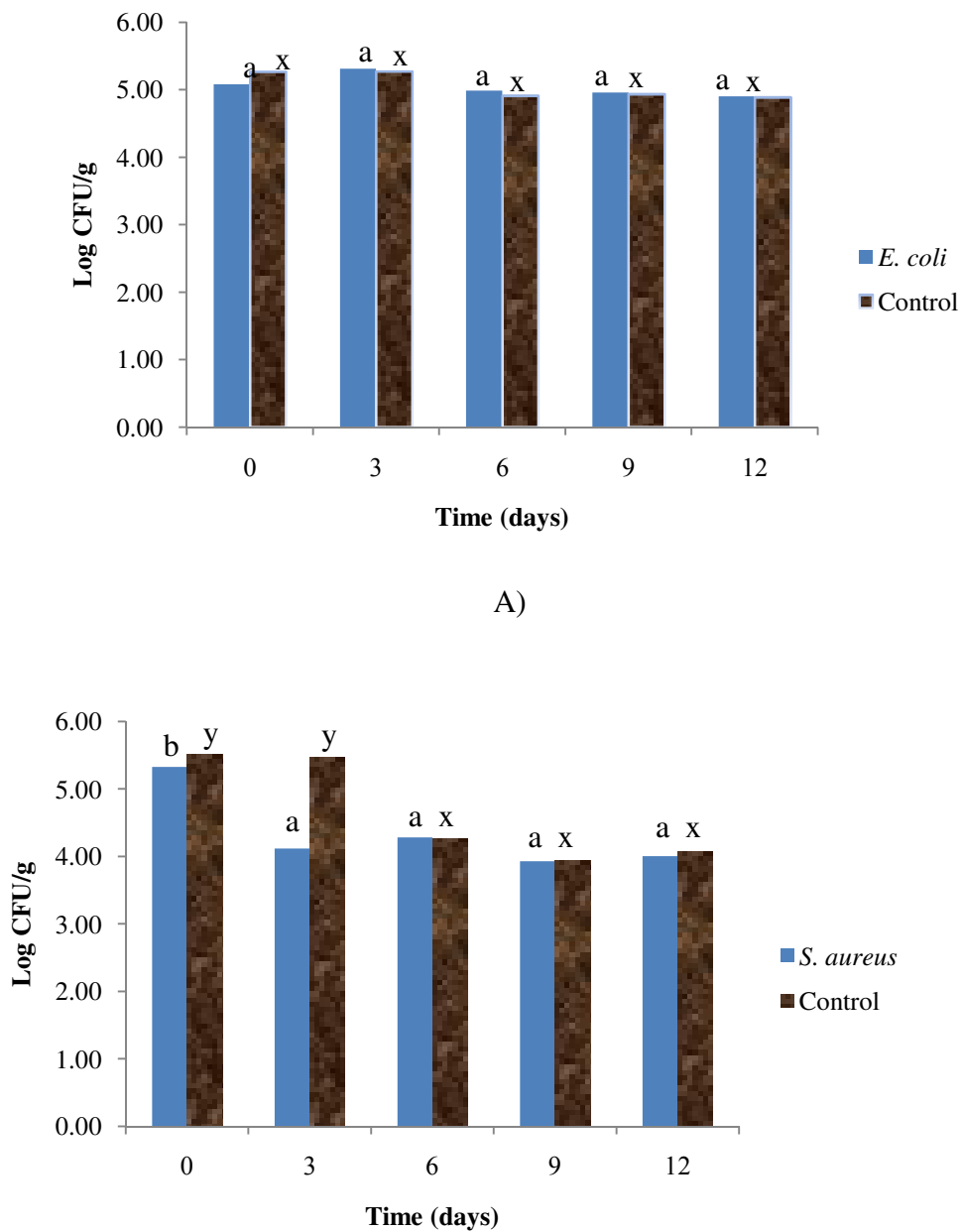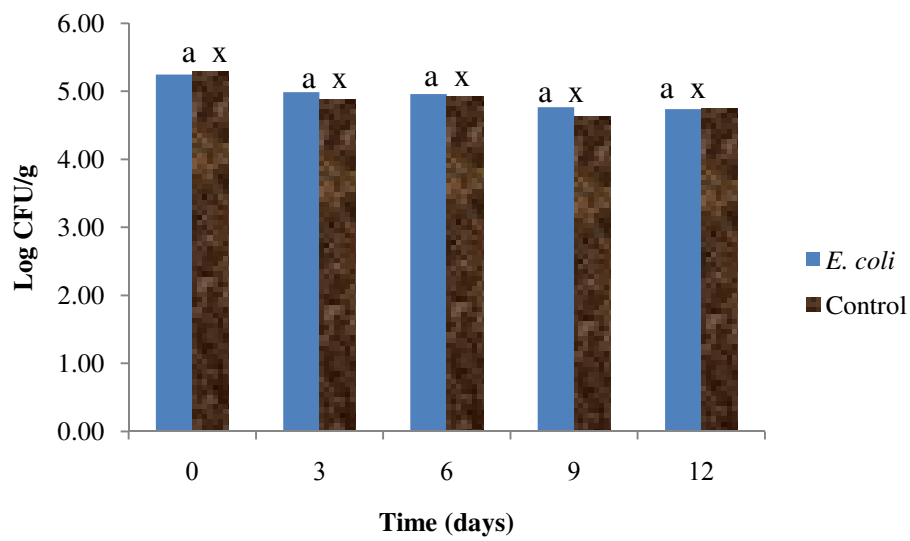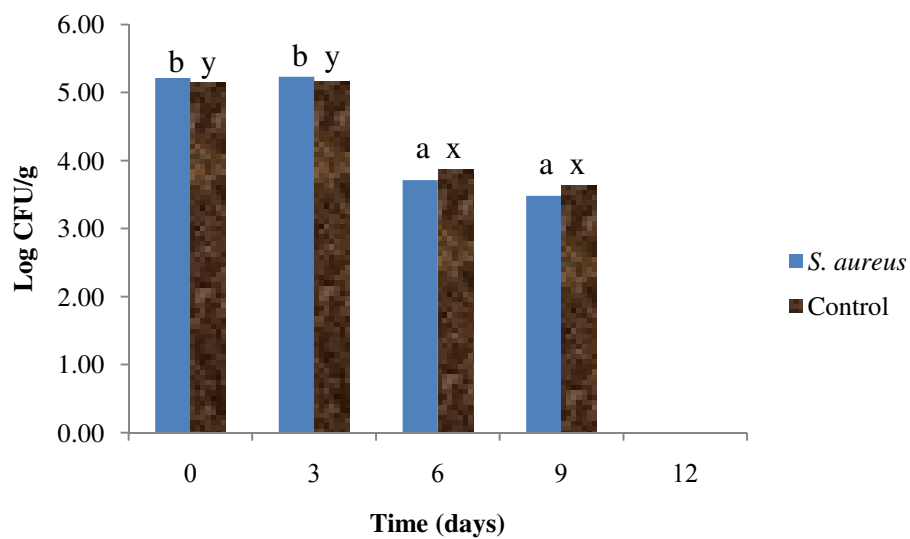
A)

Figure 20. *E. coli* and *S. aureus* populations on mangosteen pericarp extract treated fresh-cut mangosteen during storage. Each species was inoculated separately A) *E. coli* and B) *S. aureus*. The same letter (a, b, c) and (x, y, z) between storage day means no significant difference ($p > 0.05$) for each microorganisms and control, respectively.

Caution is required in the interpretation of results of experiments conducted with single microorganisms. For example, Stutzenberger and Bennett (1965) studied the sensitivity of mixed populations of *S. aureus* and *E. coli* to compounds containing mercury. *S. aureus* was found to have a higher resistance in the presence of *E. coli*. A separate experiment was therefore conducted in which the microorganisms were simultaneously inoculated onto fresh-cut mangosteen. Again, there was no evidence that the mangosteen pericarp extract could inhibit either species (Figure 21).

A)

B)

Figure 21. *E. coli* and *S. aureus* populations on mangosteen pericarp extract treated fresh-cut mangosteen during storage. Bacteria were applied as a cocktail A) *E. coli* and B) *S. aureus*. The same letter (a, b, c) and (x, y, z) between storage day means no significant difference ($p>0.05$) for each microorganisms and control, respectively.

7.5 Conclusions

Mangosteen pericarp extract positively affected the preservation of color in stored fresh-cut mangosteen. Furthermore, the extract had no negative effects on the sensory properties of the fruit. Consequently, this study provided evidence that the extract may be a useful agent for the preservation of color during refrigerated storage of fresh-cut mangosteen. Further work will be required to establish limits of use and to examine potential antimicrobial effects if higher concentrations can be used to treat the fruit before packaging.

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CHAPTER 8

EFFECT OF SEALING FILM ON THE QUALITY OF PACKAGED FRESH-CUT MANGOSTEEN

8.1 Abstract:

Three types of film (OPP/LLDPE, PET and LDPE) were used to seal rigid trays containing fresh-cut mangosteen to examine the influence of the films on the composition of the headspace and the quality of the fruit during storage. LDPE film, which has the highest OTR and CTR (2,795 and 10,500 cm^3/(m^2 day), respectively) showed the highest O_2, C_2H_4, ethanol and acetaldehyde accumulation and lowest residual CO_2 in the package. Furthermore, firmness and weight losses were higher than those of OPP/LLDPE and PET films. Film type did not affect the microbial growth of fresh-cut mangosteen. Sensory quality was negatively affected by sealing of the trays with LDPE film.

8.2 Introduction

Mangosteen harvesting in Thailand is largely concentrated, depending on the area, between April and August. Mangosteen is considered to be one of the finest tasting fruits and has earned the popular title "the queen of fruit". Fresh mangosteen remains an expensive commodity in markets outside Asia due to a relatively short shelf-life and long distances from major production zones. Shelf-life at room temperature (25-35 °C) is limited to 5-7 days. This fruit is by nature a highly perishable product. The rind hardens quickly at lower relative humidity with a rapid loss in quality (Diczbalis, 2009). Moreover, the white flesh is damaged in high temperatures and low relative humidity. Hence mangosteen is a difficult commodity to distribute in the fresh state. Consequently there is increasing interest in minimally processed products to facilitate distribution in markets with a strong demand for this unique tropical fruit.

Modified atmosphere packaging (MAP) is always used for maintain the quality of fresh-cut fruit. MAP of fresh produce relies on the modification of the atmosphere inside the package achieved by the natural interplay between two

processes, the respiration of the commodity and the permeability of the sealing films (Mangaraj *et al*., 2009). The right sealing film can contribute to create conditions in the package which can delay maturation and ageing of the commodity. It is therefore possible to retain the quality and improve the shelf-life of fresh-cut produce using this technology.

The respiration rate of fresh-cut fruit or the rate by which the oxygen is converted into carbon dioxide depends on the oxygen concentration. At low oxygen concentrations respiration is slower than at high oxygen concentrations, resulting in slower ageing and longer shelf-life. However if the O_2 content drops below critical levels respiration ceases resulting in biochemical changes that lessen quality. Sealing film for fruit and vegetables are never quite impervious to oxygen, carbon dioxide and water vapor. The rate of water or gas transmission depends on the type, thickness and area of film and temperature/pressure differences of the gasses on each side of film (Al-Ati and Hotchkiss, 2003).

Selection of a suitable film requires careful consideration of gas transmission rate. For rapid respiring products such as fresh-cut mangosteen it is necessary to select films that ensure oxygen is not depleted inside the package to avoid changes in quality.

Although the consumption of fresh-cut mangosteen has increased, very little information exists on quality retention in the stored fresh-cut product. The objectives of the present work were to evaluate the influence of various films on package atmospheres, quality and shelf-life of fresh-cut mangosteen.

8.3 Materials and methods

Plant material

An eastern cultivar of mangosteen fruit (*Garcinia mangostana*, L) was grown in an orchard in Chanthaburi province, Thailand by a commercial grower. Fruit with a mean weight of 120 ± 5 g were hand-harvested during April-May at stage 3 (reddish pink) according to the scales described by Palapol *et al*. (2009). Damaged or diseased fruit were discarded. The crop was treated with 40 ppm 1-MCP for 12 h at 25 $^{\circ}$C, then packed and transported to the laboratory by an airplane. The fruit for experiments were processed into fresh-cut format in an isolated and cleaned minimal-processing

room at 30 °C. The stalks and calyx ends were removed by with a knife and the fruit were washed by hand under tap water. An incision was made around the periphery to remove the peel, the entire white arils were removed and immediately placed in cold water (10 ^{o}C). After 5 min, the arils were dried with a handheld blower, approximately 200 g were packed in polypropylene (PP) trays (11.5×17.5×4.5 cm) and the trays were sealed with OPP/LLDPE, PET and LDPE films with a silicone sealant. The water, O_2 and CO_2 transmission rates of the films are shown in Table 18. The trays were stored at 5 °C with 85% RH in a refrigerator. Three replicates with two trays per replicate were analyzed at 0, 3, 6, 9 and 12 days of storage.

Table 18. Water and gas transmission rates of the films used to seal the trays.

Film	WTR (g/m^2 day)	OTR (cm^3/m^2 day)	CTR (cm^3/m^2 day)
OPP/LLDPE	0.29	1,160	3,150
PET	0.94	116	375
LDPE	8.60	2,795	10,500

Source: Department of Science Service, Ministry of Science and Technology, Thailand

Measurement of gas concentrations in the packages

Changes in the headspace concentrations of gases (O_2, CO_2 and C_2H_4) were measured at 3-day intervals by withdrawing air samples (1 ml) through a septum using a gas-tight syringe. The sample gas was injected into a gas chromatograph (GC) (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a thermal conductivity detector (TCD). The oven and detector temperatures were 60 and 150 ^{o}C, respectively, with helium as the carrier gas. C_2H_4 production was measured with a same but equipped with a flame ionization detector (FID) on 1 ml gas sample. The oven, injector and detector temperatures were 40, 120 and 180 ^{o}C, respectively, with helium as the carrier gas. Gas concentrations were expressed as %O_2, %CO_2 and ppm C_2H_4.

Physical changes

Firmness was evaluated using a TA-XT2i texture analyzer (Stable Micro Systems, England) with a 25 kg load cell, equipped with a 2 mm diameter cylinder stainless steel probe (P/2). Firmness measurements were taken as the maximum force value obtained during the test to penetrate the fruit 3 mm at 2.0 mm/s. Data were recorded and expressed as g/mm^2 force.

Weight losses were determined by weighing all samples with a Metler balance, model AB204 (Mettler-Toledo Inc., USA) at the beginning and end of the storage period. The difference between the values was used to calculate weight loss.

Fresh-cut color was measured using a colorimeter (Miniscan 45/0-L, Hunter Associates Laboratory, Inc., USA) calibrated with a standard white tile with the following parameters: X = 79.6, Y = 84.4, Z = 89.9 with illuminant D/65/10°, (light source used for the daylight), according to CIE L* (lightness), a* (green to red) and b* (blue to yellow) values. Numerical values of a* and b* were converted into chroma $[C = (a^{*2}+b^{*2})^{1/2}]$ and hue values $[^oH = \tan^{-1} b^*/a^*]$ (Francis, 1980).

Chemical analyses

Acetaldehyde and ethanol contents were measured using the method of Gonzalez-Aguilar *et al*. (2004). In brief, tissue (5 g) was placed in amber colored bottles with 60 ml capacity and placed in a 65 ^{o}C water-bath for 15 min. Headspace gas samples of 1 ml were injected into a gas chromatograph (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a 60 m × 0.325 mm × 0.25 µm DB-WAX column (J&W Scientific, Folsom, California). The oven, injector and detector temperatures were 60, 250 and 250 ^{o}C, respectively, with helium as the carrier gas. Retention times and standard curves of acetaldehyde and ethanol in water solutions were used for peak identification and quantification. Acetaldehyde and ethanol contents will be expressed as µl/kg fruit.

Microbiological analyses

Microbial determinations were carried out using standard methodologies (BAM, 2001). Twenty-five grams of sample were diluted in 225 ml of sterile buffered Butterfield's Phosphate and homogenized for 2 min at normal speed using a

Stomacher (Model 400 Circulator, Seward, Norfolk, England). Serial dilutions of the suspension were made and analyzed for total viable count (TVC), yeasts and moulds and *E. coli*. Another 25 g were diluted in 225 ml of buffered peptone water for the detection of *Salmonella*. TVC and yeast and mold populations were reported as cfu/g (colony forming units per gram of sample) whereas *E. coli* and *Salmonella* sp. counts were reported as MPN and detected or not, respectively.

Sensory evaluation

Twenty semi-trained panelists were asked to rank the samples for browning, off-odor, firmness, wateriness and overall visual quality (OVQ) of treated fresh-cut mangosteen against reference samples using methods described by Gomez-Lopez *et al*. (2008) with some modifications. The 5-point ratings were assigned as follows: (5) not original, (4) slightly original, (3) moderately original, (2) very original and (1) extremely original. The reference samples were prepared from untreated fresh-cut fruit immediately after cutting as a good sample and from an untreated fresh-cut after 1 day at room temperature as a bad sample. Each treatment consisted of six fresh-cut fruit served on a white plastic plate within 2-3 h of holding time at room temperature (25 $^{\circ}$C) and the same samples were evaluated by each panelist within 1 h. Scores >3 were considered to be undesirable.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and mean differences estimated by Duncan's new multiple range test (DMRT) using SPSS Statistics Standard software (IBM Corp, Sommers, NY, USA). Differences at $p \leq 0.05$ were considered significant.

8.4 Results and discussion

In-package atmospheric changes

The concentration of O_2 in trays of fresh-cut mangosteen sealed with all three films decreased rapidly (Figure 22). O_2 concentrations remained higher in trays sealed with LDPE than PET and OPP/LLDPE film ($p \leq 0.05$). There was no significant difference in O_2 concentrations in trays sealed with PET and OPP/LLDPE films

($p>0.05$). The higher O_2 transmission rate of LDPE film was likely responsible for the difference (Table 18). A similar finding was reported for fresh-cut lettuce stored in trays sealed with different films and where O_2 concentrations were greatest with films with the highest OTR (Kim *et al.*, 2005).
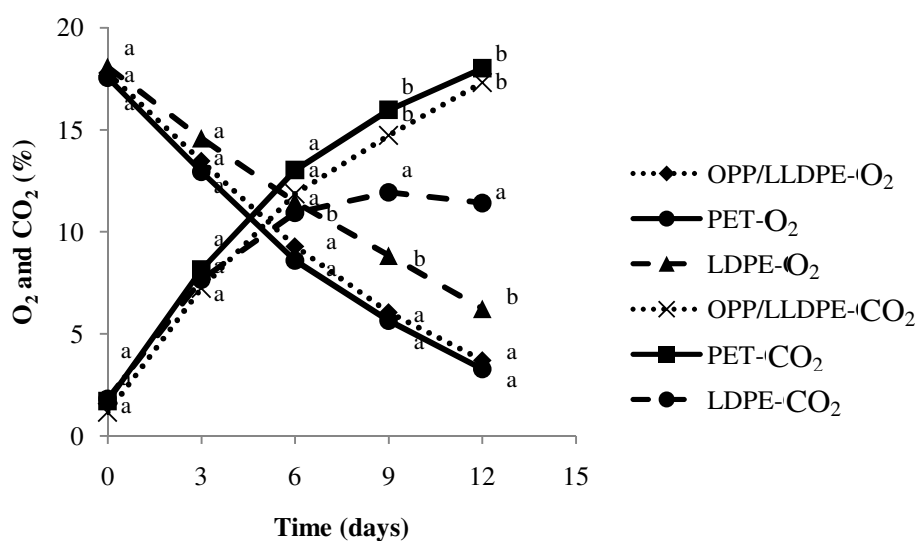

Figure 22. Changes O_2 and CO_2 concentrations in the headspace of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day in each gas means no significant difference ($p>0.05$).

CO_2 concentrations increased throughout storage in trays sealed with all the films (Figure 22). CO_2 increased in all samples but peaked at day 9 in trays sealed with PET film. This is again due to the CO_2 transmission rate, CTR, of the film, which is higher than of OPP/LLDPE and PET (Table 18).

Ethylene concentration increased during the first 6 days after processing and stabilized thereafter (Figure 23). Ethylene accumulation was higher in trays sealed with OPP/LLDPE and LDPE than PET film ($p\leq0.05$). Mangosteen is climacteric fruit with a high respiration rate during ripening. The production of ethylene can increase up to 1,000 fold from the base level during this process. OPP/LLDPE and LDPE films have higher OTRs than PET. During respiration O_2 is consumed and carbohydrates are broken down to produce energy to run cellular processes while water, CO_2, energy

and ethylene are released. Consequently higher levels of ethylene are produced in fruit packaged with OPP/LLDPE and LDPE films were expected given their higher permeability to O_2.
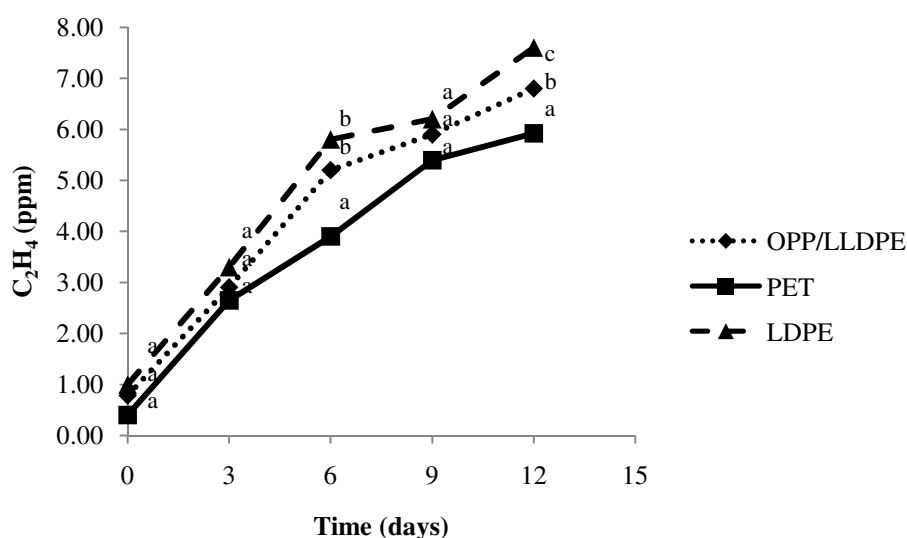

Figure 23. Changes ethylene concentrations in the headspace of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day means no significant difference ($p>0.05$).

Changes in physical properties

Firmness

The firmness of fresh-cut mangosteen decreased continuously during storage but the extent varied depending upon the film used to seal the trays (Figure 24). The decrease was significantly more pronounced with LDPE than either OPP/LLDPE or PET film ($p \leq 0.05$). Decreasing firmness during storage could be related to an increase in metabolism associated with higher residual O_2 inside trays sealed with LDPE film. Enhanced enzymatic activity associated with higher metabolic rates is known to result in loss of the firmness of fresh-cut fruit (Gonzalez-Aguilar *et al.*, 2004).
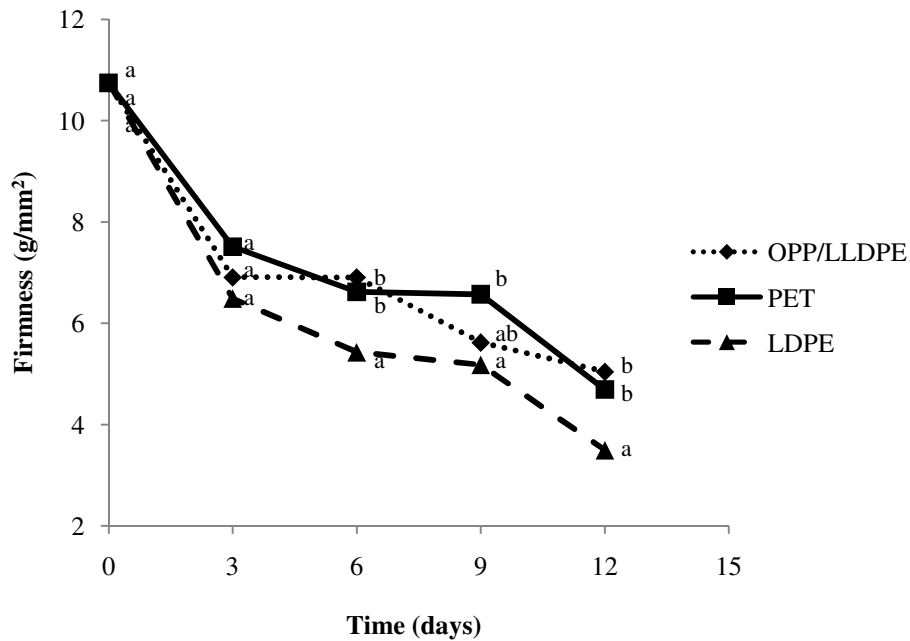
Figure 24. Flesh firmness of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day means no significant difference ($p>0.05$).

Weight loss

Weight losses increased rapidly during the first 6 days of storage of fresh-cut mangosteen packaged in trays, then either increased gradually or remained relatively stable, depending up on film type (Figure 25). Weight losses were lower with OPP/LLDPE and PET than LDPE film ($p\leq0.05$). This finding was not unexpected because the water transmission rate (WTR) of the latter was the highest thus permitting greater movement of water from the inside of the tray to the outside (Table 18).
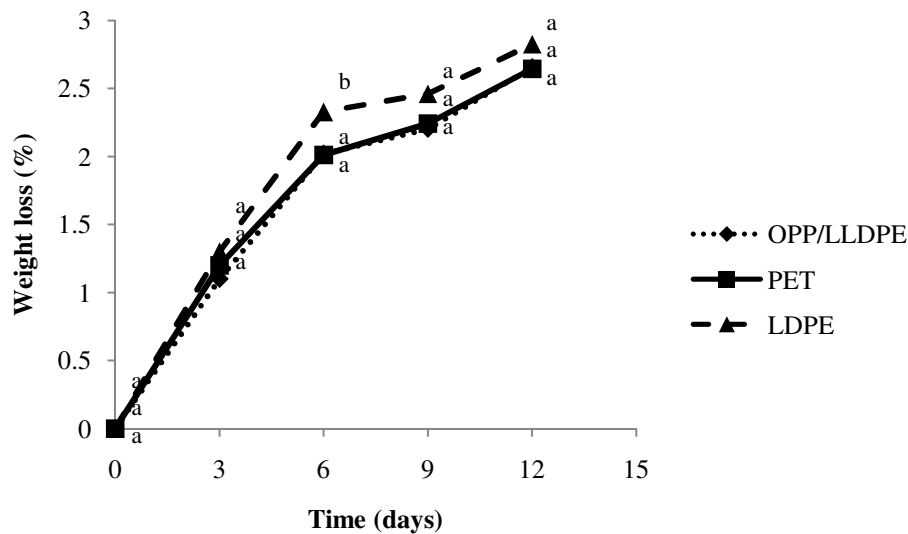
Figure 25. Weight losses of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day means no significant difference (*p*>0.05).

Changes in color

Lightness values measured for fresh-cut mangosteen packed in PP trays sealed with all three films gradually decreased throughout storage (Figure 26A). Chroma values decreased immediately after processing but remained relatively stable thereafter (Figure 26B). Hue angle measurements decreased throughout storage (Figure 26C). Statistical analysis revealed that there were no significant differences (*p*>0.05) in lightness, chroma and hue value associated with the treatments. These studies suggest that flesh color changes during storage were not influenced by the type of film used to seal the trays.
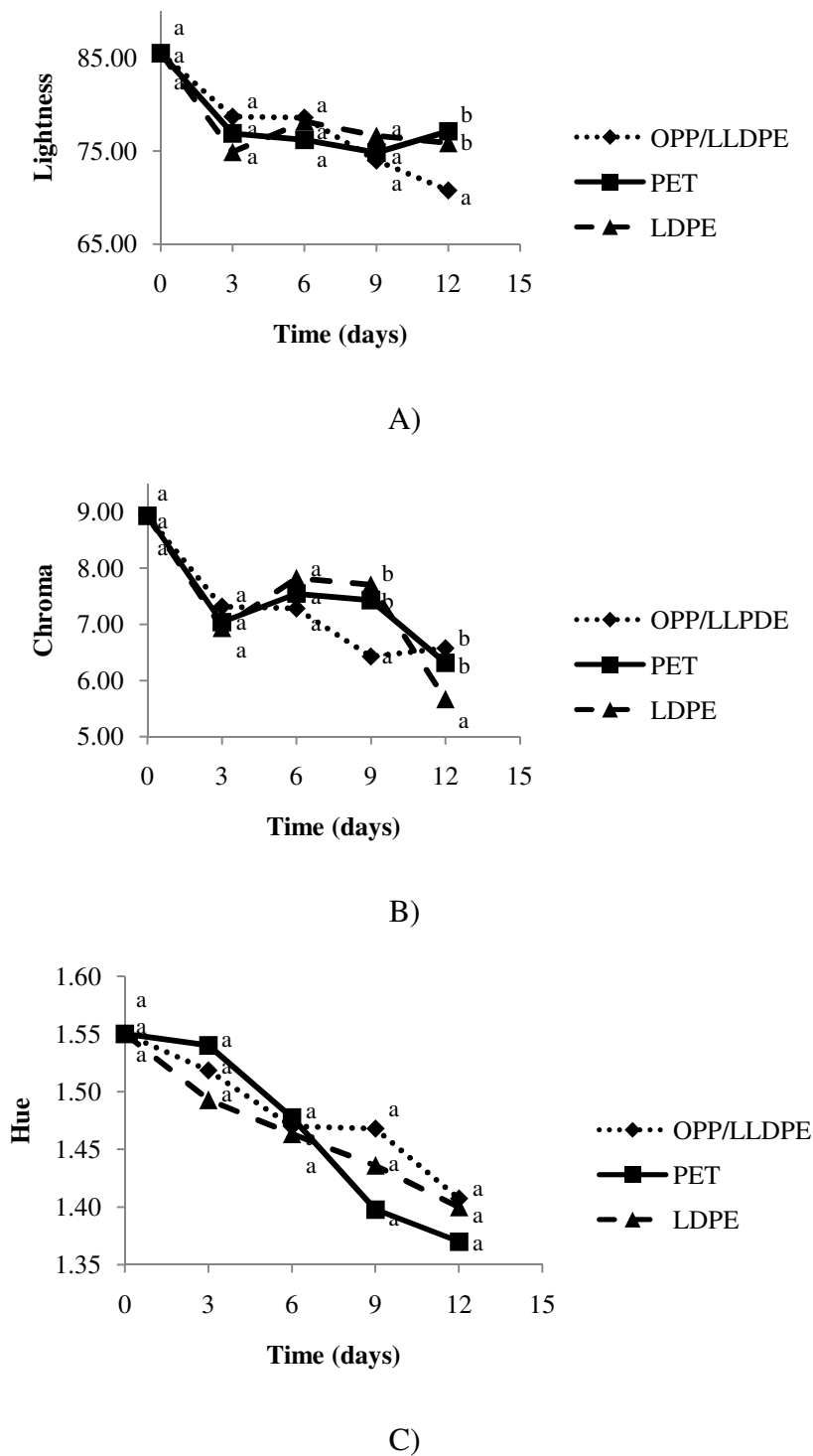
Figure 26. Color changes of fresh-cut mangosteen in PP trays sealed with three different films A) lightness B) chroma and C) hue values. The same letter within storage day means no significant difference (*p*>0.05).

Chemical changes

      Acetaldehyde and ethanol were detected in the headspace immediately after processing (Figure 27 and 28). Acetaldehyde, a natural aroma compound, is formed from pyruvate and reacts with CO_2 to form ethanol. In the present experiment acetaldehyde concentrations decreased throughout storage but there were differences associated with film type. Concentrations of acetaldehyde in trays sealed with LDPE film remained higher than in those sealed with OPP/LLDPE and PET films ($p \leq 0.05$). High acetaldehyde concentrations in LDPE film could be associated with the stress response when fruit tissues are exposed to high O_2 concentrations. Acetaldehyde may be reduced to ethanol and react further to form ethyl acetate (Jandric *et al*., 2010). That reaction, together with acetaldehyde permeation through the package material, could explain the decrease of acetaldehyde after 3 days of storage.
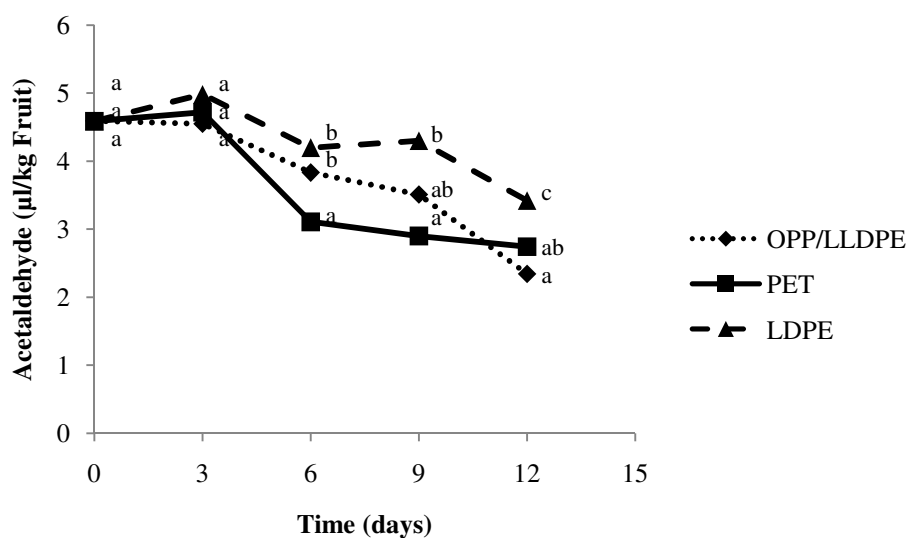

Figure 27. Acetaldehyde content of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day means no significant difference ($p > 0.05$).

Figure 28. Ethanol content of fresh-cut mangosteen in PP trays sealed with three different films. The same letter within storage day means no significant difference ($p>0.05$).

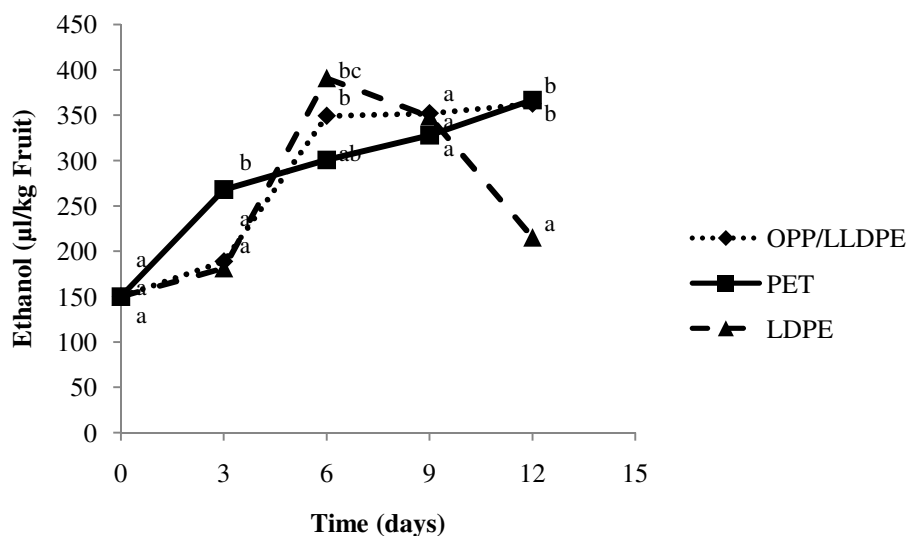Production of ethanol is an indicator of anaerobic fermentation and ethanol is responsible for development of un-pleasant off-flavors and odors in fresh-cut fruits. Ethanol concentrations in trays sealed with OPP/LLDPE and PET increased throughout storage. In contrast, concentrations in trays sealed with LDPE were higher than in those sealed with OPP/LLDPE and PET after 6 days, but decreased quickly thereafter. Reasons for this decline are unclear but could be related to the permeability of the film to ethanol.

Low concentrations of ethanol and acetaldehyde may impart floral, fruity or otherwise pleasant odors in fruit (Kim *et al*., 2005). Production of both compounds is generally associated with anaerobic respiration, which is stimulated by very low O_2 and high CO_2 atmospheres. In this study, it is unlikely that O_2 was sufficiently depleted and CO_2 elevated to the point where the fruit would enter into strictly anaerobic metabolism. Thus, the relatively low levels of acetaldehyde and ethanol detected in fresh-cut would probably not detract from the overall quality of the product.

Microbiological changes

Microbiological analysis of fresh-cut mangosteen stored at 5 $^{\circ}$C in PP trays sealed with all three films revealed no evidence of microbial growth during storage (Table 19). Generally, the development of microbial populations in fresh-cut fruit can be retarded by lowering the temperature of storage since higher temperatures can hasten many metabolic processes which stimulate their growth (Gonzalez-Aguilar *et al*., 2004). For example, Izumi and Watada (1994) found that the increase in microbial population of carrots was about 100-fold greater at 10°C than at 0 $^{\circ}$C due to the faster release of sugars from the plant tissues at the higher temperature. The fresh-cut mangosteen aril remains relatively intact during storage, which would limit the release of nutrients for microbial growth. This, in combination with the use of low storage temperature, was likely responsible for the control of microbial growth. Therefore lowering storage temperature was more critical for the control of microbial growth than the type of film used to seal the trays.

Table 19. Microbial populations of fresh-cut mangosteen packed in PP trays sealed with three different films during.

Microogarnisms	Day 0	Day 3	Day 6	Day 9	Day 12
-TVC (CFU/g)	<10	10	10	10	20
-Yeast and mold (CFU/g)	<10	<10	<10	<10	<10
-*E. coli* (MPN/g)	<3	<3	<3	<3	<3
-*S. aureus* (MPN/g)	<3	<3	<3	<3	<3
-*Salmonella* sp.	N.D.	N.D.	N.D.	N.D.	N.D.
-*L. monocytogenes*	N.D.	N.D.	N.D.	N.D.	N.D.

Note: The microbial populations in fresh-cut mangosteen in PP trays sealed with each film are the same values. N.D. (Not detected)

Sensory analysis

The scores of all sensory attributes increased over time (Table 20). Scores for browning, firmness and wateriness changed immediately after processing (a score of

>1 on a 1-5 scale). Changes in odor were first observed after 3 days of storage. The accumulation of ethanol in the trays (Figure 28) may be associated with the change in this parameter.

Table 20. Sensory scores of fresh-cut mangosteen packed in PP trays under three different films during storage.

Attributes	Film type	Time (days)				
		0	3	6	9	12
Browning	OPP/LLDPE	1.55±0.51ns*	2.63±0.95ns	2.43±0.85a	2.67±0.80a	3.02±1.00a
	PET	1.55±0.51ns	2.85±1.04ns	2.90±0.89b	2.81±0.81b	3.58±1.07b
	LDPE	1.55±0.51ns	2.60±0.94ns	2.48±0.81a	2.78±0.84b	3.32±0.89b
Off-Odor	OPP/LLDPE	1.00±0.00ns	1.65±0.81a	1.76±0.89a	2.67±1.11b	3.17±0.92ab
	PET	1.00±0.00ns	1.80±0.95a	1.81±0.93a	1.81±0.75a	3.33±1.14a
	LDPE	1.00±0.00ns	2.70±1.34b	2.81±1.25b	2.88±1.12b	2.95±1.22b
Firmness	OPP/LLDPE	1.40±0.50ns	2.15±1.18ns	2.05±0.92a	2.38±1.02ns	3.16±0.90a
	PET	1.40±0.50ns	2.11±1.10ns	1.81±0.98a	2.19±0.86ns	3.39±0.76b
	LDPE	1.40±0.50ns	2.00±1.26ns	2.30±0.75b	2.33±0.98ns	3.63±1.04b
Wateriness	OPP/LLDPE	1.25±0.44ns	1.70±0.80a	1.90±0.77ns	2.33±0.55b	3.03±0.61a
	PET	1.25±0.44ns	1.95±0.83ab	2.05±0.59ns	2.05±1.02a	3.11±0.99a
	LDPE	1.25±0.44ns	2.20±1.20b	2.05±0.67ns	3.00±0.73c	3.74±0.88b
OVQ	OPP/LLDPE	1.20±0.52ns	2.15±1.18a	2.33±1.11a	3.38±0.97a	3.53±1.26a
	PET	1.20±0.52ns	2.65±1.27b	2.85±1.02b	3.67±1.11b	4.37±1.92b
	LDPE	1.20±0.52ns	2.80±1.40b	4.19±1.54c	4.52±1.25c	4.68±1.83c

Note: * mean±SD from 20 panelists followed by the same letter within column for each attribute are not significantly different ($p>0.05$); ns (non significant, $p>0.05$)

Overall visual quality (OVQ) was influenced by type of film. Samples packed in LDPE film had the highest OVQ score, likely due to the wateriness appearance of the samples. Product from this treatment was considered unacceptable after 6 days in storage (a score >3 on a 1-5 scale) while those stored in trays sealed with OPP/LLDPE and PET film maintained higher scores until the 9th day of storage.

Hence samples packed trays sealed with OPP/LLDPE and PET films maintained higher OVQ scores than those packaged in LDPE film under the same storage conditions.

8.5 Conclusions

Film properties are crucial in fresh-cut packaging. The OTR and WTR of films used in present work influenced the quality of fresh-cut mangosteen. Fresh-Cut mangosteen packed under LDPE film, which has highest WTR and OTR, retained firmness but exuded more water than product stored under PET and OPP/LLDPE films. The color and microbial population of product were unaffected by film properties. Furthermore, the high OTR of LDPE film led to higher levels of acetaldehyde and sensory analysis suggested that the product packed under LDPE film suffered greater losses in quality. The results of this study indicate that OPP/LLDPE and PET films could be used to maintain the overall visual quality of fresh-cut mangosteen for up 9 days at 5 $^{\circ}$C. However LDPE film is not recommended for the purpose due to adverse effects on the sensory shelf-life of the product.

8.6 References

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CHAPTER 9

SELECTION OF A SEALING FILM FOR FRESH-CUT MANGOSTEEN PACKED IN PP TRAYS

9.1 Abstract

A mathematical model based on Michaelis-Menten kinetics was used to describe the effect of sealing film on the composition of gases inside packages of fresh-cut mangosteen . The Vm (maximum respiration rate) was found to be very high (714.29 ml/(kg h)), probably cutting during fresh-cut mangosteen exposes large tissue surface areas to gas exchange. The first model derived from ODE did not fit the experimental data because of a low RQ (0.64). The model was improved by plotting of R_{CO_2} between experimental and calculated data based on actual RQ. The improved model was fit properly to the experiment. The modified model was also used to estimate the effect of changing package dimension, product weight, film thickness and β ratio. The package dimension, film thickness and β ratio did not affect the model, only product weight did because of high respiration rate of product. Changing product weight is the parameter that should be considered to select film lid for fresh-cut mangosteen.

9.2 Introduction

Modified atmosphere Packaging (MAP) is one of the food preservation methods that maintain the quality of food products and extend the shelf-life (Jayas *et al*., 2002). Desirable changes in gaseous composition help to reduce microbial growth, the rate of chemical reactions and the exchange of undesirable substances with the surrounding environment. The packaging systems used in passive MAP employ impermeable films to maintain the desirable mixture of gasses around the food. The compositions of the atmospheres usually consist of high concentrations of CO_2 and lower concentrations of O_2.

Fundamentally, CO_2 and O_2 concentrations in MAP packages change as a function of produce respiration rate, weight of sample, temperature and the permeability of the film. High barrier film can lead to be excessive CO_2 leading to

anaerobic conditions, whereas low barrier film can result in insufficient CO_2 concentration in the package, a condition which may not provide maximum shelf-life of product, especially for fresh-cut produce (Gunes *et al.*, 2001).

Predictive model of MAP of fresh-cut produce is generally based on respiration rate and permeation of gases through the film. The amount of fruit, dimension of package, temperature and initial atmosphere of package can be determined for desired gas mixture in the package using a prediction model for an optimal package with known respiration rate and gas transmission equations.

As described by Exama *et al.* (1993), the transient O_2 level as a function of time can be expressed by ordinary differential equations (ODE)

$$\frac{dyiO_2}{dt} = \left(\frac{AP_{O_2}p}{VL}\right)(yeO_2 - yiO_2) - \left(\frac{WR_{O_2}}{V}\right) \tag{1}$$

where A = area of permeable film (cm^2), P_{O_2} = O_2 permeability coefficient (ml.cm/(cm^2s cm Hg)), p = pressure (1 atm), V = free volume of the package (ml), L = film thickness (cm), yeO_2 = external (atmospheric) O_2 concentration (21%), yiO_2 = internal O_2 concentration (%), W = weight of produce (kg) and R_{O_2} = O_2 consumption rate (ml/kg h).

To model the O_2 composition in a package according to Eq 1, the respiration rate (R_{O_2}) must first be modeled by assuming that respiration rate is a function of O_2 consumption and CO_2 concentration has no direct effect on respiration rate. The respiration quotient (RQ), or ratio of the rate of CO_2 produced to the rate of O_2 consumed, is 1 (Al-Ati *et al.*, 2003). In addition the model is based on Michaelis-Menten kinetics for O_2 consumption as follows:

$$R_{O_2} = \frac{Vm[O_2]}{Km+[O_2]} \tag{2}$$

where R_{O_2} = respiration rate (ml/(kg h)), Vm = maximum respiration rate (ml/(kg h)), $[O_2]$ = oxygen concentration (%) and Km = Michaelis-Menten constant.

It follows that

$$R_{CO_2} = RQR_{O_2} \tag{3}$$

Because it is assumed that RQ = 1, $R_{CO_2} = R_{O_2}$ and Eq 1 can be rewritten as

$$(dyiO_2/dt)) = (AP_{O_2}\, p/VL)(yeO_2 - yiO_2) - (w/V)((Vm[O_2])/(Km + [O_2])) \tag{4}$$

The change in gas composition inside any given flexible package of specific dimension (i.e., surface area) made from a permeable film can be mathematically simulated once the solution to Eq 4 is obtained. The O_2 level at a given time in a package (yO_2) is therefore

$$yO_2(t)) = \int(dyiO_2/dt) = \int[(APO_2p/VL)(yeO_2 - yiO_2) - (W/V)(Vm[O_2]/(Km + [O_2]] \tag{5}$$

An equation can be developed to calculate values of CO_2 concentration over time as well. With the numerical solutions for the ODEs of both O_2 and CO_2, simulation of the internal atmosphere of a given MAP system can be achieved.

The objective of this research was to investigate the effect of packaging system on O_2 consumption and CO_2 production of fresh-cut mangosteen using predictive model on the equilibrium gas composition and comparing the empirical to predicted data of the selected films on the equilibrium gas composition.

9.3 Materials and methods

Plant material

An eastern cultivar of mangosteen fruit (*Garcinia mangostana*, L) was grown in an orchard in Chanthaburi province, Thailand by a commercial grower. Fruit with a mean weight of 120±5 g were hand-harvested during April-May at stage 3 (reddish pink) according to the scales described by Palapol *et al*. (2009). Damaged or diseased fruit were discarded. The crop was treated with 40 ppm 1-MCP for 12 h at 25 °C, then packed and transported to the laboratory by an airplane. The fruit for experiments were processed into fresh-cut format in an isolated and cleaned minimal-processing

room at 30 °C. The stalks and calyx ends were removed by with a knife and the fruit were washed by hand under tap water. An incision was made around the periphery to remove the pericarp, the entire white arils were removed and immediately placed in cold water (10 ^{o}C). After 5 min, the arils were evaporated dried by small blower.

Respiration rate of fresh-cut mangosteen

Six arils were weighed and placed in each of three replicate PP trays. The trays were sealed with a lid to which a rubber septum was fastened and the trays were stored at 5 ^{o}C. Concentrations of CO_2 and O_2 inside the trays were measured in 1.0 ml gas samples removed from the head-space with a syringe inserted through the septum. The samples were injected into a gas chromatograph (GC) (AutoSystem XL, The Perkin Elmer Corporation, USA) equipped with a thermal conductivity detector (TCD). The oven and detector temperatures were 60 and 150 ^{o}C, respectively, with helium as the carrier gas.

Modified Atmosphere Model

Mangosteen fruits were processed into fresh-cut format in an isolated and cleaned minimal-processing room at 30 °C by removing the stalks and calyx ends with a knife and the fruit were washed by hand under tap water. An incision was made around the periphery to remove the pericarp, the entire white arils were removed and immediately placed in cold water (10 °C). After 5 min, the arils were evaporated dried by small blower.

Fresh-cut mangosteens were weighed and placed in PP trays as described above. Square holes (area = 80 cm^2) were made in the center of the lids to accommodate test films, three replicates were carried out. The trays were sealed with three films, OPP/LLDPE, PET and LDPE with different film thickness (cm^2), fresh-cut weight (kg), O_2 permeability coefficient (P_{O_2}, ml.cm/(cm^2 s cm Hg)), CO_2 permeability coefficient (P_{CO_2}, ml.cm/(cm^2 s cm Hg)) and β ratio as shown in Table 21. The permeation rate was calculated based on the area of film exposed to the atmosphere. The volume of fresh-cut mangosteen was estimated by measuring the volume of water displaced by six arils.

Table 21. Films and fresh-cut mangosteen weight for the study.

Film	Film area (cm^2)	Film thickness (cm)	Fresh-cut weight (kg)	Container free volume (ml)	(ml.cm/(cm^2 s cmHg))		β ratio (P_{CO_2}/P_{O_2})
					P_{O_2}	P_{CO_2}	
OPP/ LLDPE	80	0.0071	0.1979	615.46	5.97×10^{-10}	1.62×10^{-9}	2.71
PET	80	0.0043	0.1930	617.60	3.62×10^{-11}	1.17×10^{-10}	3.23
LDPE	80	0.0069	0.2016	611.78	1.40×10^{-9}	5.25×10^{-9}	3.75

To simplify the calculation of Michaelis-Menten parameters, Eq 2 was rewritten as

$$\frac{1}{R_{O_2}} = \frac{1}{V_m} + \left(\frac{K_m}{V_m}\right)\left(\frac{1}{[O_2]}\right) \tag{6}$$

R_{O_2} is rate of O_2 consumption, Km is the Michaelis-Menten constant (% O_2) and Vm is the maximum respiration rate (ml/(kg h)).

A linear regression plot of $(1/R_{O_2})$ against $(1/[O_2])$ was used to generate a line with a slope equal to (Km/Vm) and an intercept equal to (1/Vm). A plot of %O_2 and %CO_2 was also generated in order to determine R_{CO_2}.

9.4 Results and discussion

The rate of O_2 consumption (R_{O_2}) of fresh-cut mangosteen was calculated using Eq 2. Vm and Km generated from the linear regression of a plot of $1/R_{O_2}1$ and $1/[O_2]$ shown in Figure 29 were found to be 714.29 ml/(kg h) and 25.21%, respectively ($R^2 = 0.998$).
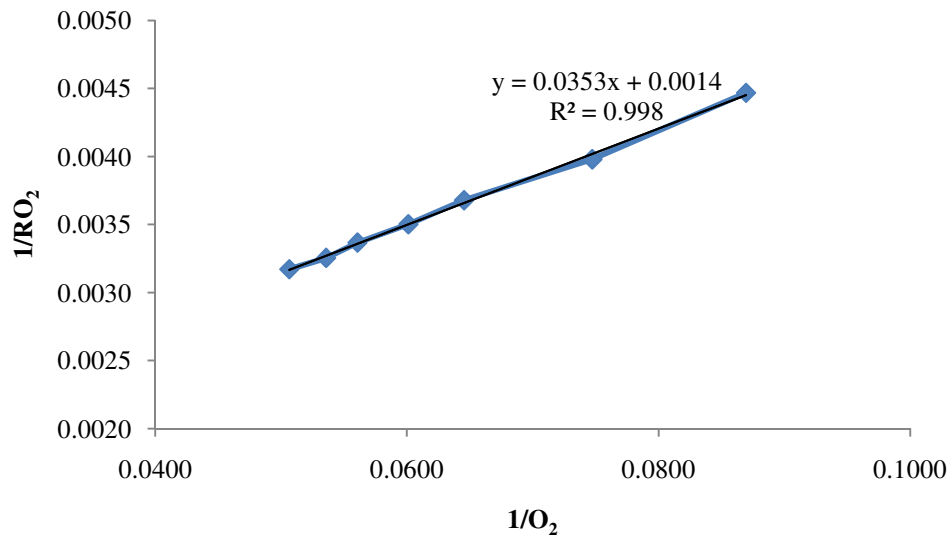
Figure 29. Linear regression of a plot of $1/R_{O_2}$ and $1/[O_2]$.

Thus, the respiration rate of fresh-cut mangosteen was

$$R_{O_2} = \frac{714.29[O_2]}{25.21 + [O_2]} \tag{7}$$

To model the effect of $[O_2]$ on $[CO_2]$ production, $\%[O_2]$ and $\%[CO_2]$ were plotted. A linear regression was used to generate a straight line equation:

$$\%O_2 = -1.56(\%CO_2) + 20.22, R^2 = 0.993 \tag{8}$$

The assumption that RQ = 1 was used to generate term of $[CO_2]$. Thus, the given model for CO_2 is

$$R_{CO_2} = \frac{714.29((-1.56)([CO_2]) + 20.22}{25.21 + ((-1.56)([CO_2]) + 20.22} \tag{9}$$

Equations 7 and 9 were used to estimate the O_2 level and CO_2 generated by fresh-cut mangosteen inside trays with selected films, as discussed previously.

To model the O_2 changes in the trays, ODEs was possible along with the incorporation with the respiration model (eq 7), which generates calculated values of the O_2 level over time. The $\%O_2$ change over time can be calculated by substituting Eq 8 into Eq 7 to yield

$$\frac{dyiO_2}{dt} = \left(\frac{AP_{O_2}p}{VL}\right)(21.0 - yiO_2) - \left(\frac{W}{V}\right)\left(\frac{714.29(yiO_2)}{25.21+(yiO_2)}\right) \tag{10}$$

Similarly, the $\%CO_2$ change over time can be calculated by substituting eq 9 into Eq 1, the following ODE simulates CO_2 production rate inside the package:

$$\frac{dyiCO_2}{dt} = \left(\frac{AP_{CO_2}p}{VL}\right)(0 - yiCO_2) - \left(\frac{W}{V}\right)\left(\frac{714.29(-1.56)(yiCO_2+20.22)}{25.21+(-1.56)(yiCO_2+20.22)}\right) \tag{11}$$

Calculation of the changes in $[O_2]$ and $[CO_2]$ due to respiration and package permeation was achieved by determining the numerical solution of Eqs 10 and 11 using the Runge-Kutta method (Scilab 5.3.3, The Scilab Consortium, France).

It was found that predicted data showed some deviations from the experimental data (Figure 30) especially for CO_2 production as indicated by $\%$ root mean square error (RMSE) of 55.34, 68.21 and 75.04% for OPP/LLDPE, PET and LDPE films, respectively. This probably could from the model used in this study based on Michaelis-Menten kinetics that the respiration quotient (RQ), or ratio of the rate of CO_2 produced to the rate of O_2 consumed, is 1 whereas the RQ of fresh-cut mangosteen was found to be 0.64 (see Appendix, p 174).
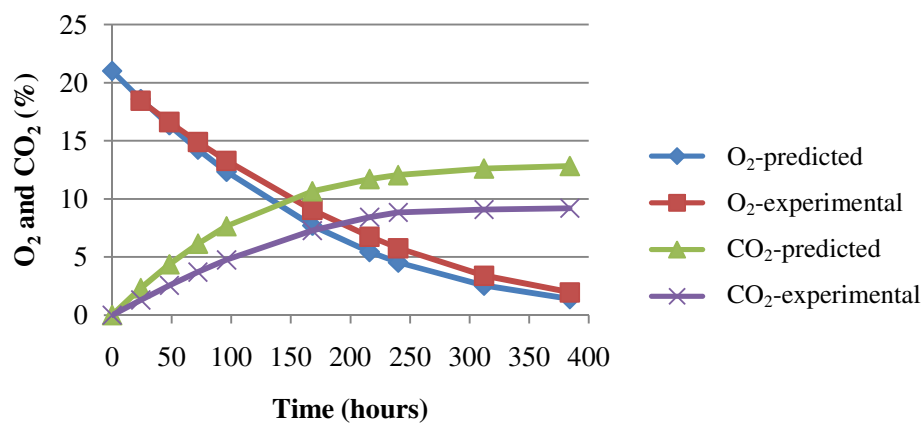
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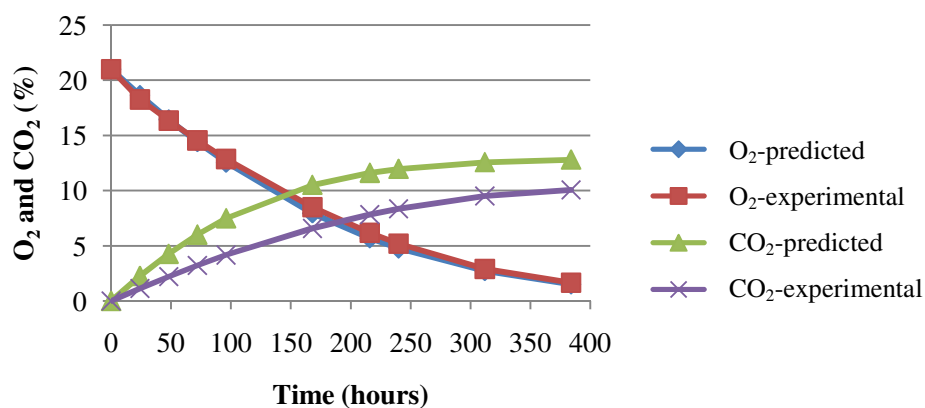
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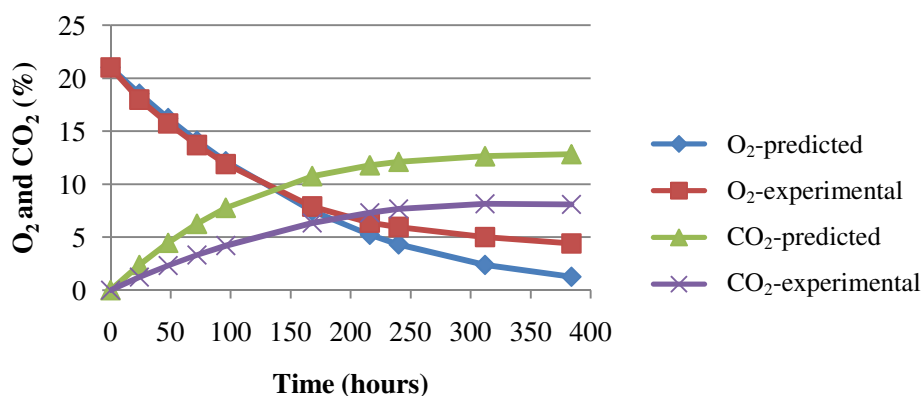
(C)

Figure 30. Predicted and experimentally determined O_2 and CO_2 concentrations inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.

Eq 11 was then improved based on the actual RQ of fruit by a plot of R_{CO_2} between experimentally and predicted values. A linear regression was used to generate a modified equation.

$$R_{CO_2}(\text{experimental}) = 0.375 R_{CO_2} \text{ (predicted)} + 54.98, R^2 = 0.999 \tag{12}$$

And the CO_2 production rate with slightly modified gave the following model:

$$R_{CO_2} = (0.375)\frac{714.29((-1.56)([CO_2])+20.22}{25.21+((-1.56)([CO_2])+20.22} + 54.98 \tag{13}$$

Thus, the ODE simulation of CO_2 production rate inside the package is

$$\frac{dyiCO_2}{dt} = \left(\frac{AP_{CO_2}P}{VL}\right)(0 - yiO_2) - \left[\left(0.375\frac{W}{V}\right)\left(\frac{714.29(-1.56)(yiCO_2+20.22)}{25.21+(-1.56)(yiCO_2+20.22)}\right) + 54.98\right] \tag{14}$$

After Eq 11 was improved to Eq 14; better prediction or less deviation of CO_2 from experimental data as indicated by %RMSE 21.28, 20.47 and 36.04% of OPP/LLDPE, PET and LDPE films, respectively, was observed (Figure 31). Fresh-cut mangosteen placed in trays sealed with films resulted in reduced O_2 level which may be an advantage with fresh-cut mangosteen because less O_2 can prevent anaerobic respiration. This is in agreement with the result of Al-Ati and Hotchkiss (2003) who studied atmospheres in fresh-cut apple slices.

The steady state levels of O_2 for OPP/LLDPE, PET and LDPE films were 3.39, 2.91 and 5.01%, respectively (Figure 31). The CO_2 accumulated in the package reached 9.07, 9.53 and 8.15% for OPP/LLDPE, PET and LDPE films, respectively. Gunes *et al*., (2001) found that the reducing $[O_2]$ and elevating $[CO_2]$ in closed packages of fresh-cut apple may be beneficial to its quality. Similar reduced $[O_2]$ and elevating $[CO_2]$ were found in the present study. Thus, it should also be beneficial to the quality of fresh-cut mangosteen.
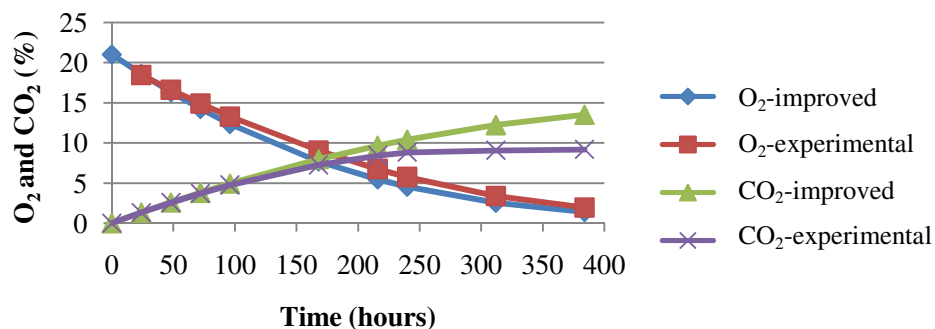
(A)
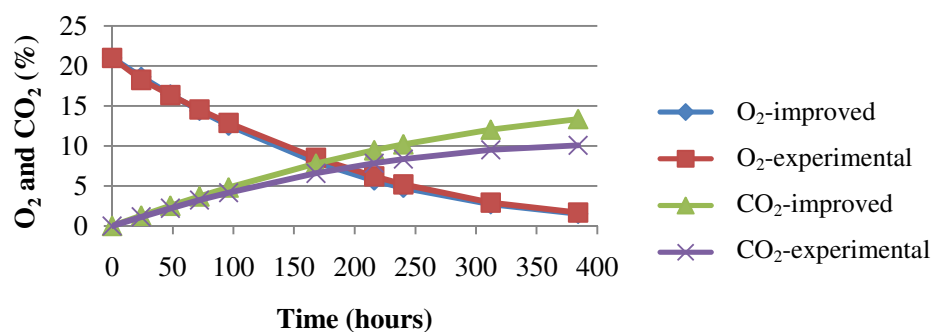
(B)
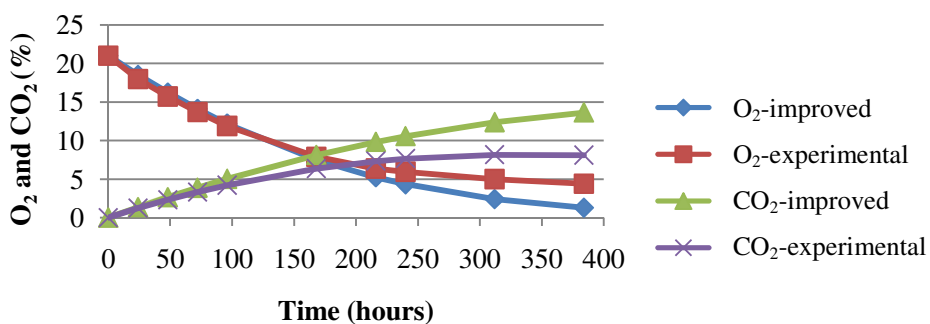
(C)

Figure 31. Improved (based on actual RQ) and experimentally determined O_2 and CO_2 concentrations inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.

PET was found to result in a lowest O_2 concentration (2.91%) compared to those of OPP/LLDPE and LDPE, which raises concern that anaerobic fermentation would occur if products were sealed in this film (Figure 31). LDPE resulted in higher O_2 levels (5.01%) but low CO_2 levels (8.15%). The time to reach equilibrium from each film was very close. While the PET film produced acceptable CO_2 levels for fresh-cut mangosteen, it failed to provide O_2 levels needed to maintain aerobic respiration. Of the films evaluated in this study, only OPP/LLDPE in which the β ratio is 2.71 (Table 21) produced both acceptable O_2 (3.39%) and CO_2 (9.07%) levels. If the same fresh-cut mangosteen mass and film areas were used, common polymeric films with β ratio close to 2.71 would likely result in O_2 levels close to 5%; CO_2 levels would be close to 9%. This condition was reported by Manurakchinakorn *et al.* (2010) that it is a good condition of MAP to maintain the shelf-life of fresh-cut mangosteen.

The improved model (Eq 14) was also used to estimate the effects of package dimension, product weight and free volume on atmosphere composition and compare the CO_2 and O_2 levels generated by fresh-cut mangosteen using film OPP/LLDPE, PET and LDPE to the experimental data (Table 22). The predicted data of CO_2 and O_2 concentration inside package due to changes in film area and product weight are expressed in Figure 32 and 33.

Table 22. Parameters used to evaluate the effect of package dimension and product mass on theoretical gas composition in packages.

| Film	Area (cm^2)	Weight (kg)	Free volume (ml)
OPP/LLDPE	80	0.198	615.46
OPP/LLDPE-½weight reduced	80	0.118	700.55
OPP/LLDPE-½area reduced	40	0.200	609.42
PET	80	0.193	617.60
PET-½weight reduced	80	0.110	712.34
PET-½area reduced	40	0.188	618.55
LDPE	80	0.202	611.78
LDPE-½weight reduced	80	0.103	715.69
LDPE-½area reduced	40	0.177	633.46

The area of all films would be expected to affect steady-state gas composition, but when the area was reduced to ½, the rate at which gas composition approached steady-state was not changed (Figure 32).
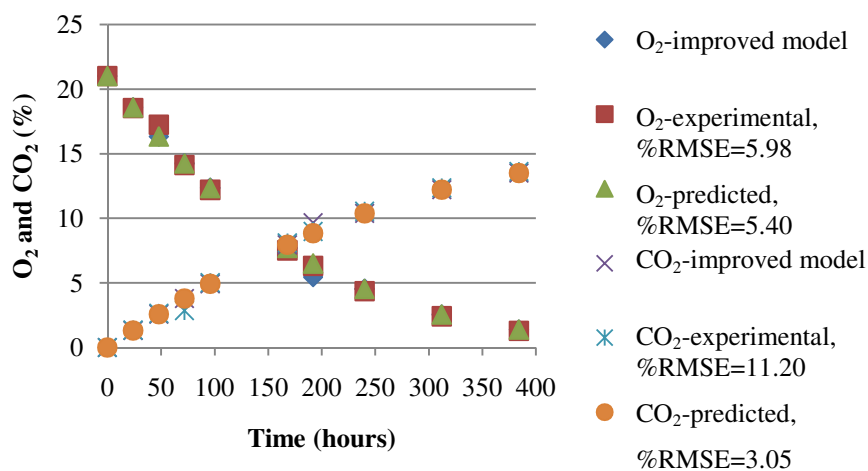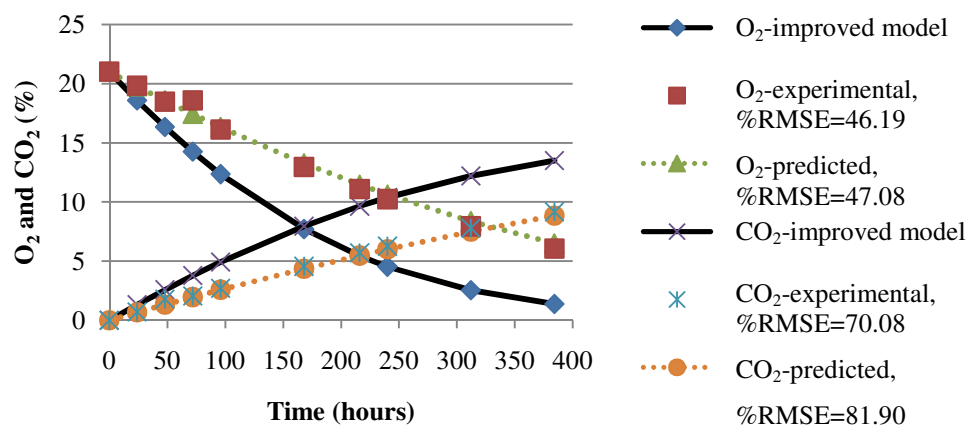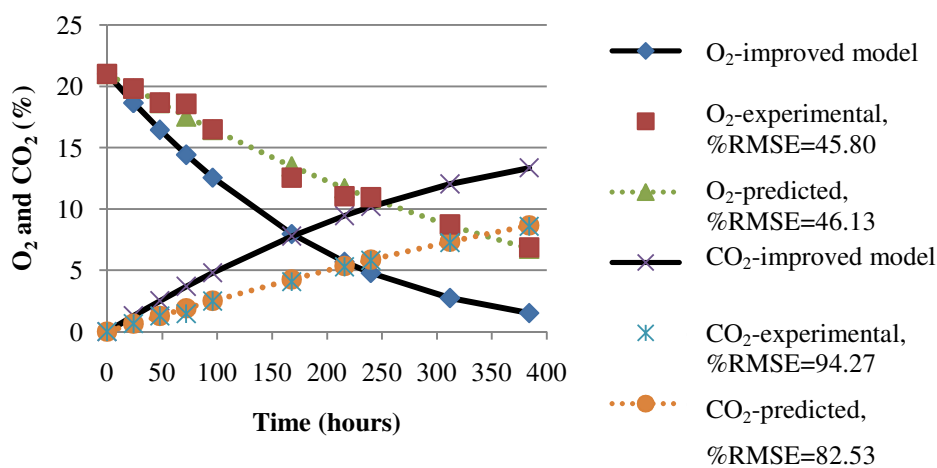
(A)
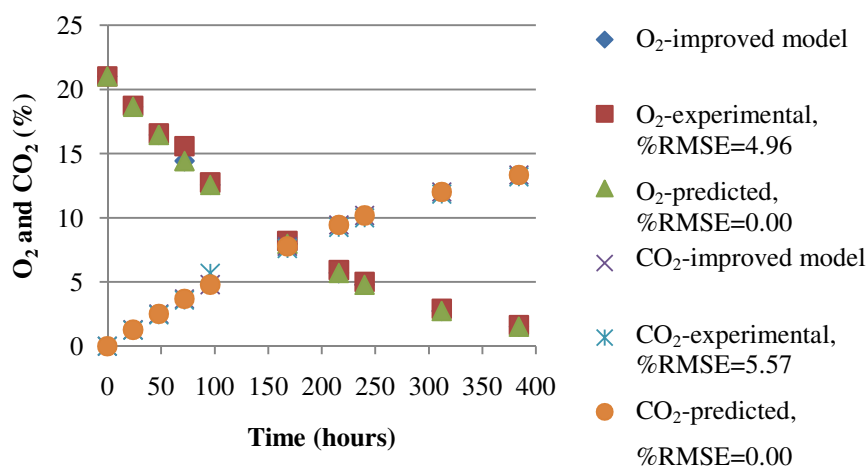
(B)
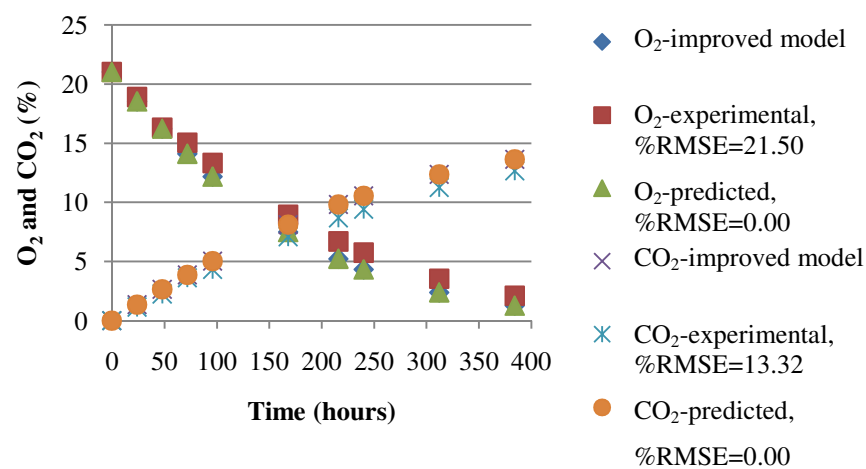
(C)

Figure 32. Effect of changing packaging dimensions on the gas composition inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.

Reducing the weight of fresh-cut mangosteen from around 200 to 100 g seems to reach the same gas concentration at steady-state as 200 g. However, it would take more time to reach the steady state, which may be the time required for 200 g of product to reach steady state (Figure 33). Increasing free volume had a similar effect on gas composition to that resulting from reducing the product weight. Free volume in this work is not focused on because it depends on product weight and could not exactly weigh the samples.

(A)

(B)

(C)

Figure 33. Effect of changing product weight on the gas composition inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.
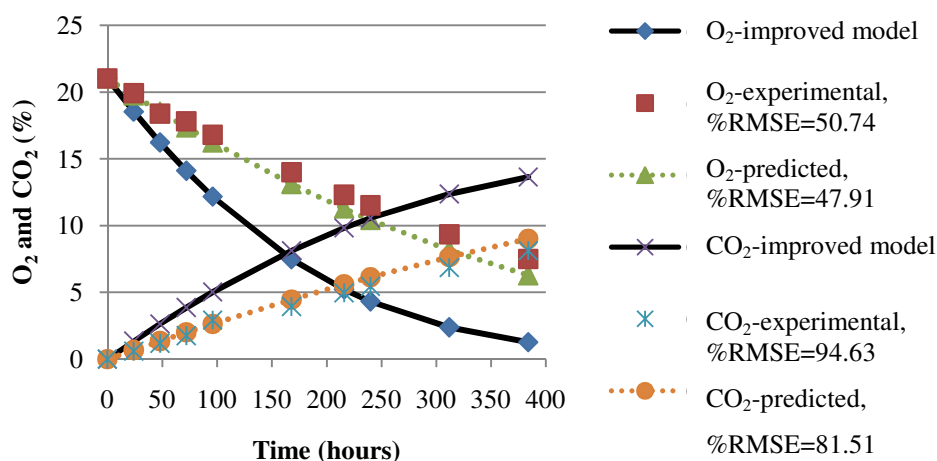
These results indicate that changing the film area could not affect the time required for $[CO_2]$ and $[O_2]$ to reach steady state. Reducing the product weight by 1/2 changed the time required for steady state. Packages with half the fresh-cut weight may require twice the time to reach steady state. From the obtained result, if the time to reach steady state must be shortened, then reducing the free volume or increasing the produce weight should be considered.

The modified model was also used to estimate the effects of changing film thickness and β ratio on atmosphere composition, CO_2 and O_2 levels using film OPP/LLDPE, PET and LDPE (Table 23). The calculated effect on equilibrium CO_2 and O_2 concentration inside package are expressed in Figure 34 and 35. Changing the film thickness of OPP/LLDPE, PET and LDPE films and β ratio of those three films had no effect on the CO_2 and O_2 levels compared to initial films as indicated by %RMSE.

Table 23. Parameters used to evaluate the effect of film thickness and β ratio on theoretical gas composition in packages.

Film	Film thickness (cm)	P_{O_2}	P_{CO_2}	β ratio (P_{CO_2}/P_{O_2})
OPP/LLDPE	0.0071	5.97×10^{-10}	1.62×10^{-9}	2.71
OPP/LLDPE with decreased thickness	0.0036	5.97×10^{-10}	1.62×10^{-9}	2.71
OPP/LLDPE with increased thickness	0.0142	5.97×10^{-10}	1.62×10^{-9}	2.71
OPP/LLDPE with increased β ratio	0.0071	5.97×10^{-10}	1.62×10^{-8}	27.10
OPP/LLDPE with decreased β ratio	0.0071	5.97×10^{-10}	1.62×10^{-10}	0.27
PET	0.0043	3.62×10^{-11}	1.17×10^{-10}	3.23
PET with decreased thickness	0.0022	3.62×10^{-11}	1.17×10^{-10}	3.23
PET with increased thickness	0.0086	3.62×10^{-11}	1.17×10^{-10}	3.23
PET with increased β ratio	0.0043	3.62×10^{-11}	1.17×10^{-9}	32.30
PET with decreased β ratio	0.0043	3.62×10^{-11}	1.17×10^{-11}	0.32
LDPE	0.0069	1.40×10^{-9}	5.25×10^{-9}	3.75
LDPE with decreased thickness	0.0035	1.40×10^{-9}	5.25×10^{-9}	3.75
LDPE with increased thickness	0.0138	1.40×10^{-9}	5.25×10^{-9}	3.75
LDPE with increased β ratio	0.0069	1.40×10^{-9}	5.25×10^{-8}	37.50
LDPE with decreased β ratio	0.0069	1.40×10^{-9}	5.25×10^{-10}	0.37

The P_{O_2} and P_{CO_2} columns are grouped under the header (ml.cm/cm^2 s cmHg).
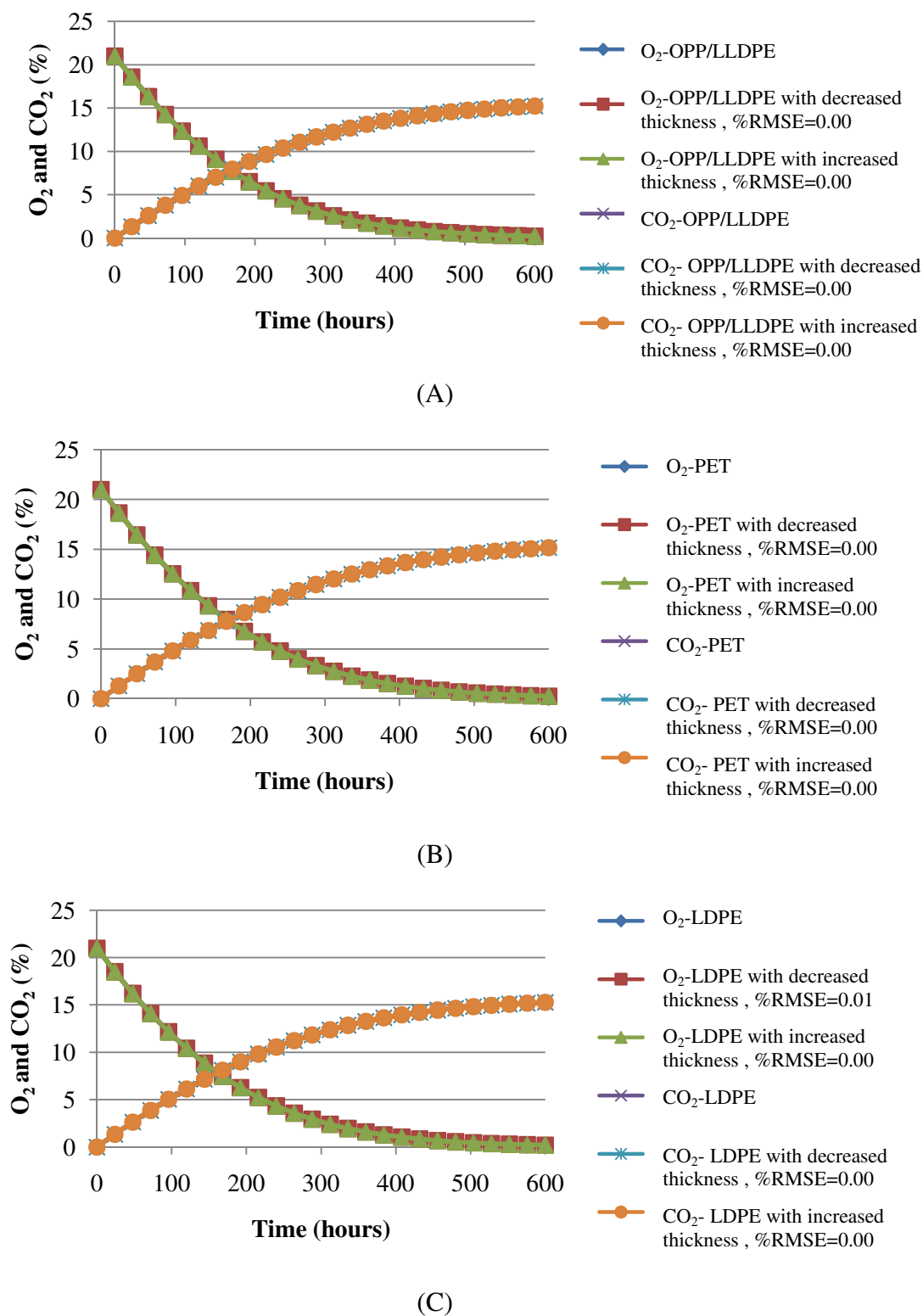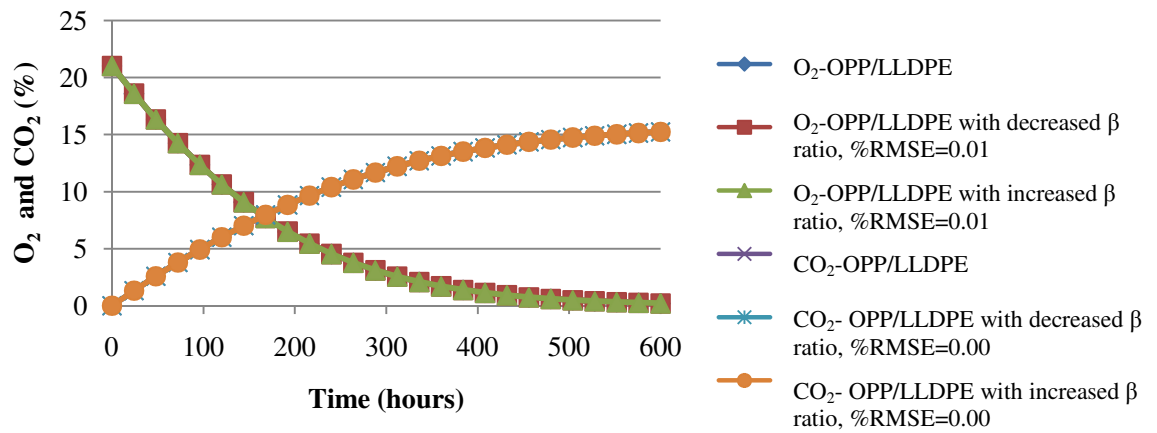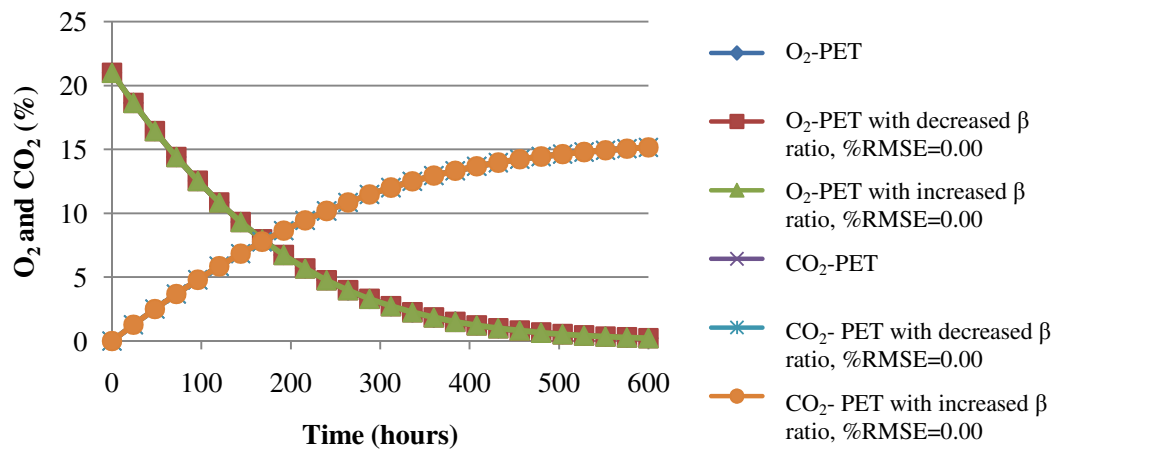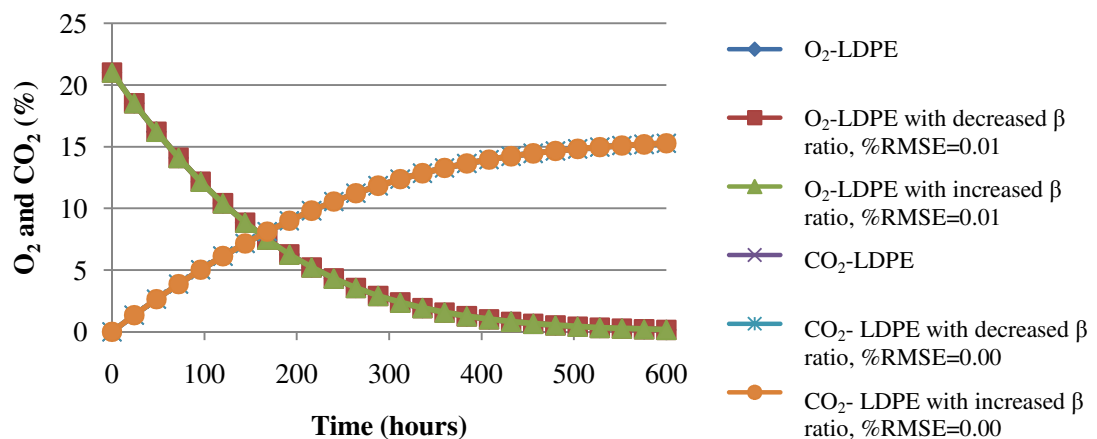
(A)

(B)

(C)

Figure 34. Effect of changing film thickness on the gas composition inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.

(A)

(B)

(C)

Figure 35. Effect of changing β ratio on the gas composition inside package sealed with A) OPP/LLDPE, B) PET and C) LDPE films.

These results are contrast with the result obtained by Al-Ati and Hotchkiss (2003) who conducted a study the effect of reduced film thickness in package of cut apple. Even though reduction in film thickness did not change the time required to reach equilibrium it increased the O_2 level and reduced the level of CO_2 and changing of β ratio also shows the effect on the CO_2 and O_2 levels. Disagreement may be described by the high respiration rate of fresh-cut fruit. The Vm of fresh-cut mangosteen is 714.29 ml/(kg h)while that of cut apple is 56.8 ml/(kg h), a difference which was likely responsible for the different results obtained with the two commodities.

Fresh-cut mangosteen respiration rates are much higher than in entire fruit form preparation and processing such as washing, peeling, trimming and cutting. These processes caused mechanical injury to the mangosteen tissue. Most published research using the Michaelis-Menten equation for modeling produce respiration involved intact fruits and vegetables or produce lightly processed to a lesser extent than the fruit in this work. Processing clearly leads to extensive injury in mangosteen fruit leading to immediate increases in respiration. In addition, the surface area of the arils is high which would favor the solubility and diffusion of O_2 and CO_2. It is likely that these factors, film thickness and β ratio hinder the application of the Michaelis-Menten model to fresh-cut manosteen.

9.5 Conclusions

Fresh-cut mangosteen has a high respiration rate and low RQ compared to other fresh-cut fruits. Consequently, it is difficult to select suitable films for MAP based on existing guidelines. However an existing model was modified to permit guide the selection of films appropriate for this purpose. The modified model was affected only by product weight while package dimension, film thickness and β ratio were not. Fresh-cut mangosteen weight inside the package appears to be the most crucial parameter to be considered.

9.6 References

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CHAPTER 10

CONCLUSIONS AND FUTURE WORKS

10.1 Conclusions

1. Fresh-cut mangosteen evidently suffers from undesirable quality changes most likely due to wound responses in the tissues. Deterioration was mainly due to softening and browning during storage.

2. 1-MCP had significant influence on the physical, chemical and sensory qualities of fresh-cut mangosteen. Fresh-cut product prepared from fruit treated with 40 ppm 1-MCP retained the best quality and this treatment may be appropriate for fresh-cut mangosteen processing.

3. Washing fresh-cut mangosteen with and ASC solution significantly reduced browning intensity during storage. Dipping in a solution containing 500 mg L^{-1} ASC is an acceptable treatment for the control of browning.

4. Mangosteen pericarp extracts have properties that make them suitable as preservative agents for the control of bacteria and oxidative changes in packaged fresh-cut mangosteen.

5. Mangosteen pericarp extracts recovered in methanol have consistently greater antimicrobial activity than those prepared with water; the extracts induce damage to the cell membrane of Gram-positive bacteria.

6. An aqueous extract prepared from mangosteen pericarp can inhibit Gram-positive bacteria, but has little activity against Gram-negative bacteria, fungi and yeast. Lowering the pH of the test medium to pH 4 can enhance the activity of the extract against Gram-positive bacteria.

7. Mangosteen pericarp extract positively affected the preservation of color in stored fresh-cut mangosteen. Furthermore, the extract had no negative effects on the sensory properties of the fruit.

8. OPP/LLDPE and PET films can be used to maintain the overall quality of fresh-cut mangosteen for up 9-12 days at 5 ^{o}C. LDPE film is not recommended for the purpose due to adverse effects on the sensory shelf-life of the product.

9. To select film overlay for fresh-cut mangosteen the product weight is the crucial parameter to be considered because fresh-cut mangosteen has high respiration rate compared to other cut fruits.

10. Combined methods improve the shelf-life of fresh-cut mangosteen. Treatment of the fruit with 40 ppm 1-MCP for 12 h before processing, dipping in a 500 ppm ASC solution and packaging in PP trays sealed with OPP/LLDPE or PET film provide benefits in this regard.

10.2 Future work

1. The effect of ASC against food-borne pathogen should be verified through studies in which fresh-cut mangosteen is inoculated with known levels of pathogens.

2. The potential value of mangosteen pericarp extracts for the control of microorganisms on fresh-cut mangosteen and other fresh-cut fruit should be investigated further; higher concentration should be considered to improve their efficacy.

3. Further characterization of the pericarp extracts should be carried out to identify bioactive components responsible for antimicrobial effects.

APPENDIX

APPENDIX

CACULATING THE *RQ* VALUES

Time (hours)	CO_2	O_2	R_{CO_2}	R_{O_2}	RQ
0	0	21			-
2	0.164	20.186	234.178	1162.464	-
6	0.406	19.744	172.585	315.245	0.547
16	1.000	18.668	169.787	307.249	0.553
24	1.466	17.836	166.189	296.970	0.560
36	2.148	16.636	162.191	285.548	0.568
48	2.809	15.494	157.394	271.841	0.579
72	4.071	13.382	150.198	251.282	0.598
96	5.253	11.500	140.604	223.869	0.628
132	6.875	9.110	128.611	189.604	0.678
168	8.315	7.238	114.219	148.485	0.769
216	9.952	5.548	97.429	100.513	0.969
			Average *RQ*		0.645

$$R_{CO_2} = \frac{\Delta CO_2 \times Free\ volume\ of\ package}{0.21 \times \Delta t}$$

$$R_{O_2} = \frac{\Delta O_2 \times Free\ volume\ of\ package}{0.21 \times \Delta t}$$

$$RQ = \frac{R_{CO_2}}{R_{O_2}}$$

Free volume of package = 599.65 ml

VITAE

Name   Mr. Choothaweep Palakawong Na Ayudhya

Student ID   5011030011

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Biology)	Khon Kaen University	1990
Master of Science (Food Technology)	Khon Kaen University	1999

Scholarship Awards during Enrolment

The Commission on Higher Education, Thailand (the program Strategic Scholarships for Frontier Research Network for the Join Ph.D. Program Thai Doctoral degree, CHE490054)

Work-Position an Address

Asst. Prof. of Faculty of Agricultural Technology, Rajabhat Maha Sarakham University, A.Muang, Maha Sarakham 44000

List of Publication and Proceeding

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