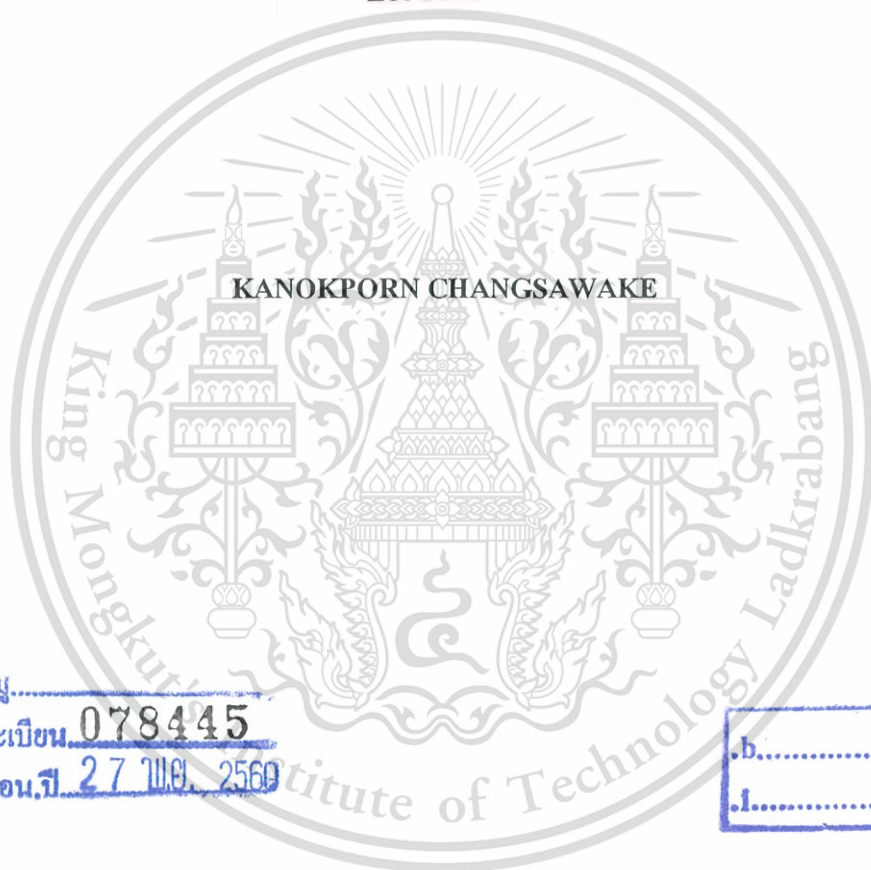


สำนักหอสมุดกลาง พระจอมเกล้าลาดกระบัง

ANTIOXIDANT PROPERTIES OF RD6 GLUTINOUS RICE VINEGAR AND
ITS VAPOR USE TO EXTEND SHELF LIFE OF KAFFIR LIME
AND SWEET BASIL



E078445



เลขหมู่.....
เลขทะเบียน 078445
รับเดือน.ปี 27 100 2560

.b.....
.j.....

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY PROGRAM IN AGRICULTURE
FACULTY OF AGRICULTURAL TECHNOLOGY
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2017

KMITL-2017-AG-D-064-010

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.



COPYRIGHT 2017

FACULTY OF AGRICULTURAL TECHNOLOGY

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

หัวข้อวิทยานิพนธ์	คุณสมบัติการต้านอนุมูลอิสระของน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 และการใช้ไอน้ำส้มสายชูหมักเพื่อยืดอายุการเก็บรักษาใบมะกรูด และใบโหระพา
นักศึกษา	นางสาวกนกพร ช้างเสวก
รหัสประจำตัว	55641051
ปริญญา	ปรัชญาดุษฎีบัณฑิต
สาขาวิชา	เกษตรศาสตร์
พ.ศ.	2560
อาจารย์ที่ปรึกษาวิทยานิพนธ์	รศ.ดร. จำรูญ เล้าสินวัฒนา
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	ผศ.ดร. มณฑินี ชีรารักษ์

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาปริมาณสารประกอบฟีนอลิกรวม กิจกรรมการต้านอนุมูลอิสระ และความสามารถในการป้องกันความเสียหายของดีเอ็นเอที่เกิดจากปฏิกิริยาเพนตันของสารสกัดน้ำจากวัตถุดิบที่ใช้ผลิตน้ำส้มสายชูหมัก (ข้าวเหนียวดิบ และข้าวเหนียวหนึ่ง) ไวน์ และน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 รวมทั้งการใช้ไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 เพื่อยืดอายุการเก็บรักษาใบมะกรูด (*Citrus hystrix* DC.) และใบโหระพา (*Ocimum basilicum* L.) การศึกษากิจกรรมการต้านอนุมูลอิสระทำการทดสอบโดยวิธีการกำจัดอนุมูล DPPH การประเมินความสามารถในการแย่งจับกับโลหะไอออน ความสามารถในการรีดิวซ์ การขจัดอนุมูลอิสระของไฮดรอกซิล การยับยั้งการเกิด lipid peroxidation และการป้องกันความเสียหายของดีเอ็นเอที่เกิดจากปฏิกิริยา Fenton's reaction ผลการทดลองแสดงให้เห็นว่า น้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 มีปริมาณสารประกอบฟีนอลิกรวมสูง (0.612 มิลลิกรัมสมมูลของกรดแกลลิกต่อ 100 มิลลิลิตร) และมีความสามารถในการต้านอนุมูลอิสระโดยวิธี DPPH free radical scavenger สูง มีค่าความเข้มข้นที่มีประสิทธิภาพในการต้านอนุมูลอิสระเป็น 50 เปอร์เซ็นต์ (IC_{50}) เท่ากับ 4.33 เปอร์เซ็นต์ ขณะที่สารละลายมาตรฐานวิตามินซี และสารละลาย BHT มีค่า IC_{50} เท่ากับ 0.0003 และ 0.0021 เปอร์เซ็นต์ เช่นเดียวกับการขจัดอนุมูลอิสระของไฮดรอกซิลที่พบสูงในน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 (IC_{50} เท่ากับ 4.11 เปอร์เซ็นต์) สารละลายมาตรฐาน mannitol มีค่า IC_{50} เท่ากับ 0.4656 เปอร์เซ็นต์ สำหรับการทดสอบความสามารถในการแย่งจับกับโลหะไอออนพบว่า สารทดสอบมีความสามารถในการแย่งจับกับโลหะไอออนต่ำ เมื่อเปรียบเทียบกับสารละลายมาตรฐาน EDTA อย่างไรก็ตามความสามารถในการรีดิวซ์เพิ่มขึ้นเมื่อความเข้มข้นของสารทดสอบเพิ่มขึ้น นอกจากนี้ยังพบว่าข้าวเหนียวหนึ่งมีความสามารถในการยับยั้งการเกิด lipid peroxidation สูง

มีค่า IC₅₀ เท่ากับ 3.24 เปอร์เซ็นต์ จากนั้นเมื่อนำไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 มาวิเคราะห์หาสารที่เป็นองค์ประกอบโดยวิธี GC-MS พบว่ามีสารประกอบสำคัญหลายชนิด ได้แก่ เอทิลอะซิเตด โพรเพน เพนทานอล และกรดอะซิติก

การทดสอบความสามารถของไอรหรือน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ที่มีกรดอะซิติกเป็นส่วนประกอบในการยืดอายุการเก็บรักษาใบมะกรูด และใบโหระพา โดยศึกษาการเปลี่ยนแปลงทางกายภาพ (การสูญเสียน้ำหนัก สีใบ การประเมินคุณภาพ และอายุการเก็บรักษา) และทางชีวเคมี (ปริมาณรงควัตถุ ที่ใช้ในการสังเคราะห์แสง การร่วไหลของเมมเบรน การเกิดลิปิดเปอร์ออกซิเดชัน และการกำจัดอนุมูล DPPH ใบมะกรูดที่รมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ที่มีกรดอะซิติก 8 เปอร์เซ็นต์ เป็นเวลา 0 (ควบคุม), 5, 10, 15 และ 20 นาที และใบโหระพา รมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ที่มีกรดอะซิติก 4 เปอร์เซ็นต์ เป็นเวลา 0 (ควบคุม), 2, 4, 6, 8 และ 10 นาที หลังจากนั้นบรรจุในถุงโพลีเอทิลีน (ขนาด 10x15 นิ้ว) ที่มีรูขนาด 0.7 เซนติเมตร จำนวน 8 รู และเก็บไว้ในที่ 12 องศาเซลเซียส ผลการทดลองพบว่ากรรมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 เป็นเวลา 15 นาที ช่วยการยืดอายุการเก็บรักษาใบมะกรูดได้นานที่สุด (32.4 วัน) เมื่อเปรียบเทียบกับวิธีควบคุม (25.2 วัน) กรรมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ช่วยเพิ่มระยะเวลาในการเก็บรักษาใบมะกรูด โดยลดการสูญเสียน้ำ การร่วไหลของเยื่อเมมเบรน และการเกิดลิปิดเปอร์ออกซิเดชัน รวมถึงรักษาคุณสมบัติในการเป็นสารต้านอนุมูลอิสระ อย่างไรก็ตามไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ไม่มีผลต่อการเปลี่ยนแปลงค่าสี แต่อาจมีส่วนช่วยในการเปลี่ยนสีใบเล็กน้อย และกรรมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 เป็นเวลา 10 นาที ยืดอายุการเก็บรักษาใบโหระพาได้นานที่สุด (25.4 วัน) เมื่อเปรียบเทียบกับวิธีควบคุม (17.8 วัน) กรรมด้วยไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 เพิ่มระยะเวลาในการเก็บรักษาใบโหระพา โดยลดการสูญเสียน้ำ ชะลอการสลายตัวของคลอโรฟิลล์ เอ และ บี รวมถึงรักษาคุณสมบัติในการเป็นสารต้านอนุมูลอิสระ อย่างไรก็ตามไอน้ำส้มสายชูหมักจากข้าวเหนียวพันธุ์ กข 6 ไม่มีผลต่อการร่วไหลของเยื่อเมมเบรน และการเกิดลิปิดเปอร์ออกซิเดชัน แต่อาจมีผลในการรักษาความสมบูรณ์ของเยื่อหุ้มเซลล์ใ้เล็กน้อย

Thesis	Antioxidant Properties of RD6 Glutinous Rice Vinegar and Its Vapor Use to Extend Shelf Life of Kaffir Lime and Sweet Basil.
Student	Miss Kanokporn Changsawake
Student ID.	55641051
Degree	Doctor of Philosophy Program
Program	Agriculture
Year	2017
Thesis Advisor	Assoc. Prof. Dr. Chamroon Laosinwattana
Thesis co-Advisor	Asst. Prof. Dr. Montinee Teerarak

ABSTRACT

The present study examined the total phenolic content, antioxidant activity and ability to protect against DNA damage induced Fenton's reagent of aqueous extracts of raw materials (grain and steamed glutinous rice), wine and fermented vinegar from RD6 glutinous rice. This study also investigated the use of ambient vapor from RD6 glutinous rice fermented vinegar (RD6 GRFV) to extend shelf life of kaffir lime (*Citrus hystrix* DC.) and sweet basil (*Ocimum basilicum* L.). Antioxidant activities were measured using the following methods: DPPH free radical scavenging activity; metal chelating activity; reducing power; hydroxyl radical scavenging activity; and inhibition of lipid peroxidation, along with DNA damage protection activity. Results showed that RD6 GRFV had high total phenolic content ($0.612 \text{ mg GAE mL}^{-1}$) and DPPH free radical scavenger activity, with the IC_{50} (half maximal inhibitory concentration) being 4.33%. In contrast, IC_{50} values of the reference standard ascorbic acid (vitamin C) and butylated hydroxytoluene (BHT) were 0.0003 and 0.0021%, respectively. Similarly, the hydroxyl radical scavenging ability of RD6 GRFV was found to be high (IC_{50} value 4.11%) compared to that of standard mannitol (IC_{50} value 0.4656%). In the metal chelating assay, all tested samples had low ability to chelate ferrous ions, compared with EDTA. However, reducing power increased with higher concentrations of test samples. Steamed glutinous rice exhibited the high lipid peroxidation inhibition, with IC_{50} value of 3.24%. Furthermore, the RD6 GRFV showed effectiveness in preventing DNA damage. The gas chromatography-mass spectrometry (GC-MS) results showed a

number of other volatiles in RD6 GRFV vapor, including ethyl acetate, propane, pentanal and acetic acid.

Regard to the ability study of ambient RD6 GRFV vapor containing acetic acid to extend the shelf life of kaffir lime and on sweet basil. Physical (weight loss, leaf color, quality evaluation and shelf life) and biochemical (photosynthetic pigment content, membrane leakage, lipid peroxidation and DPPH radical scavenging activity) properties of the leaves were recorded. Fresh leaves of kaffir lime were fumigated with RD6 GRFV vapor containing 8% acetic acid for 0 (control), 5, 10, 15 and 20 min. For sweet basil, leave were fumigated with RD6 GRFV vapor contain 4% of acetic acid for 0 (control), 2, 4, 6, 8 and 10 min. After fumigation, leaves were packed in polyethylene bags (size 10x15 in inch), each with eight holes of diameter 0.7 cm, and stored at 12 °C. Exposure to RD6 GRFV vapor for 15 min extended the shelf life of kaffir lime (32.4 days) was difference significantly compared to control (25.2 days). This exposure could be used to markedly extend the postharvest storage life of kaffir lime by helping to reduced water loss, membrane leakage, lipid peroxidation and better retention of antioxidants. Meanwhile, greenness and color values were unchanged by RD6 GRFV but they may have slightly helped in change of leaf color. For sweet basil, exposure to RD6 GRFV vapor for 10 min extended the shelf life of sweet basil (25.4 days) was difference significantly compared to control (17.8 days). This exposure could be used to markedly extend the postharvest storage life of sweet basil by helping to reduce water loss, slow the decline of chlorophylls a and b, and improve retention of antioxidants. Membrane leakage and lipid peroxidation were unchanged by RD6 GRFV, but the exposure may have slightly helped in maintaining membrane integrity.

ACKNOWLEDGEMENTS

I would like to express my great respect and deep appreciation to those who have provided helpful guidance and assisted with the whole work of this study. First, I would like to express my profound gratitude and sincere thanks to Assoc. Prof. Dr. Chamroon Laosinwattana, my advisor, and Asst. Prof. Dr. Montinee Teerarak, my co-advisor, for their precious guidance, valuable advice, kind consideration, and support and encouragement throughout the period of my thesis work and also during the whole period of my study.

This thesis is partially supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office (PERDO), Commission on Higher Education, Ministry of Education.

I would also like to give my sincerest thanks to Assoc. Prof. Dr. Warawut Krusong, the project head for the funding of this research by the King Mongkut's Institute of Technology Ladkrabang Research Fund, (KREF 025604) Thailand and especially to the Department of Plant Production Technology, as well as my co-colleagues who helped me in my research work directly or indirectly.

Finally, I am sincerely and deeply grateful towards my family and my beloved teacher who always made sacrifices to encourage and help me to see success.

Kanokporn Changsawake

TABLE OF CONTENTS

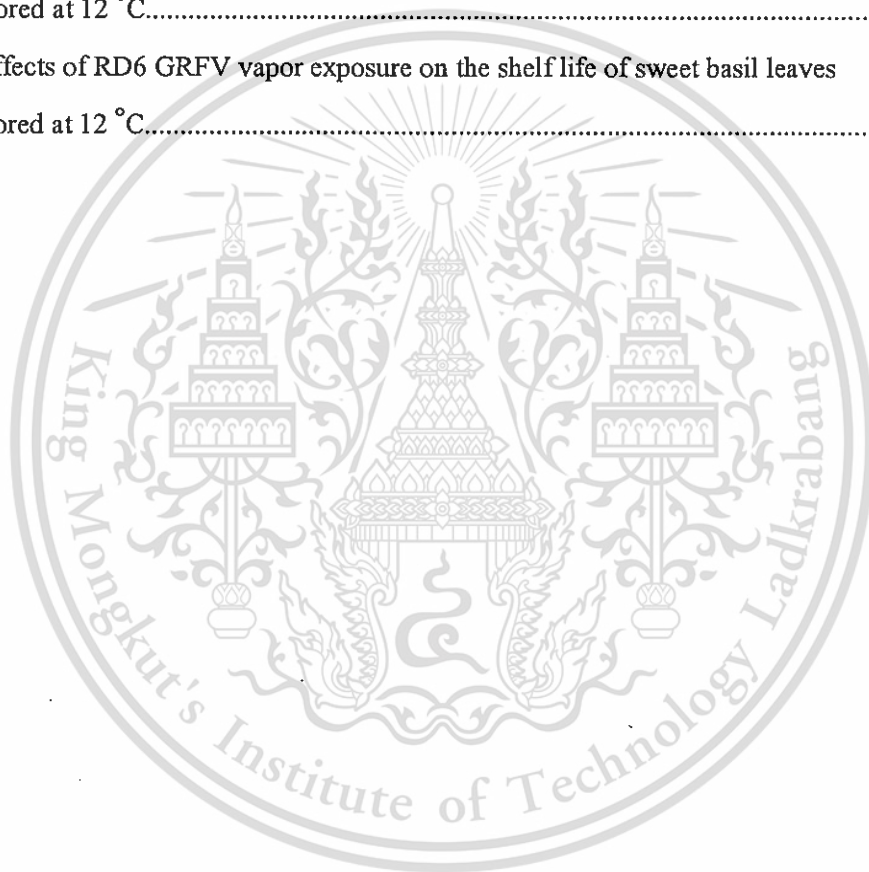
Title	Page
ABSTRACT (Thai).....	I
ABSTRACT (English).....	III
ACKNOWLEDGEMENTS.....	V
TABLE OF CONTENTS.....	VI
LIST OF TABLES.....	VIII
LIST OF FIGURES.....	IX
CHAPTER 1 INTRODUCTION	
1.1 Research Background.....	1
1.2 Objectives.....	2
1.3 Scopes of Study.....	3
1.4 The Benefits of Research.....	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Vinegar.....	4
2.2 Free Radicals and Antioxidants.....	5
2.3 Biology of Plant Test.....	13
2.4 Extending the Shelf Life of Fruits and Vegetables.....	15
CHAPTER 3 RESEARCH METHODOLOGY	
3.1 Chemicals and Instruments.....	18
3.2 Methods.....	19
CHAPTER 4 RESULTS	
4.1 Experiment 1. Study of phenolic and browning compounds in RD6 glutinous rice grain and steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV.....	31

TABLE OF CONTENTS (Continued)

Title	Page
4.2 Experiment 2. Comparison of the antioxidant properties and DNA damage protective activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV.....	32
4.3 Experiment 3. Analysis of RD6 GRFV vapor by GC-MS.....	40
4.4 Experiment 4. Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime and sweet basil.....	40
 CHAPTER 5 DISCUSSION	
5.1 Experiment 1. Study of phenolic and browning compounds in RD6 glutinous rice grain and steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV.....	64
5.2 Experiment 2. Comparison of the antioxidant properties and DNA damage protective activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV.....	65
5.3 Experiment 3. Analysis of RD6 GRFV vapor by GC-MS.....	68
5.4 Experiment 4. Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime and sweet basil.....	69
 CHAPTER 6 CONCLUSION AND SUGGESTION	
6.1 Conclusion.....	77
6.2 Suggestion.....	78
 REFERENCES.....	 79
AUTHOR BIOGRAPHY.....	102

LISTS OF TABLES

Tables	Page
4.1 The amount of total phenolic and browning compound of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV.....	32
4.2 The antioxidant properties of RD6 glutinous rice grain, steamed rice, RD6 glutinous rice wine and RD6 GRFV.....	34
4.3 Volatile compounds identified in RD6 GRFV by GC-MS.....	40
4.4 Effects of RD6 GRFV vapor exposure on the shelf life of kaffir lime leaves stored at 12 °C.....	45
4.5 Effects of RD6 GRFV vapor exposure on the shelf life of sweet basil leaves stored at 12 °C.....	57



LISTS OF FIGURES

Figures	Page
3.1 Weight loss over time during vapor production from RD6 GRFV.....	27
4.1 Inhibitory effects of aqueous extract from RD6 glutinous rice grain on DNA nicking caused by hydroxyl radicals.....	35
4.2 Inhibitory effects of aqueous extract from steamed RD6 glutinous rice on DNA nicking caused by hydroxyl radicals.....	36
4.3 Inhibitory effects of RD6 glutinous rice wine on DNA nicking caused by hydroxyl radicals.....	36
4.4 Inhibitory effects of RD6 GRFV on DNA nicking caused by hydroxyl radicals.....	37
4.5 Densitometric analysis of open circular and supercoiled plasmid DNA upon treatment by Fenton's reaction in the presence of RD6 glutinous rice grain or steamed RD6 glutinous rice extracts.....	38
4.6 Densitometric analysis of open circular and supercoiled plasmid DNA upon treatment by Fenton's reaction in the presence of RD6 glutinous rice wine or RD6 GRFV.....	39
4.7 Change in fresh weight (%) of kaffir lime leaves during storage at 12 °C after treatment with ambient vapor from undiluted RD6 GRFV for periods ranging from 0 to 20 min.....	41
4.8 Changes in color of kaffir lime leaves during storage at 12 °C after treatment with ambient vapor from diluted RD6 GRFV for periods ranging from 0 to 20 min, measured as (A) L* value, (B) a* value and (C) b* value.....	42
4.9 Visual quality score during storage at 12 °C of kaffir lime leaves treated with ambient vapor from diluted RD6 GRFV.....	43
4.10 Visual quality of kaffir lime leaves treated with ambient vapor from RD6 GRFV during storage at 12 °C for 35 days.....	44
4.11 Photosynthetic pigment content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min); (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll.....	47

LISTS OF FIGURES (Continued)

Figures	Page
4.12 Carotenoid content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	48
4.13 Electrical conductivity (EC) of a bathing medium after 1 h (A), 2 h (B) and 3 h (C) of floating of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	49
4.14 Lipid peroxidation expressed as the malondialdehyde (MDA) content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	50
4.15 DPPH radical scavenging activity of kaffir lime leaves stored at 12°C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	51
4.16 Change in fresh weight (%) of sweet basil leaves stored at 12 °C after treatment with ambient vapor from undiluted RD6 GRFV for periods ranging from 0 to 10 min.....	52
4.17 Changes in color of sweet basil leaves during storage at 12 °C after treatment with ambient vapor from diluted RD6 GRFV for periods ranging from 0 to 10 min, measured as (A) L* value, (B) a* value and (C) b* value.....	54
4.18 Visual quality score during storage at 12 °C of sweet basil leaves treated with ambient vapor from diluted RD6 GRFV.....	55
4.19 Visual quality of sweet basil leaves treated with ambient vapor from RD6 GRFV during storage at 12 °C for 25 days.....	56
4.20 Photosynthetic pigment content of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times. (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll.....	59
4.21 Carotenoid content of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	60
4.22 Electrical conductivity (EC) of a bathing medium after 1 h (A), 2 h (B) and 3 h (C) floating of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	61

LISTS OF FIGURES (Continued)

Figures	Page
4.23 Lipid peroxidation expressed as the malondialdehyde (MDA) content of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	62
4.24 DPPH radical scavenging activity of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times.....	63



CHAPTER 1

INTRODUCTION

1.1 Research Background

Better understanding of the importance of healthy eating has increased consumer demand for fresh plant products. More people turn to consume fruits and leafy vegetables, as they believe it is safer than the use of drugs and also a major source of antioxidants such as vitamin C, vitamin E, beta-carotene and phenolic compounds (Soares *et al.*, 1997; Saha *et al.*, 2008). Accordingly, production volumes of these items have risen and supply chains have lengthened. This has put pressure on the industry infrastructure to adopt more sophisticated storage and transport systems that better maintain product quality through to the end user. Like many green, leafy vegetables, fresh herbs deteriorate rapidly after harvest, imposing major marketing limitations (Cantwell and Reid, 2002). Therefore, it is important to reduce postharvest losses, especially of highly perishable leafy vegetables. Usually, vegetables deteriorate considerably during postharvest storage and transportation. After harvest, leafy vegetables are stored in darkness or extremely low irradiance, which induces postharvest senescence (Ella *et al.*, 2003). Degradation of chlorophyll and protein, ethylene production and antioxidant loss are characteristic changes that take place during leafy vegetable senescence (Grozeff *et al.*, 2010; Hassan and Mahfouz, 2012; Sun, 2012). Among several green herbs, the consumption of kaffir lime (*Citrus hystrix* DC.) and sweet basil (*Ocimum basilicum* L.) leaves continuously increases, despite problems with storability and short-shelf life. Major postharvest losses of both herbs occur due to bacterial or fungal infection, chilling injury and physiological disorders. Soonthornvipat (2012) reported that modified atmosphere packing is effective in delaying postharvest senescence of bergamot leaves. A number of techniques have been trailed with a view to extending the postharvest life of sweet basil such as 1-methylcyclopropene (Hassan and Mahfouz, 2010), light and packaging (Anderson *et al.*, 2011), exposure to low-intensity light (Costa *et al.*, 2013) and atmosphere packaging (Lange and Cameron, 1998; Jirapong *et al.*, 2010).

Natural antioxidants present in many plant foods such as vegetables, fruit as well as cereals. Vinegar is a fermented food produced from a variety of cereals. Vinegar has been used for preservation, flavoring and pickling and has been drunk as a health food. It has also been reported to have antioxidative properties (Su and Chien, 2007; Sakanaka and Ishihara, 2008; Aili

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

et al., 2012). Fermented vinegar contains numerous trace components which contribute to its smell and taste. The total mass of these trace components is usually less than 10% and their nature depends on the raw material from which the alcohol is derived, fermentation process, brewing conditions and storage time (Zhang *et al.*, 2006). Glutinous rice vinegar is produced by the fermentation of ethanol by acetic acid bacteria. Antioxidant activities of glutinous rice was reported previously (Jansom *et al.*, 2010; Tananuwong and Tewaruth, 2010; Ngamdee *et al.*, 2016; Rahim *et al.*, 2016). Vitamins and minerals, including iron, from the glutinous rice (raw material) are extracted into the fermented vinegar. Acetic acid, the main organic component of fermented vinegar, has been known for its safe and antimicrobial properties. In postharvest applications, vinegar has been shown to reduce postharvest decay in stone fruits, strawberries and apples (Sholberg *et al.*, 2000); to suppress the development of anthracnose rot in tomatoes (Tzortzakis, 2010); and to improve storage of strawberry fruit (Krusong *et al.*, 2015a) and coriander leaves (Krusong *et al.*, 2015b). Past studies report that the use of this vapor at high temperature can have negative effects on fruit quality such as: discoloration, shriveling and loss of lustre (Wszelaki and Mitcham, 2003); skin browning (Woolf and Laing, 1996); heat injury and mass loss (Zhou *et al.*, 2002); and accelerated fruit ripening (Jacobi *et al.*, 2001). Therefore, the use of vapor at room temperature has the potential to cause less damage while providing a useful postharvest control strategy for fruit and vegetables, including decreasing consumer nervousness surrounding the use of synthetic chemicals in the food. In the present study, the action of Kor Khor 6 (RD6) glutinous rice fermented vinegar (RD6 GRFV); raw material (grain and steamed glutinous rice) and wine against free radicals were studied. In addition, the potential of postharvest treatment with ambient vapor from RD6 GRFV for qualities of kaffir lime and sweet basil leaves was investigated.

1.2 Objectives

The research has been carried out to achieve the following objectives:

1.2.1 To investigate the antioxidant properties of RD6 glutinous rice raw materials (grain and steamed glutinous rice), RD6 glutinous rice wine and RD6 GRFV.

1.2.2 To evaluate the effects of postharvest treatment with RD6 GRFV ambient vapor on the quality and shelf life of kaffir lime and sweet basil.

1.2.3 To study the physical and biochemical changes in kaffir lime and sweet basil leaves after postharvest treatment with ambient RD6 GRFV vapor.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

1.3 Scope of the Study

1.3.1 To investigate total phenolic and browning compounds of RD6 glutinous rice raw materials (grain and steamed glutinous rice), RD6 glutinous rice wine and RD6 GRFV.

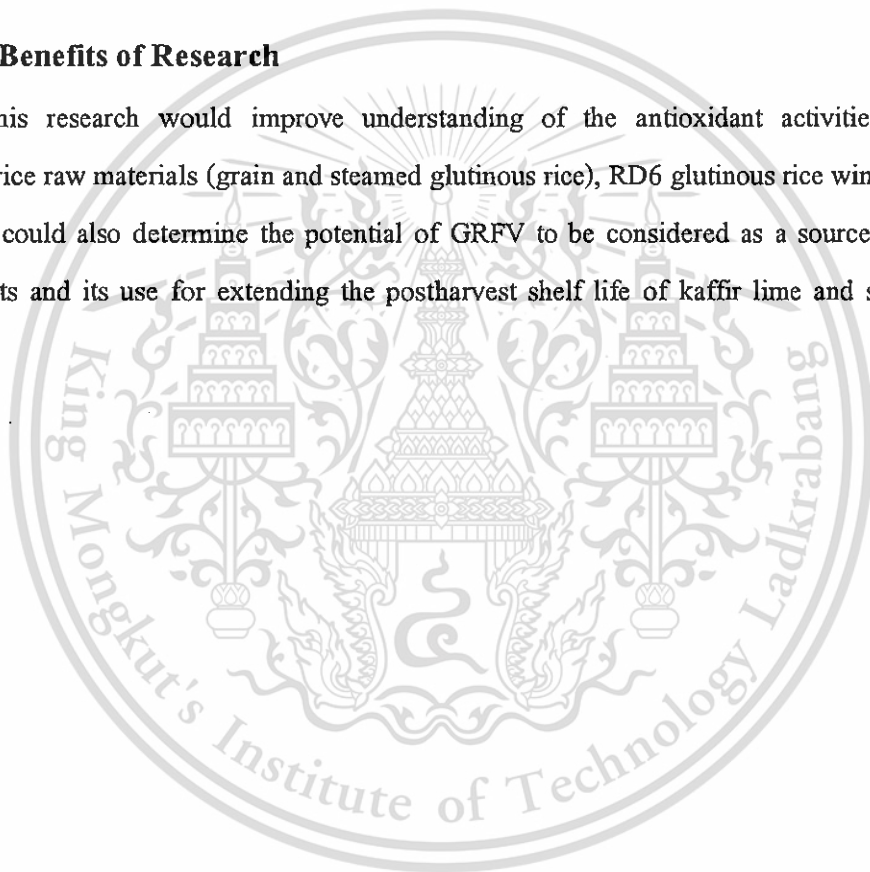
1.3.2 To study the antioxidant properties and DNA damage protective activity of RD6 glutinous rice raw material (grain and steamed rice), RD6 glutinous rice wine and RD6 GRFV.

1.3.3 To determine the chemical composition of RD6 GRFV vapor by GC-MS.

1.3.4 To study the physical and biochemical changes of kaffir lime and sweet basil leaves after postharvest treatment with ambient RD6 GRFV vapor.

1.4 The Benefits of Research

This research would improve understanding of the antioxidant activities of RD6 glutinous rice raw materials (grain and steamed glutinous rice), RD6 glutinous rice wine and RD6 GRFV. It could also determine the potential of GRFV to be considered as a source of natural antioxidants and its use for extending the postharvest shelf life of kaffir lime and sweet basil leaves.



CHAPTER 2

LITERATURE REVIEW

2.1 Vinegar

Vinegar is a sour liquid substance consisting mainly of about 5-20% acetic acid (CH_3COOH), water and other trace chemicals which may include flavorings. The acetic acid is produced through the fermentation of ethanol by acetic acid bacteria (*Acetobacter*). Commercial vinegar is produced either by fast or slow fermentation processes and vinegar can be produced from any alcoholic material ranging from alcohol-water mixtures to various fruit wines (Peppler and Beaman, 1967). The color and aroma of vinegar are greatly dependent on the material from which it is made (Kehrer, 1921). Vinegar is now mainly used as a cooking ingredient or in pickling. Historically, as the most readily available mild acid, it had a great variety of industrial, medical and domestic uses, some of which (such as its use as a general household cleanser) are still practiced today.

2.1.1 Types of vinegar

Vinegar can be made from almost any fermentable carbohydrate source, for example fruits, vegetables, syrups and wines. The basic requirement for vinegar production is a raw material that will undergo alcoholic fermentation. Apples, pears, grapes, honey, syrups, cereals, hydrolyzed starches, beer and wine are all ideal substrates for the production of vinegar. Each variety of vinegar has a distinctive color and flavor, depending on the material from which it was produced (Ministry of Public Health, 2003). Vinegar can be classified into three types as follows:

(1) Fermented vinegar is a product derived from the natural fermentation of cereal, fruit, or sugar by vinegar bacteria and yeast.

(2) Distilled vinegar is a product derived either from the fermentation of distilled alcohol by vinegar bacteria, or by distillation of fermented vinegar.

(3) Artificial vinegar is a product derived from the dilution of acetic acid (final concentration about 4-7%).

2.1.2 Varieties of vinegar

Vinegar made from various sources and they are good for different remedies. Most vinegar contains healthful antioxidants and mineral depend on the raw material used. Varieties of vinegar such as apple cider, balsamic, malt, sherry, red wine and rice vinegars. Rice vinegar is

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

made from fermented rice and is the most commonly used vinegar (Crisco Company, 2005).

2.1.3 Uses of vinegar

2.1.3.1 Antimicrobial activity

Vinegar containing 5% acetic acid showed bactericidal activity attributed to its acidity (Medina *et al.*, 2007). The following microorganisms were used in the study: *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* 0157:H7, *Shigella sonnei* and *Yersinia* sp. The active ingredient in vinegar, acetic acid, can effectively kill mycobacteria, as shown with drug-resistant tuberculosis bacteria and other mycobacteria (Entani *et al.*, 1998).

2.1.3.2 Cleaning

White vinegar, diluted with water, is often used as a natural household cleaning agent because it contains acetic acid which can dissolve mineral deposits from glass, coffee makers and other smooth surfaces. Vinegar can be used for polishing brass or bronze. Vinegar is also one of the best ways to restore color to upholstery like curtains and carpet (Peltier, 2016).

2.1.3.3 Agricultural application

Vinegar can be used as an herbicide, as shown by scientific trials reported by the United States Department of Agriculture (Comis, 2002). The trials showed that a number of common weeds could be effectively controlled using vinegar with five percent to 20 percent acetic acid (lower concentrations were less effective). Acetic acid is not absorbed into root systems, so vinegar will kill top growth but perennial plants will reshoot.

2.2 Free Radicals and Antioxidants

2.2.1 Free radicals

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. One of the well-known principal mechanisms of cytotoxicity leading to cell death is the production of reactive oxygen species (ROS). ROS include oxygen radicals (superoxide and hydroxyl) as well as some non-radical derivatives of molecular oxygen (O₂) such as superoxide anions and hydrogen peroxide (Halliwell, 1999). It is widely acknowledged that damage from free radicals is involved in various human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis as well as adversely affecting cellular components including DNA (Sies, 1997; Mahmood *et al.*, 2008). Under normal conditions, the body can handle free radicals, but if

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

antioxidants are unavailable, or if free radical production becomes excessive, cellular damage can occur.

2.2.2 Antioxidants

Antioxidant substances are of great importance in terms of protection against and therapeutics of diseases involving free radicals (Soares *et al.*, 1997; Saha *et al.*, 2008), where antioxidants from external sources become mandatory. Therefore, natural antioxidants have attracted considerable attention and demand because of positive perceptions from consumers about their safety. Antioxidants act at different levels in the body's defense systems, including prevention (which suppress the formation of free radicals), scavenging active radicals to suppress chain initiation and/or breaking chain propagation reactions, cellular repair and synthesis of de novo antioxidants (Lobo *et al.*, 2010).

2.2.3 Types of antioxidants

Antioxidants can be divided into two major groups, enzymatic antioxidants and non-enzymatic antioxidants.

2.2.3.1 Enzymatic antioxidants

Superoxide dismutase is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. Superoxide dismutase converts superoxide radicals to H_2O_2 (hydrogen peroxide), which catalase goes on to convert into H_2O and O_2 (Subash and Subramanian, 2009). Superoxide dismutase is widely distributed to protect cells against the toxic effects of superoxide anion (Rao *et al.*, 2009). Superoxide dismutase is found in barley grass, broccoli, Brussels sprouts, cabbage, wheatgrass and most green plants.

Catalase is produced naturally within the body. It helps the body to convert hydrogen peroxide into water and oxygen, thus preventing the formation of carbon dioxide bubbles in the blood (Turkseven *et al.*, 2005). Catalase is found in a number of fruits and vegetables in large enough quantities to transform toxic oxidants to substances useful to the body. Pineapples, cherries, apricots, bananas, watermelons, kiwis and peaches have the highest levels of catalase among fruits when eaten fresh.

Glutathione peroxidase is an antioxidant enzyme "family" and is an enzyme dependent on the micronutrient selenium. Glutathione peroxidase plays a critical role in the scavenging and inactivation of hydrogen and lipid peroxides, potential substrates for the Fenton's reaction, thereby protecting the body against oxidative stress. If glutathione peroxidase activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage. Glutathione

This material is reserved for educational use only, not allowed for commercial use.

peroxidase is related to but not the same as glutathione, the master antioxidant. Glutathione peroxidase is found in almost all fruits and vegetables (Shazia *et al.*, 2012a).

2.2.3.2 Nonenzymatic antioxidants

Vitamin A is a group of fat-soluble compounds that can be divided into two categories, depending on the food sources. Vitamin A found in foods that come from animals is called preformed vitamin A or retinol. The richest food source of preformed vitamin A is liver, with considerable amounts also found in egg yolk, whole milk, butter and cheese. Vitamin A found in fruits and vegetables is called provitamin A carotenoid, which can be cleaved into retinol in the body. Provitamin A carotenoid is found in carrots, yellow and dark green leafy vegetables (e.g. spinach, broccoli), pumpkins, apricots, melons and palm oil (Shazia *et al.*, 2012b).

Vitamin C or **ascorbic acid** is a monosaccharide antioxidant found in both animals and plants that cannot be synthesized in humans and must be obtained from the diet. Ascorbic acid is a reducing agent that can reduce and thereby neutralize reactive oxygen species, such as hydrogen peroxide (Linster and Van Schaftingen, 2007). This vitamin is found in citrus fruits like oranges and limes, as well as green peppers, broccoli, green leafy vegetables, strawberries and tomatoes.

Vitamin E is a fat-soluble vitamin existing in eight different forms. The main function of Vitamin E is to protect against lipid peroxidation (Pryor, 2000) and there is also evidence to suggest that α -tocopherol and ascorbic acid function together in a cyclic process. During this reaction, α -tocopherol is converted to an α -tocopherol radical by the donation of a labile hydrogen to a lipid or lipid peroxy radical and the α -tocopherol radical can therefore be reduced to the original α -tocopherol form by ascorbic acid (Kojo, 2004). Vitamin E is found in almonds, other nuts and seeds, whole grains, wheat germ, green leafy vegetables, broccoli, mangos, vegetable oils and soybean oil.

Selenium, zinc and copper are trace elements that have indirect antioxidant properties and are essential in enzymatic defense systems against ROS-induced cellular damage. Lowered selenium status has been linked to neurodegenerative and cardiovascular diseases as well as to an increased risk of cancer (Brenneisen *et al.*, 2005). The key role of selenium in human metabolism is attributed to its presence in glutathione peroxidase, an antioxidant enzyme. Selenium is found in red meat, fish and shellfish, eggs, chicken, grains and garlic. The biological roles of zinc and copper are related to their participation in the structures and functions of many enzymes, e.g. copper and zinc superoxide dismutase which has antioxidant and anti-inflammatory

This material is reserved for educational use only, not allowed for commercial use.

activity. Copper and zinc are also essential co-factors for enzymes involved in the synthesis of various bone matrix constituents and could be important in the elderly since they may play an important role in reducing bone loss in osteoporosis (Lowe *et al.*, 2002). Many kinds of fish and shellfish are rich in copper and zinc, as are foods including meat, eggs, dairy products and legumes.

Beta-carotene is a fat soluble member of the carotenoids, which are considered provitamins because they serve as precursors of vitamin A (converted to vitamin A). Beta-carotene is converted to retinol, which is essential for vision (Sies and Stahl, 1995). It is a strong antioxidant and the best quencher of singlet oxygen. Beta-carotene, besides being a precursor to vitamin A, has potent antioxidant properties as it removes singlet oxygen and thus protects against free radical attack (Goulson and Warthesen, 1999). Beta-carotene is present in liver, egg yolks, milk, butter, spinach, carrots, tomatoes, grains, cantaloupes, pumpkins, apricots, squash and some green leafy vegetables (kale and spinach).

Carotenoids are plant pigments responsible for bright red, yellow and orange hues in many fruits and vegetables. These pigments play an important role in plant health. Carotenoids have received considerable attention for their possible clinical uses in diseases associated with reactive oxygen species such as cancer (Goto *et al.*, 2001). Therefore, carotenoids have been regarded to be of value not only as effective nutrients for the eyes, but also as antioxidants (Nissen *et al.*, 2003). Carotenoids are found in many plants, algae and bacteria.

Polyphenols are aromatic secondary plant metabolites and are widely spread throughout the plant kingdom; these compounds have been associated with color, sensory qualities and the nutritional and antioxidant properties of food. There are several mechanisms for their antioxidant activity, but it is believed that radical scavenging via hydrogen atom donation is the predominant mode. Polyphenols are considered chemopreventive agents because they can quench or prevent the formation of ROS and reactive nitrogen species, which play important roles in carcinogenesis (D'Angelo *et al.*, 2009). Foods which are rich in phenols are onion, tea, red wine, cocoa and virgin olive oil. Phenol compounds can directly scavenge H_2O_2 . Flavonoids or polyphenols are found in soy, red wine, purple or concord grapes, acai berries, pomegranates, blueberries, cranberries and tea.

2.2.4 Methods for testing antioxidant activity and phenolic compound content

2.2.4.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical is usually a dark-colored, powdered organic compound composed of stable free-radical molecules with many crystalline

This material is reserved for educational use only, not allowed for commercial use.

forms (Kiers *et al.*, 1976; Teruaki, 1981). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of foods (Hou *et al.*, 2001). DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom (an antioxidant), this gives rise to the reduced, non-radical form diphenylpicrylhydrazine (DPPH-H). The reduced form loses the violet color and as a consequence its absorbance is decreased compared to that of DPPH. The decolorization is more pronounced with respect to the number of electrons captured.

The scavenging reaction between DPPH and an antioxidant (H-A) can be written as:

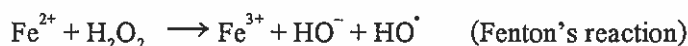


2.2.4.2 Hydroxyl ($\bullet\text{OH}$) radical scavenging activity. The hydroxyl radical is the neutral form of the hydroxide ion (OH^-). Hydroxyl radicals are highly reactive (easily becoming hydroxyl groups) and consequently short-lived; however, they form an important part of radical chemistry (Ohkawa *et al.*, 1979). The hydroxyl radical is one of the most potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cells (Halliwell and Gutteridge, 1981). Hydroxyl radicals generated from a Fe^{2+} -ascorbate-EDTA- H_2O_2 system (Fenton's reaction) can be estimated by the degradation of deoxyribose, resulting in thiobarbituric acid reactive substances (TBARS) (Elizabeth and Rao, 1990).

The first step involves reduction of ferric into ferrous ion:



The second step is the Fenton's reaction:



The sugar deoxyribose, on exposure to hydroxyl radicals generated by the Fenton's reaction model system, degrades into fragments. Upon heating with thiobarbituric acid (TBA) at low pH, this solution generates a pink chromogen that can be measured spectrophotometrically at 532 nm.

2.2.4.3 Anti-lipid peroxidation. Lipid peroxidation proceeds by a free-radical-mediated chain reaction that includes initiation, propagation and termination reactions. The chain

reaction is initiated by the abstraction of a hydrogen atom from a methylene group of an unsaturated fatty acid. Lipid peroxidation, a well-established mechanism of cellular injury in plants and animals, is used as an indicator of oxidative stress in cells and tissues (Ohkawa *et al.*, 1979). Lipid peroxides are unstable and decompose to form a complex series of compounds, including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) upon decomposition and the measurement of MDA has been used as an indicator of lipid peroxidation (Janero, 1990).

2.2.4.4 Metal chelating activity. Ferrozine can chelate with Fe^{2+} and form a red colored complex which can be quantitated. This reaction is limited in the presence of other chelating agents, resulting in less of the red ferrozine- Fe^{2+} complex (Dinis *et al.*, 1994). The absorbance of the ferrozine- Fe^{2+} complex is measured at 562 nm (Soler-Rivas *et al.*, 2000). The antioxidants present in plant extracts form a coordinate complex with metal ions (chelating activity) and inhibit the transfer of electrons. Thus the oxidation reaction is arrested and no free radicals are produced.

2.2.4.5 Reducing power. This method is based on measuring an increase in the absorbance of reaction mixtures, which indicates an increase in antioxidant activity (Oyaizu, 1986). Substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric-ferrous complex that has an absorption maximum at 700 nm.

Antioxidant



2.2.4.6 Plasmid DNA nicking. This assay tests the ability of extracts to protect plasmid DNA against damage caused by hydroxyl (OH) radicals, as evaluated by DNA nicking assay. Hydroxyl radicals produced by Fenton's reagent react with the nitrogenous bases of DNA to create base radicals and sugar radicals. The base radicals in turn react with the sugar moiety, causing breakage of the nucleic acid's sugar-phosphate backbone and resulting in a strand break (von Sonntag, 1987). The plasmid's initial supercoiled configuration changes to open circular and nicked linear forms that present altered electrophoretic mobility properties on an agarose gel (Kitts *et al.*, 2000).

2.2.4.7 Phenolic compounds. Phenolic compounds are among the most important natural antioxidants for preventing oxidation in food and the human body. These compounds are

involved in food browning, a chemical reaction that occurs in many kinds of fruits and vegetables and can occur in the post-harvest period, during processing and during storage of raw materials. The browning reaction can result from either enzymatic or non-enzymatic processes; non-enzymatic processes can involve a combination of phenolic compounds and metal ions, decomposition of vitamin C, or the Maillard reaction. The Maillard reaction is a chemical reaction between an amino acid and a reducing sugar, protein, or some other nitrogen compound and usually requires the addition of heat. This reaction produces brown substances known as melanoidins, which exhibit antioxidant properties (Robards *et al.*, 1999).

2.2.4.8 Antioxidant substances in rice, wine and vinegar. Compounds in vinegar can vary differently due to the production processes including alcoholic fermentation, acetic fermentation, thermal processing and aging as well as raw materials including a range of fruits and cereals. Upland rice vinegar is made from glutinous rice. The antioxidative action and the chemical component of various rice varieties have been reported by many researchers (Jun *et al.*, 2011; Tanaka *et al.*, 2011; Hansakul *et al.*, 2011; Zubair *et al.*, 2012; Muthal *et al.*, 2015; Pengkumsri *et al.*, 2015). Tananuwong and Tewaruth (2010) investigated the antioxidant properties of black glutinous rice flour, which was extracted with 70:30 acetone-water (v/v) at pH 2 or 6.8 for total extraction times of 2, 4, or 8 h. The results revealed that crude extract with pH 6.8 solvent and 4 h extraction time had the highest antioxidant activity as analyzed by both ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays (significant at $p \leq 0.05$), although its total phenolic content and total monomeric anthocyanin content were not the greatest. Walter *et al.* (2013) reported that rice grains with red or black pericarp had higher soluble phenolic compounds and antioxidant activity values. Parboiling reduced soluble phenolic concentration in the grains due to loss in the processing water, thermal decomposition and possibly interactions with other components. Brazilian rice cultivars showed high amounts of unsaturated fatty acids such as linoleic and oleic acid. They found that the DPPH and FRAP assays showed the highest correlations (Palombini *et al.*, 2013). Sripum *et al.* (2016) investigated the effects of storage conditions on the antioxidant capacity and total phenolic content of parboiled germinated brown Thai jasmine rice (Khao Dok Mali 105). They found that storage temperature did not significantly change either antioxidant activity (ferric reducing antioxidant power values of 3.48-3.71 $\mu\text{mol TE g}^{-1}$ and oxygen radical antioxidant values of 24.75-28.12 $\mu\text{mol TE g}^{-1}$, DPPH values of 62-66 %), or total phenolic content (62-64 mg GAE g^{-1}). Their results also suggested that parboiled germinated brown rice exhibited higher

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

antioxidant activity and total phenolic content than germinated brown rice, brown rice, or white rice. Moreover, wines and vinegars derived from *different* plant foods have been reported to have antioxidative actions (Tagliazucchi *et al.*, 2008; Ortiz *et al.*, 2013; Jagtap and Bapat, 2014; Ageeva *et al.*, 2015). For instance, jackfruit wine was effective in DPPH radical scavenging ($69.44 \pm 0.34\%$), ferric reducing (0.358 optical density value, O.D.), N, N-dimethyl-p-phenylenediamine radical scavenging ($78.45 \pm 0.05\%$) and nitric oxide scavenging ($62.46 \pm 0.45\%$). By high performance liquid chromatography coupled to a diode array detector (HPLC-DAD), two phenolic compounds (gallic acid and protocatechuic acid) were identified. Moreover, the jackfruit wine was also able to protect pBR322 plasmid DNA against damage induced by H_2O_2 + UV radiation and γ -radiation (100 Gy) (Jagtap *et al.*, 2011). Pinsiromdom *et al.* (2010) investigated the quality of nine samples of wine vinegar derived from fruit and grain. All wine vinegar samples contained greater levels of phenolic compounds (80.3 - $973.4 \mu\text{g mL}^{-1}$) and greater antioxidant strength (22 - $3,148 \mu\text{g AAeq mL}^{-1}$, 15 - $2,107 \mu\text{g TLeq mL}^{-1}$ and 140 - $19,880 \mu\text{g BHTeq mL}^{-1}$ for DPPH scavenging activity and 462 - $15,362 \mu\text{g AAeq mL}^{-1}$, 270 - $8,991 \mu\text{g TLeq mL}^{-1}$ and 194 - $6,455 \mu\text{g BHTeq mL}^{-1}$ for H_2O_2 scavenging activity), compared to distilled vinegar. Among the nine samples, balsamic vinegar exhibited the strongest antioxidant capacity, which correlated to the highest level of phenolic compounds. Antioxidant activities of vinegars derived from blackberry, red ginseng (Kim *et al.*, 2012) *Akebia quinata* fruit (Lee *et al.*, 2014) apple and grape (Bakir *et al.*, 2016) was reported previously. The physicochemical properties of various commercially available vinegar drinks consumed in the Korean market included pH, acidity, sugar, total soluble sugar, total acid, total amino acid content and antioxidant capacity. The pH values ranged from 2.81 to 3.20 and total acidity ranged from 1.95% to 2.34%. Sugar content ranged from 31.63 to 38.75 °Bx and the total amino acid content ranged from 781 to 982 mg L⁻¹. The highest total anthocyanin content was 13.21 mg% in blackberry vinegar and the lowest was 0.06 mg% in red ginseng vinegar. Blackberry vinegar was also found to have the highest antioxidant activity and highest total polyphenol content (Kim *et al.*, 2012). Lee *et al.* (2014) investigated the physicochemical properties and antioxidant activity of vinegar added with different levels (0, 1, 3, 5 and 7%) of *Akebia quinata* fruit during two-step fermentation. During acid fermentation, total acidities of vinegar increased. However, total polyphenol contents of vinegar added with 0-7% *Akebia quinata* fruit significantly increased and vinegar added with 7% *Akebia quinata* fruit showed the highest total polyphenol content (4.15 mg g^{-1}) after 13 days of fermentation. Bakir *et al.* (2016) studied the effects of different processing steps on the

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

antioxidant, phenolic compound and flavonoid contents of apple and grape vinegars using spectroscopic methods and high-performance liquid chromatography (HPLC). Their HPLC results showed that metabolites such as gallic acid are lost in later stages of vinegar processing. Similarly, spectrophotometric methods indicated a strong loss of antioxidant phenolic compounds during the transition from fruit wine to fruit vinegar.

2.3 Biology of Plant Tested

2.3.1 Kaffir lime

Botanical name: *Citrus hystrix* DC., Family Rutaceae (Citrus family); also known as Makrut leaves. Kaffir lime is from Southeast Asia and popular in Thailand, Cambodia, Malaysia and Indonesia. Kaffir lime has aromatic leaves, which are evergreen and have a distinctive structure with a winged petiole (leaf stem) that is similar in size to the leaf itself, giving the appearance of a laterally divided leaf. The leaves are aromatic, used as a spice and for various flavoring purposes. The fruit is bitter and not very palatable, but the rinds and leaves are widely used in Thai cooking. Oil is also extracted from the rind for use in cosmetics and beauty products (Katzner, 2002). The stem barks of *C. hystrix* showed methanolic extract of leaves were used as mosquito repellent (Tawatsin *et al.*, 2001) and to inhibit the herpes virus (Fortin *et al.*, 2002).

Benefits: Fresh kaffir lime leaves are regularly rubbed on the teeth and gums to aid in dental health. Essential oils are often extracted from limes and leaves to produce a vast variety of products such as air fresheners, deodorants, detergents, soaps and shampoos. Some of the oils produced are used in shampoos for the treatment of both hair and scalp; one wash with the shampoo will not only leave hair squeaky clean, but also help in reviving the scalp. The oil is also infused in deodorants and body sprays for extra zing and used in tonics which aid in digestion and purifies the blood.

Main constituents: The compound responsible for kaffir lime's characteristic aroma was identified as (-)-(S)-citronellal, which comprises up to 80% of the leaf oil; minor components are citronellol (10%), nerol and limonene. Kaffir lime peel contains an essential oil comparable to lime peel oil; its main components are limonene and β -pinene (Ng *et al.*, 2001; Nurhani, 2013).

Extending the shelf life of kaffir lime: In the treatments to extend the shelf life has previously been reported by Ratanachinakorn *et al.* (2007) investigated the effect of temperature on storage life of fresh-cut fruits and vegetables. The result found that kaffir lime leaves were stored at 10 °C extended the shelf life of 24 days.

2.3.2 Sweet basil or Thai basil

Botanical name: *Ocimum basilicum* L., Lamiaceae family. Thai basil is also known as sweet basil or Asian basil and the Thai name is *bai horapa*. These tropical varieties of basil are slightly sweeter and have more stable flavor when cooked than Mediterranean basil. Sweet basil is an erect annual, up to 35 cm in height, with ovate, toothed, or entire leaves which are up to 8 cm in length. The flowers are white or purple-tinged, around 1 cm long and borne in simple terminal racemes. Some varieties have partly red or entirely purple leaves (Vaughan and Geissler, 1997). A great diversity of sweet basil varieties have been developed (Simon *et al.*, 1999).

Benefits: Sweet basil has antibacterial properties and contains high amounts of beta-carotene. It is full of vitamins and minerals such as vitamin A, vitamin B, vitamin C, calcium, iron and phosphorus. It can be boiled with filtered water and drunk day and night for antibacterial, anti-cancer and anti-fungal effects as well as lowering blood pressure and blood sugar. Sweet basil contains powerful antioxidants to potentially protect the body from damage caused by toxins and free radicals. Basil helps prevent memory loss associated with old age and helps in the treatment of constipation, stomach cramps, indigestion and flatulence when used as a tea.

Main constituents: Sweet basil oil is made up of d-linalool, estragole (methyl chavicol) and methyl cinnamate. The oil also contains other compounds such as 1,8-cineole, eugenol, borneol, ocimene, geraniol, anethol, 10-cadinols, β -caryophyllene, α -terpineol, camphor, 3-octanone, methyleugenol, safrole, sesquithujene and 1-epibicyclosesqui-phellandrene. It also contains juvocimene 1 and juvocimene 2. Take note that variations on these chemicals may exist depending on the source of the plant (Re: Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics).

Extending the shelf life of sweet basil: In the treatments to extend the shelf life has previously been reported by Berry *et al.* (2010) investigated the effect of 1-methylcyclopropene (1-MCP) on basil shoot quality during storage at temperatures at 5 or 10 °C. The result found that shoots from all treatments retained good to excellent quality when stored up to 9 d at 5 or 10 °C. Similar to Hassan and Mahfouz (2010) investigated the effect of 1-MCP on shelf-life and postharvest quality of sweet basil detached leaves. 1-MCP treatment significantly retarded the degradation of chlorophyll and protein content of detached leaves during storage and decreased leaf ethylene production. Furthermore, sweet basil leaves induced by ethylene under modified atmosphere packaging and packed in 60 μ m polyethylene (PE) bags showed best condition in maintaining the quality of basil for 9 days (Jirapong *et al.*, 2010). It also has light and packaging

This material is reserved for educational use only, not allowed for commercial use.

(Anderson *et al.*, 2011) and chilling or non-chilling temperatures (Fратиanni *et al.*, 2017)

2.4 Extending the Shelf Life of Fruits and Vegetables

Usually, vegetables deteriorate considerably during postharvest storage and transportation. Extending shelf life is important for improved transport systems that better maintain product quality through to the end user. Methods to extend the shelf life of fruits and vegetables are as follows.

2.4.1 Cold storage

Deterioration of fruits and vegetables during storage depends largely on temperature. One way to slow down this change and so increase the length of time fruits and vegetables can be stored, is by lowering the temperature to an appropriate level. All fruits and vegetables have a 'critical temperature' below which undesirable and irreversible reactions or 'chill damage' takes place. The storage temperature always has to be above this critical temperature. Cold storage postharvest treatments are performed for cherry tomatoes (Fagundes *et al.*, 2015) and peppermint (Barbosa *et al.*, 2016).

2.4.2 Controlled atmosphere storage

Controlled atmosphere (CA) storage is a technique whereby the level of oxygen is reduced and CO₂ is increased. Long-term storage of vegetables and fruit involves inhibiting the ripening and ageing processes, thus retaining flavor and quality. This occurs as a result of modifying gas conditions in the cool cell so that respiration of the fruit and vegetables is reduced. Quality and freshness of fruit and vegetables are retained under CA conditions without the use of any chemicals and many products can be stored for two to four times longer than usual. Cantin *et al.* (2011) investigated the effect of 1-MCP treatment on softening of 'Hayward' kiwifruit under ethylene-free and ethylene-contaminated atmospheres during storage. Oliveira *et al.* (2015) investigated the application of modified atmosphere packaging to fresh-cut fruit and vegetables as a safety approach. Including D'Aquino *et al.* (2016) report the effect of modified atmosphere packaging on postharvest quality of cherry tomatoes

2.4.3 Wax

Fruit waxing is the process of covering fruits such as *Ortanique tangor* (Machado *et al.*, 2012) and in some cases vegetables, with waxing material. Natural wax is removed first, usually by washing. Applied waxing materials may be either natural or petroleum-based. The primary reasons for waxing are to prevent water loss and thus retard shrinkage and spoilage, and to

This material is preserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

improve appearance. Dyes may be added to further enhance appearance and sometimes fungicides.

2.4.4 Ozone

Ozone gas is already widely employed as a surface sterility agent. Ozone is becoming an important alternative to chlorine for disinfection of wash water for fruits and vegetables. It has been evaluated for postharvest disease control and other storage uses. Some commercial use has occurred with a few commodities such as apples, cherries, carrots, onions and potatoes. In general, ozone treatments in postharvest storage have the greatest economic benefit when stored produce will be sorted prior to shipment or re-packed following distribution and short-term storage to remove decayed product. Ozone destruction of ethylene in air filtration systems has been linked to the extended storage life of diverse ethylene-sensitive commodities including strawberries (Pérez *et al.*, 1999) and persimmons (Salvador *et al.*, 2006).

2.4.5 Sulfur dioxide (SO₂)

Sulfur dioxide gas is one of the oldest antimicrobial agents and is used as a fumigant for postharvest decay control. It is also commonly used to prevent enzymatic browning in fruit and vegetables due to its inhibition of polyphenol oxidase enzyme activity. Sulfur dioxide fumigation is used in the postharvest handling of fresh longan and lychee for export to foreign markets. Venditti *et al.* (2012) investigated the quality of table grapes (*Vitis vinifera* L. cv. 'Taloppo') after storage with acetic acid (AC) treatment, comparing in addition the effects of AC and SO₂ treatments. Results suggested that all treatments reduced gray mold incidence, with respect to untreated fruit.

2.4.6 Packaging films

Barrier packaging can often help control or extend shelf life. When moisture content is a mechanism for product degradation, the use of packaging with a low moisture vapor transmission rate, along with desiccants, helps keep moisture in the package within acceptable limits. When oxidation is the primary concern, packaging with a low oxygen transmission rate and oxygen absorbers can help extend the shelf life. Produce and other products with respiration often require packaging with controlled barrier properties. The use of a modified atmosphere in the package can further extend shelf life for some products. Some active packaging with antibacterial properties is also available. Packaging films are used for postharvest storage of crops such as mushrooms (Xing *et al.*, 2008) and atemoya (Yamashita *et al.*, 2002).

2.4.7 Heat treatment

Postharvest heat treatment of fruits and vegetables has been used for many years for disease control and insect disinfection. Heat treatment is a quarantine process that does not use any chemicals, but uses hot saturated water vapor to sterilize fruit like mangoes and papayas. During this process, the temperature and humidity are strictly controlled so the fruit remains naturally fresh and undamaged. Postharvest heat treatments have also been used alone or in combination with other physical treatments: hot water (Wszelaki and Mitcham, 2003; Kaewsuksaeng *et al.*, 2015), hot acetic acid (Farahnaky and Afshari Jouybari, 2011), hot acetic acid vapor (Radi *et al.*, 2010) and hot air (Yahia *et al.*, 2007; Shao *et al.*, 2014; Belović *et al.*, 2015).

2.4.8 Acetic Acid treatment

Chemically, acetic acid is also known as ethanolic acid, ethylic acid included vinegar acid, among others. It is widely that acetic acid possesses antibacterial and antifungal properties (Mari *et al.*, 2016). This compound has valid consumer friendly and can reduce toxic residues. The efficacy of this compound was investigated on a variety of fruits such as apples (Sholberg *et al.*, 2001), bananas (Perera Niranjala and Karunaratne, 2001), citrus and table grapes (Venditti *et al.*, 2009; Venditti *et al.*, 2017) and kiwifruit (Lagopodi *et al.*, 2009). In addition to acetic acid treatment, the use of fermented vinegar containing acetic acid was found to extend the shelf life of tomatoes (Tzortzakis *et al.*, 2011), fresh lettuce (Krusong *et al.*, 2012), strawberries (Krusong *et al.*, 2015a), coriander leaves (Krusong *et al.*, 2015b) and pepper fruit (Tzortzakis *et al.*, 2016)

2.4.9 Other methods

In addition to the above treatments, other methods of extending the shelf-life of agricultural produce have been used such as vapor treatment, hexanal and ethanol treatment. Many authors have reported on the effectiveness of these treatments in preserving postharvest quality, either alone or used in combination with other physical treatments. Aghdam *et al.* (2011) investigated the effects of methyl salicylate (MeSA) vapor exposure at 20°C for 16 h on the postharvest quality of kiwifruit (*Actinidia deliciosa* 'Hayward'). They found that ethylene production was decreased significantly by the use of MeSA. Jin *et al.* (2013) reported that ethanol vapor treatments markedly delayed the senescence of harvested oriental sweet melons. In addition, the use of water vapor (Feng *et al.*, 2002), ethanol vapor (Asoda *et al.*, 2009; Mori *et al.*, 2009; Fukasawa *et al.*, 2010; Krusong *et al.*, 2016), hexanal vapor (Thavong *et al.*, 2010).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Chemicals and Instruments

3.1.1 Chemicals and reagents

The chemicals and reagents used were as follows: Vitamin C (ascorbic acid) (Sigma-Aldrich, China), butylated hydroxytoluene (BHT) (Sigma-Aldrich, China), ferrous sulfate (FeSO_4) (Ajax Finechem, New Zealand), ferrozine, (Sigma-Aldrich, China), ethylene diamine tetraacetic acid (EDTA) (Merck KGaA, Germany), Folin-Ciocalteu reagent (Merck KGaA, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, China), gallic acid (Sigma-Aldrich, China), potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (Ajax Finechem, New Zealand), trichloroacetic acid (TCA) (Loba Chemie, India), thiobarbituric acid (TBA) (Sigma-Aldrich, China), sodium chloride (NaCl) (Ajax Finechem, New Zealand), potassium chloride (KCl) (Merck KGaA, Germany), disodium hydrogenphosphate (Na_2HPO_4) (Ajax Finechem, New Zealand), monosodium phosphate (NaH_2PO_4) (Ajax Finechem, New Zealand), potassium dihydrogen phosphate (KH_2PO_4) (Ajax Finechem, New Zealand), ferric chloride (FeCl_3) (Sigma-Aldrich, China), hydrogen peroxide (H_2O_2) (Merck KGaA, Germany), mannitol (Sigma-Aldrich, China), tris (Ajax Finechem, New Zealand), glacial acetic acid (Merck KGaA, Germany), sodium hydroxide (NaOH) (Merck KGaA, Germany), sodium carbonate (Na_2CO_3) (Ajax Finechem, New Zealand), 2-deoxyribose (Sigma-Aldrich, China), citrate buffer, bromophenol blue, agarose gel (Favorgen Biotech Corp, Tai- wan), ethidium bromide, glycerol, xylene, cyanol, 99% ethanol (Merck KGaA, Germany) and 80% acetone (Merck KGaA, Germany).

3.1.2 Scientific instruments

The scientific instruments used were as follows: UV-VIS Spectrophotometer (Spectronic™ GENESYS 20 spectrophotometer, ThermoFisher Scientific, USA), auto pipette (BioPette™, Labnet, Poland), autoclave (Hirayama HV-85, Artisan Technology Group, USA), water bath (WNB14, Memmert, Germany), centrifuge (Universal 320R, Hettich Lab Technology, Germany), gel documentation system (Syngene, USA), hotplate and magnetic stirrer (ST0707V2, Favorit, PLT Scientific, Malaysia), UV transilluminator and Consort manual power supply (C830, Consort, Belgium).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

3.1.3 Raw materials

Sweet basil leaves (*Ocimum basilicum* L.) and kaffir lime leaves (*Citrus hystrix* DC.) were purchased from local commercial vegetable producers in Pathumthanee province. Glutinous rice was obtained at supermarkets in Ladkrabang and RD6 glutinous rice wine and RD6 GRFV were obtained from the Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang.

3.1.4 Other materials

Egg, pUC19 vector in *E. coli*, distilled water, hot pot, camera, polyethylene bags of size 20 x 40 cm, gas stove, 0.6 and 0.7 cm diameter puncher, scissors, pruning shears and other miscellaneous materials were also used refrigerator.

3.2 Methods

3.2.1 Experiment 1. Study of phenolic and browning compounds in RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

3.2.1.1 Sample preparation

Test samples were RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV. Aqueous extract from RD6 grain and steamed rice was prepared by soaking 20 grams of sample with 80 mL of distilled water in a refrigerator for 3 days. Afterwards, the extract was filtered through Whatman No. 1 filter paper, diluted to a final concentration of 20 % and stored in a refrigerator at 4 °C until assays were performed. For assays, extracts were diluted with distilled water in the following ratios: 0.3125% (1:64), 0.625% (1:32), 1.25% (1:16), 2.5% (1:8), 5% (1:4), 10% (1:2) and 20% (1:0) (v/v). RD6 glutinous rice wine and RD6 GRFV were diluted with distilled water to the following concentrations: 3.125% (1:32), 6.25% (1:16), 12.5% (1:8), 25% (1:4), 50% (1:2), 75% (3:4) and 100% (1:0) (v/v).

3.2.1.2 Analysis methods

(1) Total phenolic content

The Folin-Ciocaltu method was used to determine the total phenolic content of samples (Ebrahimzadeh *et al.*, 2008; Nabavi *et al.*, 2008). One mL of sample was added to 4.5 mL distilled water and 0.5 mL of 2N Folin-Ciocaltu reagent. The reaction mixture was vortexed for 5 sec and added 4 mL of 1 M Na₂CO₃. Subsequently, the mixture was vortexed for 15 sec and incubated for 60 min at room temperature for color development. The mixture was centrifuged for

5 min at 6,000 rpm at 25 °C. The absorbance was read at 765 nm using a spectrophotometer. Total phenolic compounds were determined using a calibration curve based on gallic acid and expressed as milligrams of gallic acid equivalents per 100 gram of weight.

(2) Browning compounds

The sample was diluted with distilled water as described above and browning compounds were determined by measuring the absorbance at 420 nm using a spectrophotometer. The results were expressed as absorption units (AU) (Tagliazucchi *et al.*, 2008).

3.2.1.3 Statistical analysis

The results of all experiments performed were expressed as mean \pm SD of triplicate analysis.

3.2.2 Experiment 2. Comparison of antioxidant properties and DNA damage protection of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

3.2.2.1 Sample preparation

All samples were prepared as previously described in the experiment 1 (section 3.2.1.1) to study the properties of antioxidants (DPPH free radical scavenger, metal chelating activity, anti-lipid peroxidation, hydroxyl radical scavenging activities, reducing power and DNA damage protection induced by Fenton's reaction).

3.2.2.2 Analysis methods

(1) DPPH free radical scavenging activity

The DPPH radical is widely used to evaluate the free-radical scavenging capacity of antioxidants (Shimada *et al.*, 1992). DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either hydrogen or electron donation. Substances in samples that show the ability to perform this reaction can be considered radical scavengers and therefore antioxidants (Dehpour *et al.*, 2009). Aliquots (2 mL) of different concentrations of test samples were mixed to of a 100 μ M ethanol solution of DPPH radical (2, 2-diphenyl 2-picrylhydrazyl) (Ebrahimzadeh *et al.*, 2010). The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min. Absorbance of the solution was then measured at 517 nm using a spectrophotometer. Vitamin C and BHT were used as standards. The inhibition of DPPH radicals by samples was calculated according to the following equation:

$$\text{DPPH-scavenging activity (\%)} = [(A-B) - (C-D) / (A-B)] \times 100$$

Where

A = the absorbance of DPPH without test sample

B = the absorbance of ethanol

C = the absorbance of DPPH after reacting with the test sample

D = the absorbance of the test sample in ethanol

The sample concentration providing 50% inhibition (IC_{50}) was calculated by linear regression from a graph of inhibition (%) vs. test sample concentration. All tests were carried out in triplicate.

(2) Metal chelating activity

The ability of test samples to chelate ferrous ions was estimated by the method of Dinis *et al.* (1994) with slight modification and compared with EDTA as a standard. Briefly, 1 mL of different concentrations of test samples was added to 1.5 mL of distilled water and 50 μ L of 2 mM ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$). The reaction was initiated by the addition of 100 μ L of 5 mM Ferrozine, after which the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The ability of extracts to chelate ferrous ions was calculated using the formula:

$$\text{Ferrous ion chelating ability (\%)} = [(A-B) - (C-D) / (A-B)] \times 100$$

Where

A = Absorbance of ferrozine without test sample

B = Absorbance of distilled water

C = Absorbance of ferrozine after reacting with the test sample

D = Absorbance of the test sample in distilled water

The sample concentration providing 50% chelation ability (EC_{50}) was calculated from a graph plotting ferrous ion chelating ability against sample concentration.

(3) Reducing power

The reducing power of test samples was determined according to the method previously described by Oyaizu *et al.* (1986). One mL of various concentrations of test sample was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was then incubated at 50 °C for 20 min. Afterwards, 2.5 mL of 10% trichloroacetic acid was added and the mixture centrifuged at 3,000 rpm for 10 min at 25 °C. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

0.1% FeCl₃ then incubated at room temperature for 30 min. The absorbance was measured at 700 nm by using a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a standard. The EC₅₀ value is the effective concentration at which the absorbance was 0.5 for reducing power and obtained by interpolation from a linear regression analysis.

(4) Hydroxyl radical scavenging activity

Hydroxyl radicals were evaluated by the deoxyribose method as previously described (Halliwell *et al.*, 1987). The reaction mixtures contained 450 µL of sodium phosphate buffer (0.2 M, pH 7.6), 150 µL of 2-deoxy-D-ribose (100 µM), 150 µL of 200 µM FeCl₃ and 200 µM EDTA (1:1 v/v), 600 µL extract solution of varied concentration and 150 µL of H₂O₂ (10 mM). The mixture was incubated at 37 °C for 4 hr, after which 750 µL of 2.8% TCA (w/v in water) and 750 µL of 1% TBA (w/v) were added and the solution boiled in a water bath at 100 °C for 15 min. After cooling, absorbance was measured at 520 nm using a spectrophotometer. Mannitol was used as the standard. Percentage inhibition was calculated with the formula:

$$\text{Hydroxyl radical scavenging ability (\%)} = [(A-B) - (C-D) / (A-B)] \times 100$$

Where

A = Absorbance of H₂O₂ without test sample

B = Absorbance of distilled water

C = Absorbance of H₂O₂ after reaction with the test sample

D = Absorbance of the test sample in distilled water

The sample concentration providing 50% inhibition (IC₅₀) was calculated from the plot of hydroxyl radical scavenging ability against sample concentration.

(5) Inhibition of anti-lipid peroxidation

A modified TBARS assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid-rich media, as described by the method of Pandey *et al.* (2007). Briefly, 0.5 mL of egg yolk homogenate emulsified with phosphate buffered saline (10%, pH 7.45) was added to 1 mL of test sample of different concentrations. Thereafter, 0.5 mL of ferrous sulfate (FeSO₄, 24 mM) was added and the mixture was incubated at 37 °C for 15 min. After incubation, the mixture was treated with 0.5 mL of 20% TCA and 1.0 mL of 0.8% TBA. The reaction tubes were incubated in a boiling water bath at 100 °C for 15 min. After cooling, the tubes were centrifuged at 3,500 rpm for 20 min at 25 °C to remove precipitated protein. The

absorbance of the organic upper layer was measured at 532 nm using a spectrophotometer.

Vitamin C was used as the standard. Percentage inhibition was calculated with the formula:

$$\text{Inhibition of anti-lipid peroxidation (\%)} = [(A-B) - (C-D) / (A-B)] \times 100$$

Where

A = Absorbance of FeSO₄ without the test sample

B = Absorbance of distilled water

C = Absorbance of FeSO₄ after reaction with the test sample

D = Absorbance of the test sample in distilled water

The sample concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition of anti-lipid peroxidation percentage against sample concentration.

(6) DNA damage protection induced by the Fenton's reaction

(A) Preparation of buffer solution (Lee *et al.*, 2002)

Preparation of 50x TAE electrophoresis buffer (1 L)

2.0 M Tris base (MW = 121)	242 g
Glacial acetic acid	57.1 g
0.5 M EDTA stock solution (pH 8.0)	100 mL

**Adjust the final volume to 1000 L with deionized water.

Preparation of 0.5 M EDTA stock solution (pH 8.0)

EDTA	186.1 g
Deionized water	800 mL
NaOH	20 g

**Add 5N NaOH to adjust the solution pH to 8.0 and then adjust the final volume to 1000 L with deionized water.

** Sterilize the solution by autoclaving (15 min at 15 psi from 121-124 °C on liquid cycle).

Preparation of 6X DNA loading dye

Bromophenol blue	0.25% (w/v)
Xylene cyanol FF	0.25% (w/v)
Glycerol in water	30% (v/v)

** Aliquot and freeze at -20 °C for long-term storage.

(B) Preparation of agarose gel (1%)

The concentration of agarose needed in a gel will depend on the sizes of DNA fragments to be separated, with most gels ranging between 0.5-2%. The buffer volume should not be greater than 1/3 of the flask capacity.

Agarose (1 g) was suspended in 100 mL 0.5x TAE buffer and heated in a microwave oven until the solution boiled and the agarose was completely dissolved, leaving the solution clear and free of particles. After cooling the solution at room temperature to about 55 - 60 °C, the agarose was slowly poured into the gel mold with a slot-forming comb inserted. Small air bubbles were removed and the agarose allowed to solidify. After the agarose was solid (about 30 min), the comb was removed, using care not to rip the bottom of the wells. The gel mold was then inserted into an electrophoresis apparatus filled with TAE buffer.

(C) Preparation of DNA

The free radical scavenging activities of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV, fractions thereof were analyzed using a DNA nicking assay modified from Kitts *et al.* (2000). pUC19 is a plasmid cloning vector commonly used in *E. coli*. The molecule is a small double-stranded circle, 2,686 base pairs in length and has a high copy number. pUC19 carries a 54 base-pair multiple cloning site polylinker that contains unique sites for 13 different hexanucleotide-specific restriction endonucleases (Yanisch-Perron *et al.*, 1985). The molecular weight of pUC19 is 1.75×10^6 Daltons.

DNA nicking assay was performed using plasmid DNA as described by the method of Lee *et al.* (2002). A mixture of 8 μ L of test sample at different concentrations and 2 μ S L plasmid DNA (0.25 μ S) was incubated for 10 min at room temperature. This was followed by addition of 5 μ L of Fenton's reagent (30 mM H₂O₂, 50 μ M ascorbic acid and 80 μ M FeCl₃) and water to a final volume of 20 μ L, and incubation for 45 min at 37 °C. The reaction was terminated by the addition of 3 μ L of 6 \times DNA loading dye. The DNA samples were electrophoresed on a 1% agarose gel and stained with ethidium bromide (0.5 mg L⁻¹) for 10 min at room temperature. After washing, the gel was visualized on a UV-transilluminator and photographed with the Gel Documentation System (Syngene, USA). DNA quantities were determined using the Gene Tool Analysis Software (Syngene, USA).

3.2.2.3 Statistical analysis

The results of all experiments performed were expressed as mean \pm SD of triplicate assays. The test of significance was applied wherever necessary and any results with $p \leq 0.05$ were considered statistically significant.

3.2.3 Experiment 3. Analysis of RD6 GRFV vapor by GC-MS.

GC-MS analyses were performed using an Agilent Technologies 6890 GC-Agilent Technologies 5973 Inert MS (Agilent Technology, Palo Alto, USA) coupled to a Finnigan MAT quadruple ion trap detector, operating at 70 eV with an ion source temperature of 200 °C. Gas chromatography analysis of RD6 GRFV vapor was performed on a Dani Educational gas chromatograph fitted with a Carbowax column (30 m \times 0.25 mm i.d., film thickness 0.25 μ m). The oven temperature was held at 40 °C for 3 min following injection and then raised to 200 °C at 4 °C min⁻¹ before remaining isothermal at 200 °C for 10 min. The RD6 GRFV vapor in the headspace of the bottle was delivered to the box for 60 min and vapor samples were taken. Typically, a 10 mL sample was injected with a split ratio of 1:20. Injector and detector were maintained at 200 °C. The carrier gas was helium at a pressure of 4.7 psi. Peak area and retention time were calculated with a Shimadzu CR6A data processor, while the Kovlats retention indices were determined on a series of n-alkanes. For MS determination, an electron ionization mode was employed with ion source temperature of 200 °C, scan mass range of 30-400 amu, and MS transfer line at 200 °C with 0 min solvent delay time. Identification of RD6 GRFV vapor components was based on retention times and mass spectra fragmentation patterns and compared with the Wiley, 275.L data library for the GC-MS system.

3.2.4 Experiment 4. Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime and sweet basil

3.2.4.1 Sample preparation

(1) Plant samples

Leaves of kaffir lime and bunches of sweet basil shoots were purchased from local commercial vegetable producers in Pathumthanee province, Thailand and taken within about 2 h of harvest to the laboratory at the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand where the treatments were carried out. Only top-quality leafy sweet basil shoots with no signs of visible damage or of yellowing (length of about 15-20 cm) were used. Kaffir lime leaves

were separated from stalks and stem, washed thoroughly, and weighed. Only kaffir lime leaves with no signs of visible damage or of darkened areas were used.

(2) RD6 GRFV vapor treatment

A plastic box (25x30x25 cm) with a vent valve to prevent pressure buildup during vapor treatment (Krusong *et al.*, 2015a) was designed especially for this study. We produced a relative humidity (RH) of $80 \pm 2\%$, measured by a thermo-hygrometer (Model TH-302, Diichi, Japan), by pump bubbling external air through distilled water and then directing the water vapor into the plastic box. The RD6 GRFV vapor was prepared by bubbling external air through RD6 GRFV (acetic acid content 8% or 4%) contained in a 1000 mL bottle at room temperature (25 °C). RD6 GRFV vapor in the headspace of the bottle was delivered to the box, as described in Krusong *et al.* (2015a).

3.2.4.2 Analysis methods

(1) Treatment with ambient RD6 GRFV vapor

The rate of vapor production was calculated from the rate of weight loss of the diluted RD6 GRFV, as 0.032 ± 0.002 g RD6 GRFV min^{-1} (Figure 3.1). After that, sweet basil shoots (about 25 g) and kaffir lime leaves (5 leaves) each were washed with distilled water and placed in a plastic box. Fresh leaves of kaffir lime were fumigated with RD6 GRFV vapor containing 8% acetic acid for 0 (control), 5, 10, 15 and 20 min. For sweet basil, leaves were fumigated with RD6 GRFV vapor containing 4% of acetic acid for 0 (control), 2, 4, 6, 8 and 10 min. Samples were packed in polyethylene bags (size 10x15 in inch), each with eight perforated holes (diameter 0.7 cm). All experiments consisted of four replicate plastic bags for each treatment and each sampling time. All packed samples were stored in darkness at 12 °C and RH $80 \pm 2\%$ until shelf-life assessments were made.

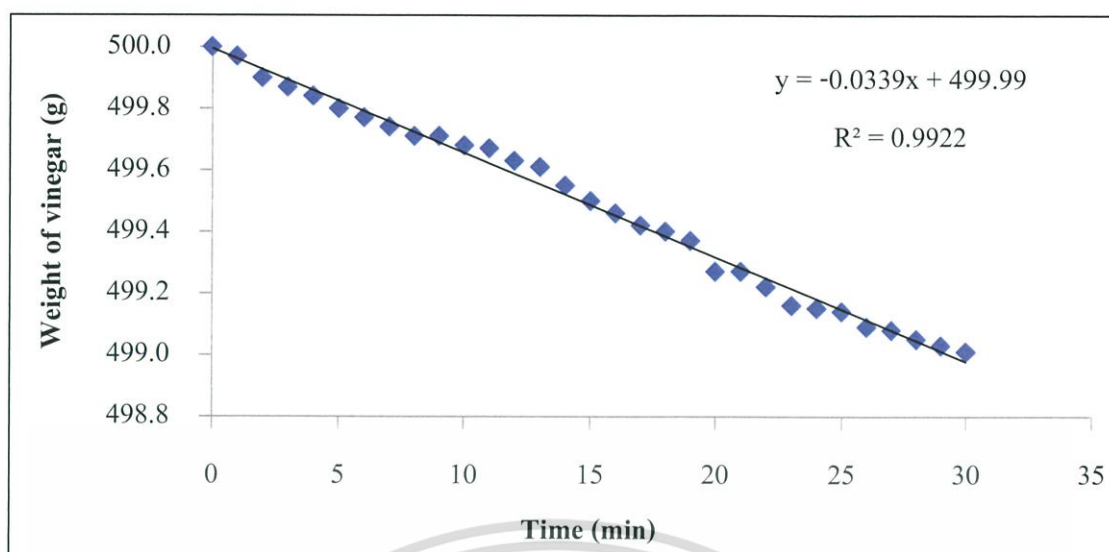


Figure 3.1 Weight loss over time during vapor production from RD6 GRFV.

(2) Physical analysis of kaffir lime and sweet basil postharvest quality

(A) Change in fresh weight

Leaves were initially weighed before packing and sealing in the polyethylene bags. These bags were subsequently analyzed for fresh weight changes during storage. The fresh weight was recorded in 2 days intervals till the end of the experiment. Leaf weight relative to fresh weight was calculated by the formula (Gharezi *et al.*, 2012):

$$\text{Relative leaf weight (\%)} = \frac{(\text{Initial weight} - \text{Weight on the day of observation}) \times 100}{\text{Initial weight}}$$

(B) Change in leaf color

Colors of kaffir lime and sweet basil leaves were determined by measuring L^* , a^* and b^* values and hue angle using the Royal Horticultural Society color chart, which covered an area of 8 mm, in intervals till the end of the experiment. The L^* (ranging from 0=black to 100=white), a^* (+red, -green) and b^* (+yellow, -blue) color coordinates were determined according to the CIELAB coordinate color space system. Color values were calculated from L^* , a^* , b^* values by the formula (Kim *et al.*, 2002):

$$L^* = 10\sqrt{Y}$$

$$a^* = 17.5\{1.02(x-y)\} / \sqrt{y}$$

$$b^* = 7.0 (Y-0.847z) / \sqrt{y}$$

$$z = 1-x-y$$

(C) Evaluation of visual quality and shelf-life

The shelf-life quality of leaves was evaluated based on visible changes in freshness and the gradual appearance of darkened, pitted lesions. Overall appearance – wilting, leaf color (changes from dark green to brown), necrosis (darkened areas) – was assessed daily. For each assessment, subjective ratings were assigned on the following scale: 5 = no damage; 4 = several dark spots; 3 = black stains on 30% of the leaf area; 2 = black stains on 30-50% of the leaf area; and 1 = black stains on more than 50% of the leaf area (Meir *et al.*, 1997). Leaf samples were considered to have reached the limit of marketability when they showed black stains on 30% of the leaf area (score 3) and the limit of edibility when they showed black stains on 30-50% of the leaf area (score 2).

(3) Biochemical analysis of kaffir lime and sweet basil postharvest quality

(A) Photosynthetic pigment content

Samples of leaves were taken for chlorophyll determination at days 0, 5, 10, 15, 20 and 25. A total of 13 leaf discs (0.6 diameter) for each treatment were frozen in liquid nitrogen, ground using a mortar and pestle, and extract produced by incubation for three hours in 5 mL of 80% acetone. Afterwards, the suspension was filtered through a Whatman No. 1 paper filter. Absorbances of filtered extracts were determined spectrophotometrically at three wavelengths: 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for carotenoids. Calculations were completed using Lichtenthaler's equation (Lichtenthaler, 1987) and expressed as mg cm^{-2} .

$$\text{Chl a (mg L}^{-1}\text{)} = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$\text{Chl b (mg L}^{-1}\text{)} = 21.50 \times A_{647} - 5.10 \times A_{663}$$

$$\text{Carotenoids (mg L}^{-1}\text{)} = (1000 \times A_{470} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b})/198$$

(B) Electrolyte leakage

Electrolyte leakage was measured in discs cut out of leaves fresh at days 0, 5, 10, 15, 20 and 25. For each treatment, ten replicate leaf discs (diameter 0.6 cm, fresh weight (FW) 70-80 mg) were cut with a cork borer, carefully avoiding the main vein. Discs were floated on 10 mL of deionized water at room temperature (25 °C). Electrolyte leakage into the solution was measured after 1, 2 and 3 h using a conductivity meter (Consort, C830, Belgium). Total conductivity was expressed as $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW).

(C) Lipid peroxidation (determination of MDA content)

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content as per Heath and Packer (1968). Fresh leaves (nearly 0.5 g) were homogenized in 5 mL of 0.1% (w/v) TCA and centrifuged at 10,000 g for 20 min at 25 °C. A subsample of 1 mL of the supernatant was added to 4 mL of TBA (0.5% w/v in 20% w/v TCA) and 0.4 mL of 4% (w/v) BHT. The mixture was heated at 95 °C for 30 min, cooled in an ice bath and then centrifuged at 6,000g at 25 °C for 15 min. Absorbance was measured at 532 nm and corrected for nonspecific absorbance at 600 nm using a spectrophotometer. MDA content was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nmol g⁻¹ (FW) by the following formula:

$$\text{MDA contents (nmol g}^{-1}\text{ FW)} = [(A_{532} - A_{600})/155,000] \times 10^6$$

(D) DPPH radical scavenging activity

The antioxidant activity of leaf samples was determined by evaluating DPPH radical scavenging activity (Ebrahimzadeh *et al.*, 2010). A sample of 1 g of fresh leaves was extracted with 95% ethanol 99 mL at room temperature for 72 h. The resulting solution was filtered through four layers of cheesecloth to remove any fiber debris, followed by a second filtration through Whatman No. 1 filter paper, and was set as the original concentration (1%). Aliquots (2 mL) of a range of dilutions (0.1-1%) of the original extract were added to 2 mL of DPPH solution (100 μM in ethanol) and incubated at room temperature for 30 min. Absorbance was measured at 517 nm using a spectrophotometer. Vitamin C and BHT were used as standards. Inhibition of DPPH radicals by the samples was calculated according to the following equation:

$$\text{DPPH-scavenging activity (\%)} = [(A-B) - (C-D) / (A-B)] \times 100$$

Where

A = the absorbance of DPPH without test sample

B = the absorbance of ethanol

C = the absorbance of DPPH after reacting with the test sample

D = the absorbance of the test sample in ethanol

The leaf extract concentration giving 50% inhibition (IC₅₀) was calculated by linear regression from a graph of inhibition (%) vs. extract concentration. All tests were carried out in triplicate.

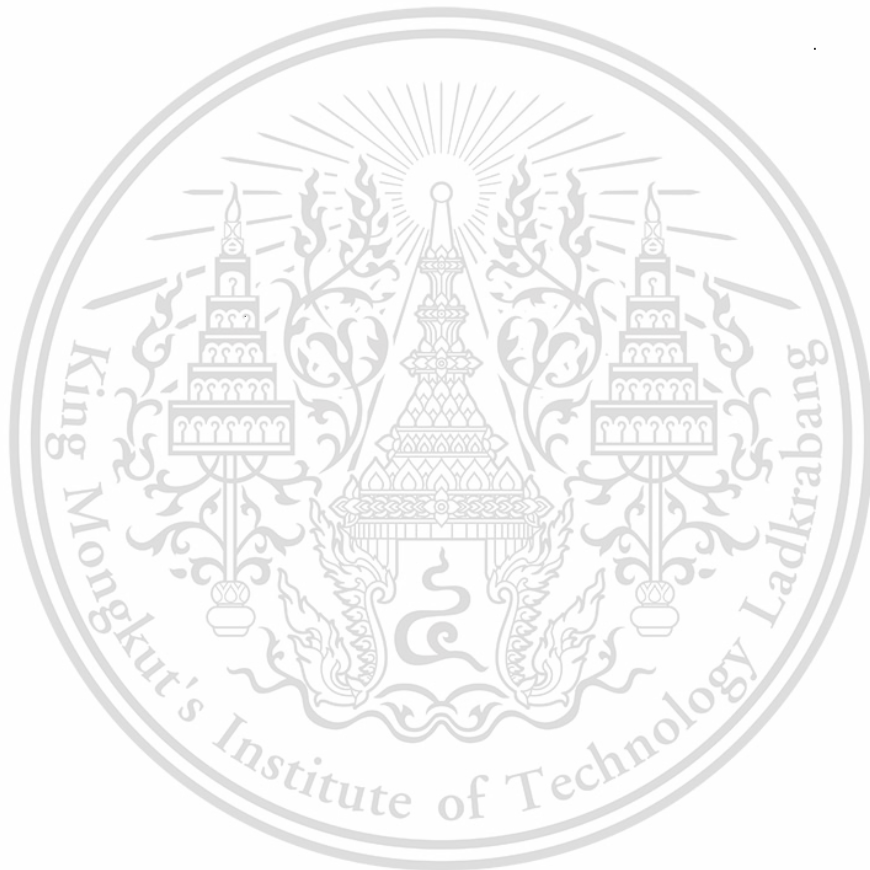
3.2.4.3 Statistical analysis

For weight loss, color measurement, chilling injury assessment, and shelf life evaluation, leaf samples were taken every 2 days till the end of the experiment. For biochemical

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

analyses including electrolyte leakage, chlorophyll content, lipid peroxidation (determination of malondialdehyde content), and antioxidant activity by DPPH radical scavenging assay, leaf samples were taken every 5 days (kaffir lime) and 7 days (sweet basil) till the end of the experiment. All treatment of four replicates was imposed in a completely randomized design (CRD). Analysis of variance was calculated for all data and comparisons between treatments were made at probability $p \leq 0.05$ using Tukey's Studentized Range Test. Meanwhile, the means of four replications were reported along with appropriate standard deviations (mean \pm SD).



CHAPTER 4

RESULTS

4.1 Experiment 1. Study of phenolic and browning compounds in RD6 glutinous rice grain and steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

4.1.1 Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu reagent and expressed as milligrams of gallic acid equivalents (GAE). The results showed that RD6 GRFV contained the high amount of phenolic acids. The total phenolic contents of RD6 glutinous rice grain and steamed rice were 0.055 and 0.012 mg GAE g⁻¹ weight, while the total phenolic contents of RD6 glutinous rice wine and RD6 GRFV were 0.415 and 0.612 mg GAE mL⁻¹, respectively (Table 4.1). The results indicated that high phenolic compounds linked to antioxidant activity, which shown to possess high antioxidant activities.

4.1.2 Browning compounds

Browning compounds were measured as a convenient index of the development of Maillard reaction products (MRPs), offering a visual estimate (absorbance at A₄₂₀ nm). The results showed that RD6 GRFV had a greater amount of browning compounds (1.647 a.u.) than RD6 glutinous rice wine (0.389 a.u.), steamed rice (0.065 a.u.) and RD6 glutinous rice grain (0.049 a.u.); this is the same trend was observed for total phenolic content (Table 4.1). In this study, the amount of browning compounds increased during fermentation. Thus, vinegar fermentation of RD6 glutinous rice might have effects on the rates of Maillard reactions that produce MRPs. The final products of nonenzymic browning are known as melanoidins, which exhibit antioxidant properties.

Table 4.1 The amount of total phenolic and browning compound of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

Sample test	Total phenolic contents	Browning compounds ^b
	(mg GAE ^a)	(a.u.)
RD6 glutinous rice grain	0.055 ± 0.000	0.065 ± 0.002
Steamed RD6 glutinous rice	0.012 ± 0.001	0.049 ± 0.001
RD6 glutinous rice wine	0.415 ± 0.007	0.389 ± 0.004
RD6 GRFV	0.612 ± 0.014	1.647 ± 0.006

Each experiment was performed in triplicate and the results are mean ± SD

^aGAE = Gallic acid equivalents in mg g⁻¹ weight of rice extract and mg mL⁻¹ of wine and RD6 GRFV

^bBrowning compounds were measured as absorbance at 420 nm.

4.2 Experimental 2. Comparison of the antioxidant properties and DNA damage protective activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

4.2.1 DPPH free radical scavenging activity

The DPPH radical scavenging assay was employed to evaluate the antioxidant properties of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV. The free radical scavenging activity of samples increased with increasing concentration. The IC₅₀ values of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV were 6.58, 14.09, 16.10 and 4.33%, respectively (Table 4.2). The IC₅₀ of standard compounds, vitamin C and BHT were 0.0003 and 0.0022%, respectively. Lower IC₅₀ indicated higher antioxidant capacity of the sample.

4.2.2 Metal chelating activity

The ability of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV to chelate Fe²⁺ was evaluated and compared with EDTA. For all samples, chelating activity was not detectable, while the EC₅₀ (The effective concentration of sample required to ability to metal chelate by 50%) of EDTA was 0.0018% (Table 4.2).

4.2.3 Reducing power

In the reducing power assay, increasing absorbance at 700 nm indicates an increase in reductive ability, specifically converting the oxidized form of iron (Fe^{+3}) in ferric chloride to ferrous iron (Fe^{+2}). Increasing absorbance also indicates greater antioxidant content. Table 4.2 shows reducing power values for RD6 glutinous rice wine and RD6 GRFV. The abilities of RD6 glutinous rice grain and steamed RD6 glutinous rice extract to reduce ferrous ions were not detectable (Table 4.2). The EC_{50} of standard compound, vitamin C was 0.0046 %. It was found that the reducing power of test samples increased with their concentrations.

4.2.4 Hydroxyl radical scavenging activity

The IC_{50} values for hydroxyl radical scavenging activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV were found to be 6.76, 18.32, 4.30 and 4.11%, respectively (Table 4.2). Scavenging activities were concentration dependent. The standard mannitol showed an IC_{50} value of 0.4656%.

4.2.5 Inhibition of anti-lipid peroxidation

The abilities of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV to inhibit lipid peroxidation induced by ferrous sulphate in egg-yolk homogenates are shown in Table 4.2. The IC_{50} values for inhibition of lipid peroxidation by RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV were 6.28, 3.20, 8.76 and 8.37%, respectively while that of the standard vitamin C was 0.0624%.

Table 4.2 The antioxidant properties of RD6 glutinous rice grain, steamed rice, RD6 glutinous rice wine and RD6 GRFV

Sample test / Positive control	DPPH scavenger (IC ₅₀) ^a	Metal chelator (EC ₅₀) ^b	Reducing power (EC ₅₀) ^c	Hydroxyl radical (IC ₅₀) ^a	Anti-lipid peroxidation (IC ₅₀) ^a
RD6 glutinous rice grain (%)	6.58 ± 0.78 c*	ND	ND	6.76 ± 0.08 b*	6.28 ± 0.34 b*
Steamed RD6 glutinous rice (%)	14.09 ± 0.21 b	ND	ND	18.32 ± 0.04 a	3.20 ± 0.33 c
RD6 glutinous rice wine (%)	16.10 ± 0.61 a	ND	74.60 ± 0.34 b*	4.30 ± 0.12 c	8.76 ± 0.19 a
RD6 GRFV (%)	4.33 ± 1.15 d	ND	95.37 ± 4.69 a	4.11 ± 0.10 c	8.37 ± 0.85 ab
Positive controls					
Vitamin C (%)	0.0003 ± 0.00 e	-	0.0046 ± 0.00 c	-	0.0624 ± 0.00 d
BHT (%)	0.0022 ± 0.00 c	-	-	-	-
EDTA (%)	-	0.0018 ± 0.00	-	-	-
Mannitol (%)	-	-	-	0.4656 ± 0.003 d	-

*Values (mean ± SD) followed with the same letter are not significantly different within each column according to Tukey's test ($p \geq 0.05$).

^aIC₅₀: The concentration of sample giving 50% inhibition of free radicals.

^bEC₅₀: The effective concentration of sample required to chelate by 50%.

^cEC₅₀: The effective concentration at which the absorbance is 0.5.

ND : : None detected.

4.2.6 DNA damage protection induced by the Fenton's reaction

DNA damage protective activity was measured by conversion of plasmid DNA between forms. When electrophoresed on an agarose gel, the faster-moving band represents the native form of supercoiled circular DNA and the slower-moving band corresponds to the open circular form (Russo *et al.*, 2001). Normal pUC19 (lane 1) showed one band, the supercoiled form, while DNA exposed to Fenton's reagent (lane 2) showed two bands, the supercoiled form and open circular form (nicked DNA) (Figures 4.1-4.4). This indicates that the supercoiled form was decreased by conversion into the open circular form. When pUC19 DNA was incubated with Fenton's reagent and various concentrations of samples (lanes 3-8), the amount of open circular form was slightly decreased by steamed RD6 glutinous rice extract and RD6 glutinous rice wine (Figures 4.2-4.3). However, RD6 GRFV showed an amount of supercoiled form almost equal to the untreated control (Figure 4.4).

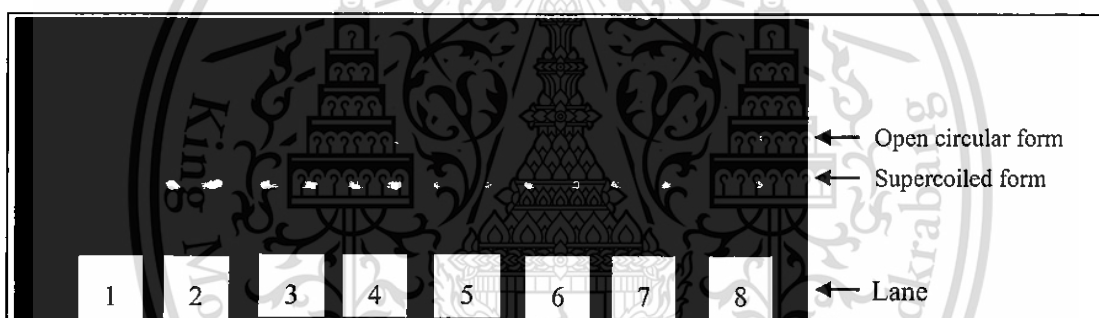


Figure 4.1 Inhibitory effects of aqueous extract from RD6 glutinous rice grain on DNA nicking caused by hydroxyl radicals. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + RD6 glutinous rice grain extract of different concentrations (0.625, 1.25, 2.5, 5, 10 and 20%, respectively). All lanes contain 0.25 μ g plasmid DNA.

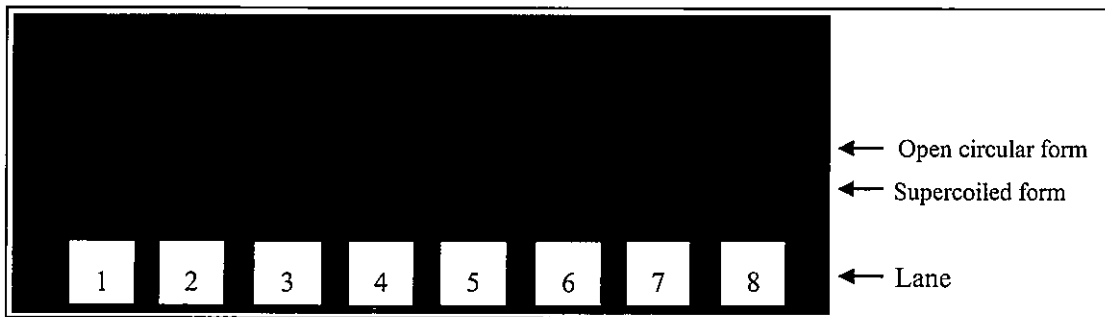


Figure 4.2 Inhibitory effects of aqueous extract from steamed RD6 glutinous rice on DNA nicking caused by hydroxyl radicals. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + steamed RD6 glutinous rice extract of different concentrations (0.625, 1.25, 2.5, 5, 10 and 20%, respectively). All lanes contain 0.25 μ g plasmid DNA.



Figure 4.3 Inhibitory effects of RD6 glutinous rice wine on DNA nicking caused by hydroxyl radicals. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + RD6 glutinous rice wine of different concentrations (0.361, 0.781, 1.563, 3.125, 6.25 and 12.5%, respectively). All lanes contain 0.25 μ g plasmid DNA.

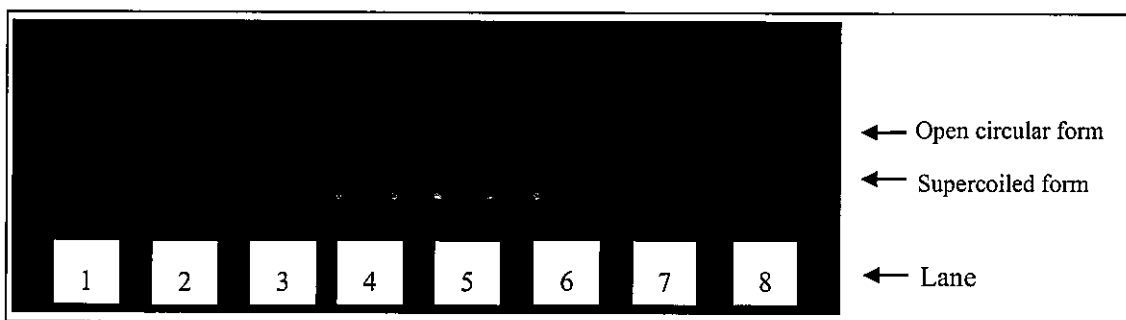


Figure 4.4 Inhibitory effects of RD6 GRFV on DNA nicking caused by hydroxyl radicals. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + RD6 GRFV of different concentrations (0.361, 0.781, 1.563, 3.125, 6.25 and 12.5%, respectively). All lanes contain 0.25 μ g plasmid DNA.

Quantitation of DNA in bands was determined using Gene Tool Analysis Software (Syngene, USA). Figure 4.5-4.6 shows the protective effects of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV against DNA nicking. The quantity of supercoiled DNA in untreated plasmid is equal to 100%. The intensity of the supercoiled DNA band increased with increasing concentrations of sample, as compared with Fenton's reagent treatment alone. Results showed that the intensity of supercoiled DNA from RD6 glutinous rice grain (0.625, 1.25, 2.5, 5, 10 and 20%) was 51.09, 50.93, 58.58, 69.65, 72.92 and 74.70%, respectively; in contrast, the supercoiled band treated with only Fenton's reagent had an intensity of 44.63% (Figure 4.5A). The steamed RD6 glutinous rice extract showed slightly increased presence of supercoiled DNA; the intensities of supercoiled bands from steamed RD6 glutinous rice (0.625, 1.25, 2.5, 5, 10 and 20%) were 56.20, 57.61, 59.40, 64.36, 66.19 and 71.42%, respectively, while plasmid DNA with only Fenton's reagent had an intensity of 57.44% (Figure 4.5B). For RD6 glutinous rice wine at concentrations of 0.361, 0.781, 1.563, 3.125, 6.25 and 12.5%, supercoiled bands had intensities of 80.50, 83.56, 84.27, 87.86, 88.28 and 91.70%, respectively, while plasmid DNA treated with only Fenton's reagent was alone had an intensity of 62.26% (Figure 4.6A). However, RD6 GRFV showed the highest ability to prevent DNA damage protection from Fenton's reagent. At the highest concentration (12.5%), the intensity of the supercoiled DNA band was 96.18%. At the other concentrations tested (0.361, 0.781, 1.563, 3.125 and 6.25%) the intensities of supercoiled bands were 62.42, 75.94, 79.55, 80.02 and 91.19%, respectively. In contrast, with Fenton's reagent treatment alone the intensity of supercoiled DNA was 56.56% (Figure 4.6B). These results indicated that RD6 glutinous rice

This material is reserved for educational use only, not allowed for commercial use.

grain, steamed rice, rice wine and RD6 GRFV all show effectiveness in preventing DNA damage protection from hydroxyl radicals.

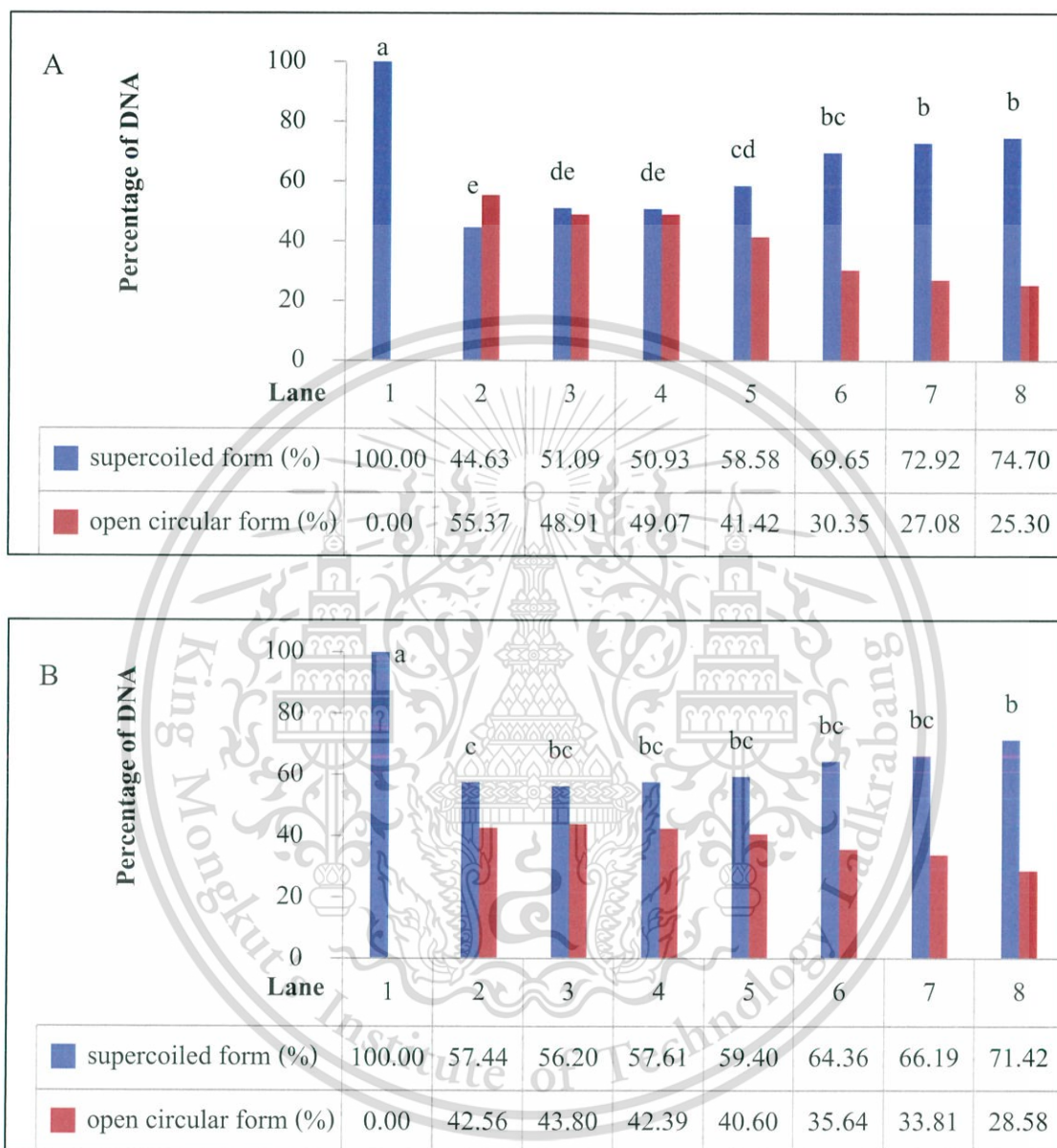


Figure 4.5 Densitometric analysis of open circular and supercoiled plasmid DNA upon treatment by Fenton's reaction in the presence of RD6 glutinous rice grain or steamed RD6 glutinous rice extracts. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + RD6 glutinous rice grain (A) or steamed RD6 glutinous rice (B) extract of different concentrations (0.625, 1.25, 2.5, 5, 10 and 20%, respectively). All lanes contained 0.25 μ g plasmid DNA. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

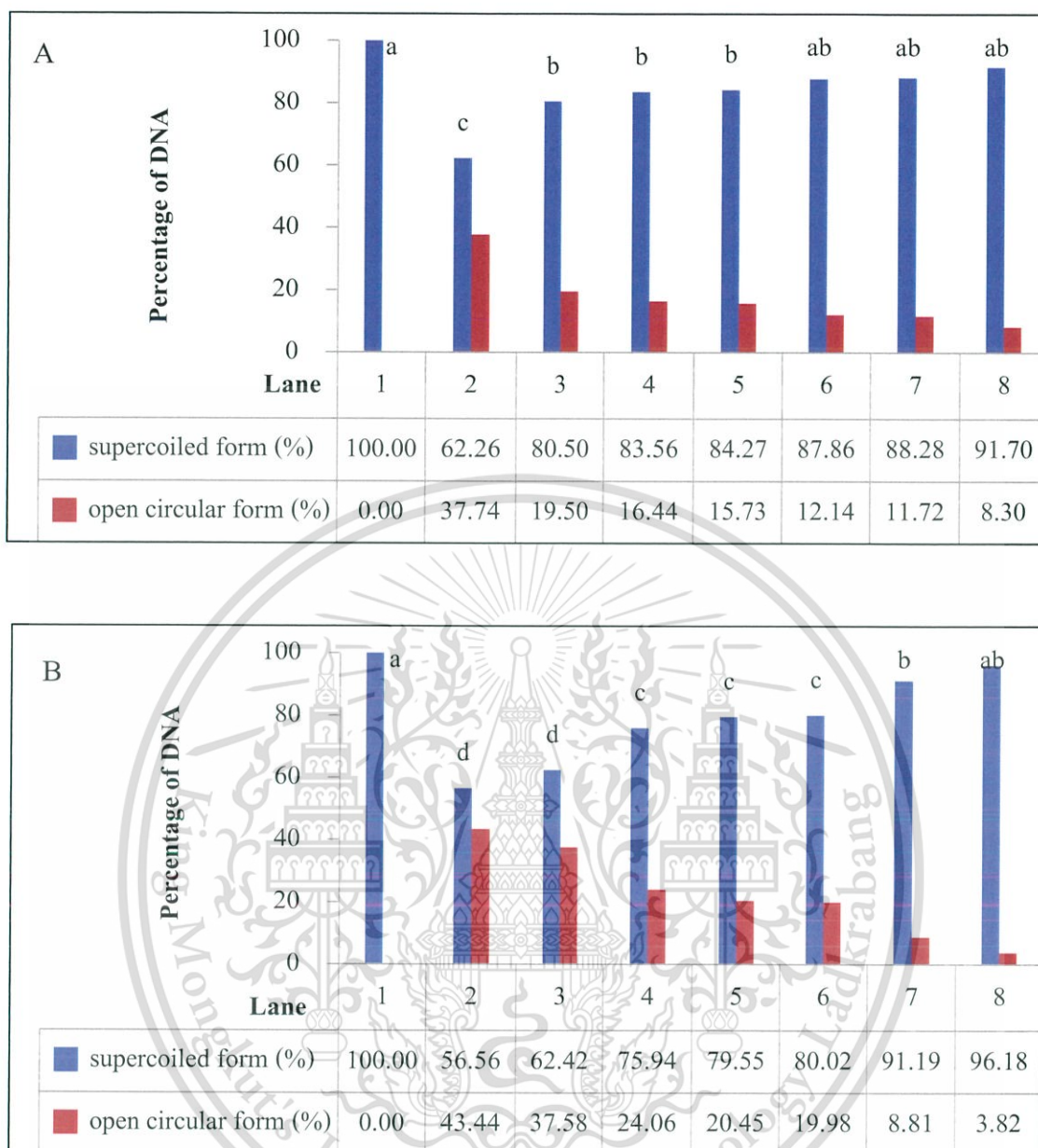


Figure 4.6 Densitometric analysis of open circular and supercoiled plasmid DNA upon treatment by Fenton's reaction in the presence of RD6 glutinous rice wine or RD6 GRFV. Lane 1, native pUC19 DNA; lane 2, pUC19 DNA + Fenton's reagent; lanes 3 to 8, pUC19 DNA + Fenton's reagent + RD6 glutinous rice wine (A) or RD6 GRFV (B) of different concentrations (0.361, 0.781, 1.563, 3.125, 6.25 and 12.5%, respectively). All lanes contained 0.25 μ g plasmid DNA. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

4.3 Experiment 3. Analysis of RD6 GRFV vapor by GC-MS.

The volatile compounds in RD6 GRFV vapor were analyzed by GC-MS. Table 4.3 lists the observed compounds, including their linear retention indices. The major volatile compounds (>3%) in RD6 GRFV vapor were found to be ethyl acetate (77.78%), propane (12.70%), pentanal (5.49%) and acetic acid (3.82%).

Table 4.3 Volatile compounds identified in RD6 GRFV by GC-MS.

R.T. (min)	Area (%)	IUPAC name	Formula	CAS number	MW	Chemical group
1.35	12.70	Propane	C ₃ H ₈	74-98-6	44.096	Organic compound
2.34	77.88	Ethyl Acetate	C ₄ H ₈ O ₂	141-78-6	88.11	Ester
2.44	5.49	Pentanal	C ₅ H ₁₀ O	110-62-3	86.13	Aldehyde
3.26	3.82	Acetic acid	C ₂ H ₄ O ₂	64-19-7	60.05	Organic compound
3.42	-	Butan-1-ol	C ₄ H ₁₀ O	71-36-3	74.12	Primary alcohol
3.58	0.11	Hexanal	C ₆ H ₁₂ O	66-25-1	100.159	Aldehyde

4.4 Experiment 4. Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime and sweet basil

4.4.1 Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime

4.4.1.1 Physical changes in treated kaffir lime

(1) Changes in fresh weight

Fresh weight of kaffir lime leaves was expressed as a percentage of that at the beginning of the experiment. All groups exhibited a pattern of decrease during the whole storage period (Figure 4.7). Leaves treated with RD6 GRFV vapor for 15 min had the greatest delay in weight loss compared to other treatments. At the end of the storage period, no other treatment was significantly different in weight from the control. At 35 days of storage, control leaf weight reached a minimum of 69.29% fresh weight, while the weights of leaves treated with RD6 GRFV vapor for 5, 10, 15 and 20 min (containing 0.0113-0.0455 mmol L⁻¹ Acetic Acid (AA)) reached values of 71.23, 74.63, 78.13 and 74.17%, respectively.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

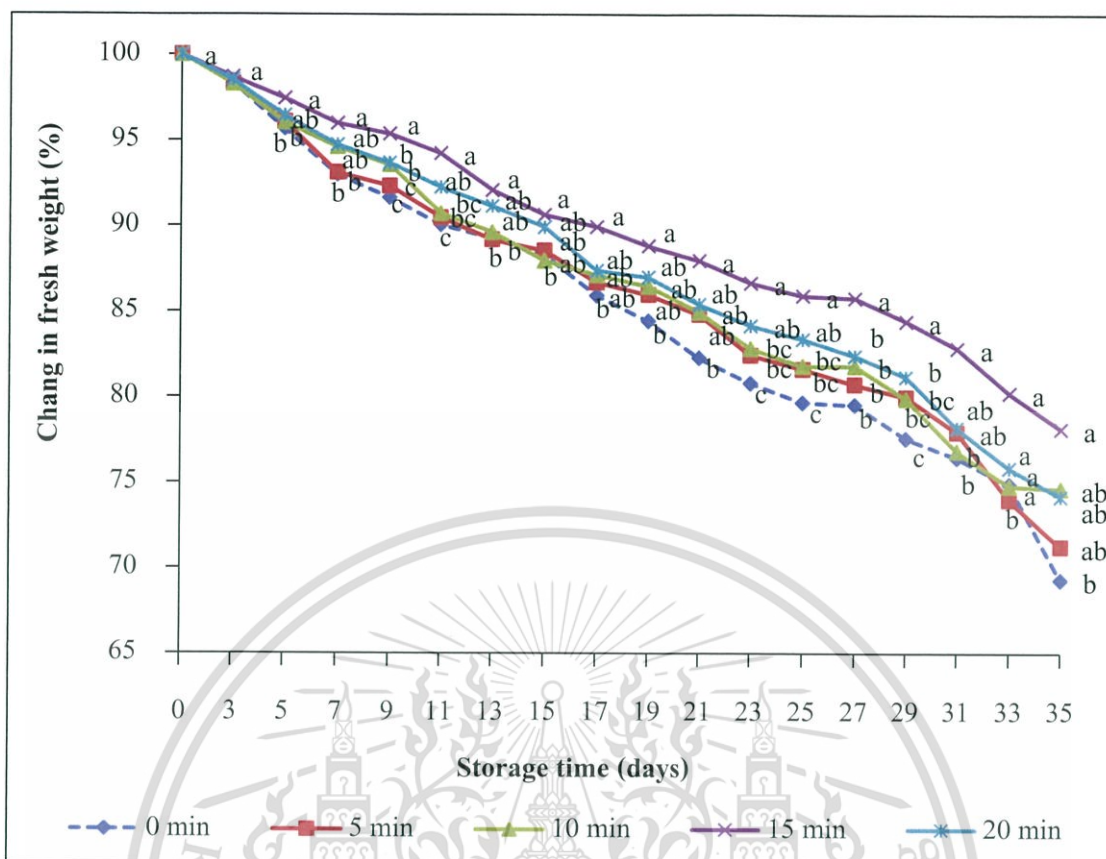


Figure 4.7 Change in fresh weight (%) of kaffir lime leaves during storage at 12 °C after treatment with ambient vapor from RD6 GRFV for periods ranging from 0 to 20 min. Fresh weight values are the means of four replicates. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

(2) Changes in leaf color during storage

Color values of kaffir lime were expressed as CIELAB color space units and the mean values for lightness (L^*), red-greenness (a^*) and blue-yellowness (b^*) parameters were calculated for each treatment.

L^* values of kaffir lime treated with RD6 GRFV vapor for 5, 10, 15 and 20 min (containing 0.0113-0.0455 mmol L⁻¹ AA) all decreased (increased darkness) during storage (Figure 4.8A). At 35 days of storage, the L^* values were 22.26, 23.78, 24.54 and 23.78 for 5, 10, 15 and 20 min of RD6 GRFV vapor treatment, respectively. No treatment was significantly different from the control (22.26).

a^* values of kaffir lime treated with RD6 GRFV vapor in all treatment groups increased (decreased greenness) during storage (Figure 4.8B). At 35 days of storage, the a^* values

were -0.33, -1.13, -1.21 and -1.13 for 5, 10, 15 and 20 min of RD6 GRFV vapor treatment, respectively. No treatment was significantly different from the control (-0.33).

b^* values of kaffir lime treated with RD6 GRFV vapor in all treatment groups increased (yellowish color) during the first 7 days of storage, then started to decrease (Figure 4.8C) at 28 days of storage, continuing until the end of storage. At 35 days of storage, the obtained b^* were 0.99, 1.13, 1.21 and 1.13 for 5, 10, 15 and 20 min of RD6 GRFV vapor treatment, respectively. No treatment was significantly different from the control (0.99).

Overall, the treated kaffir lime samples presented lower L^* and b^* values (increased darkness and yellowish color), while a^* values were generally higher (decreased greenness), though no differences was significant.

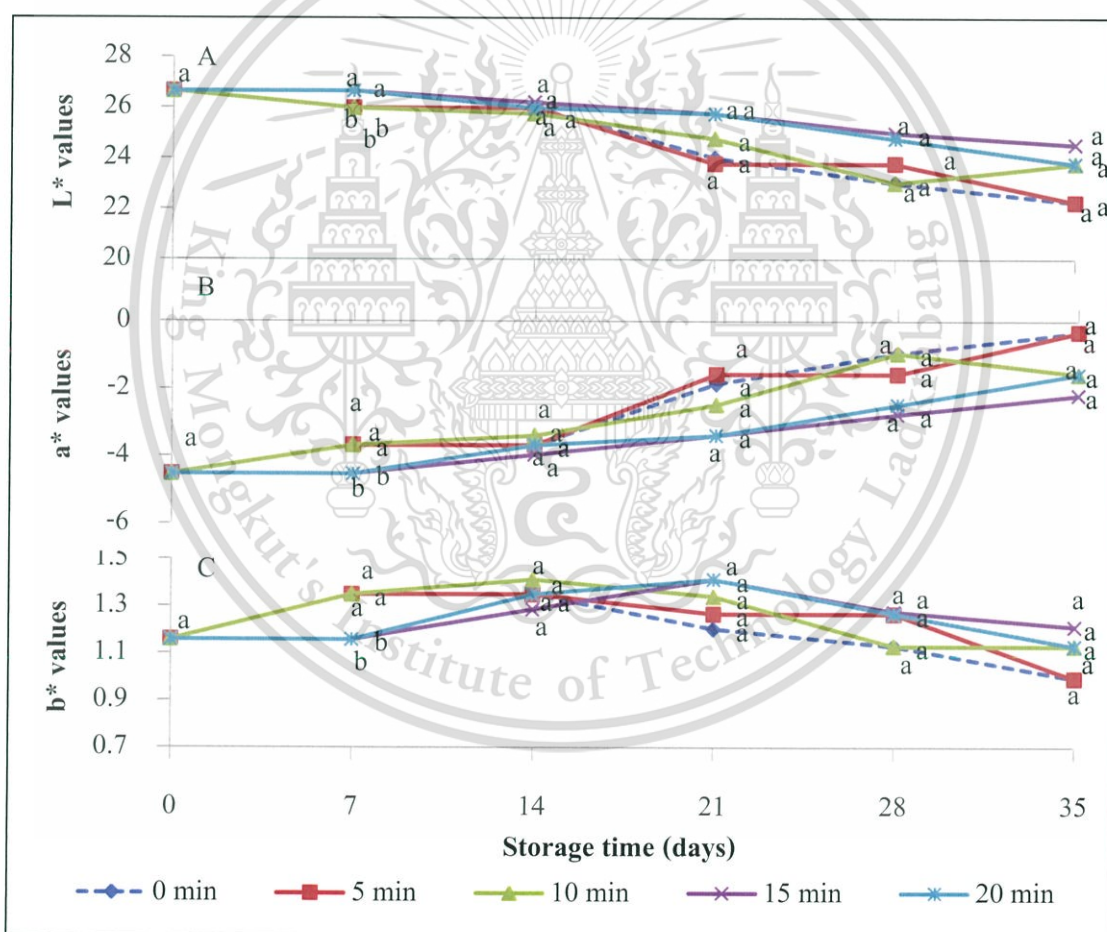


Figure 4.8 Changes in color of kaffir lime leaves during storage at 12 °C after treatment with ambient vapor from RD6 GRFV for periods ranging from 0 to 20 min, measured as (A) L^* value, (B) a^* value and (C) b^* value. Values are the means of four replicates. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

(3) Evaluation of visual quality and shelf-life

Visual appearance scores of kaffir lime decreased steadily with increasing storage time (Figure 4.9). For all treatments, scores did not begin to decrease until the 13th day of storage; after that point, untreated controls and leaves treated with RD6 GRFV vapor for 5-10 min decreased in score more rapidly than leaves treated with RD6 GRFV vapor for 15-20 min. By day 29, quality scores of controls and leaves treated for 5-10 min had decreased to below the limit of marketability (score 3). However, at the end of the storage period (day 35), leaves treated with RD6 GRFV vapor for 15 min (containing $0.0341 \pm 0.0001 \text{ mmol L}^{-1}$ AA) had visual quality scores higher (3.20 points) and untreated leaves had visual quality scores lower (1 points). RD6 GRFV vapor treated kaffir lime leaves for 15 min showed a slight black stain on leaf (Figure 4.10).

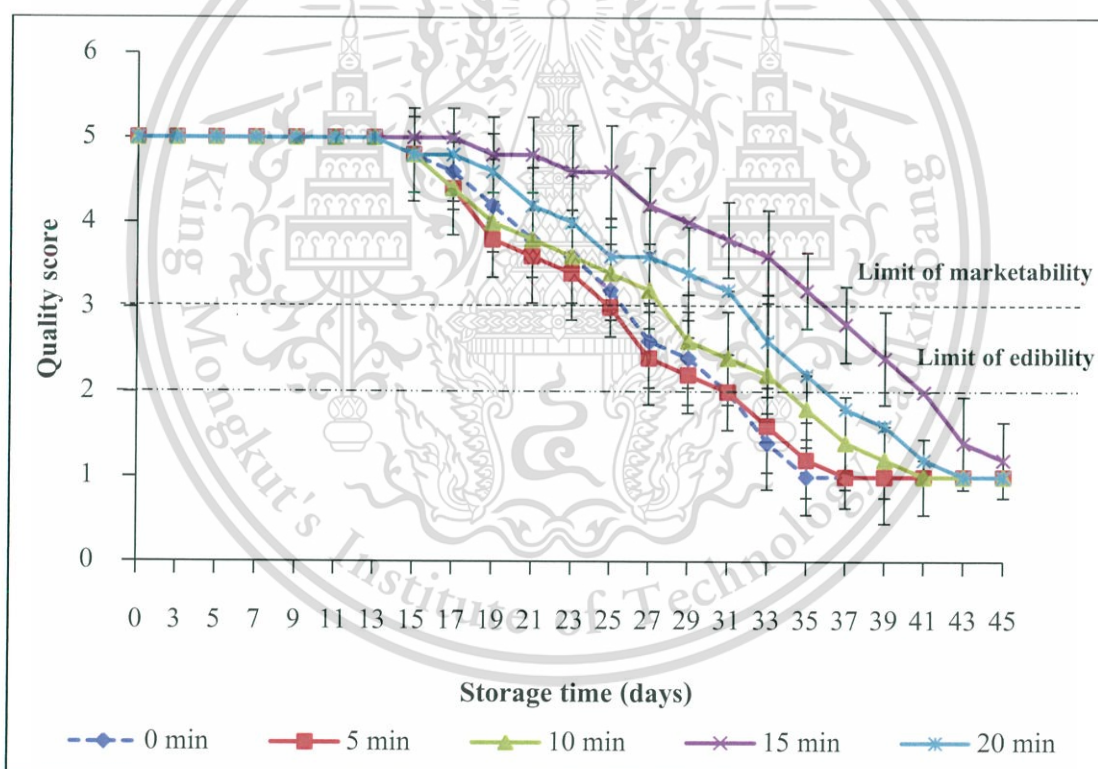


Figure 4.9 Visual quality score during storage at 12°C of kaffir lime leaves treated with ambient vapor from RD6 GRFV. Values are each the mean of four replications. Quality scores indicate degree of leaf darkening; 3 = black stains on 30% of the leaf area (limits of marketability); 2 = black stains on 30-50% of the leaf area (limits of edibility); and 1 = black stains on more than 50% of the leaf area.

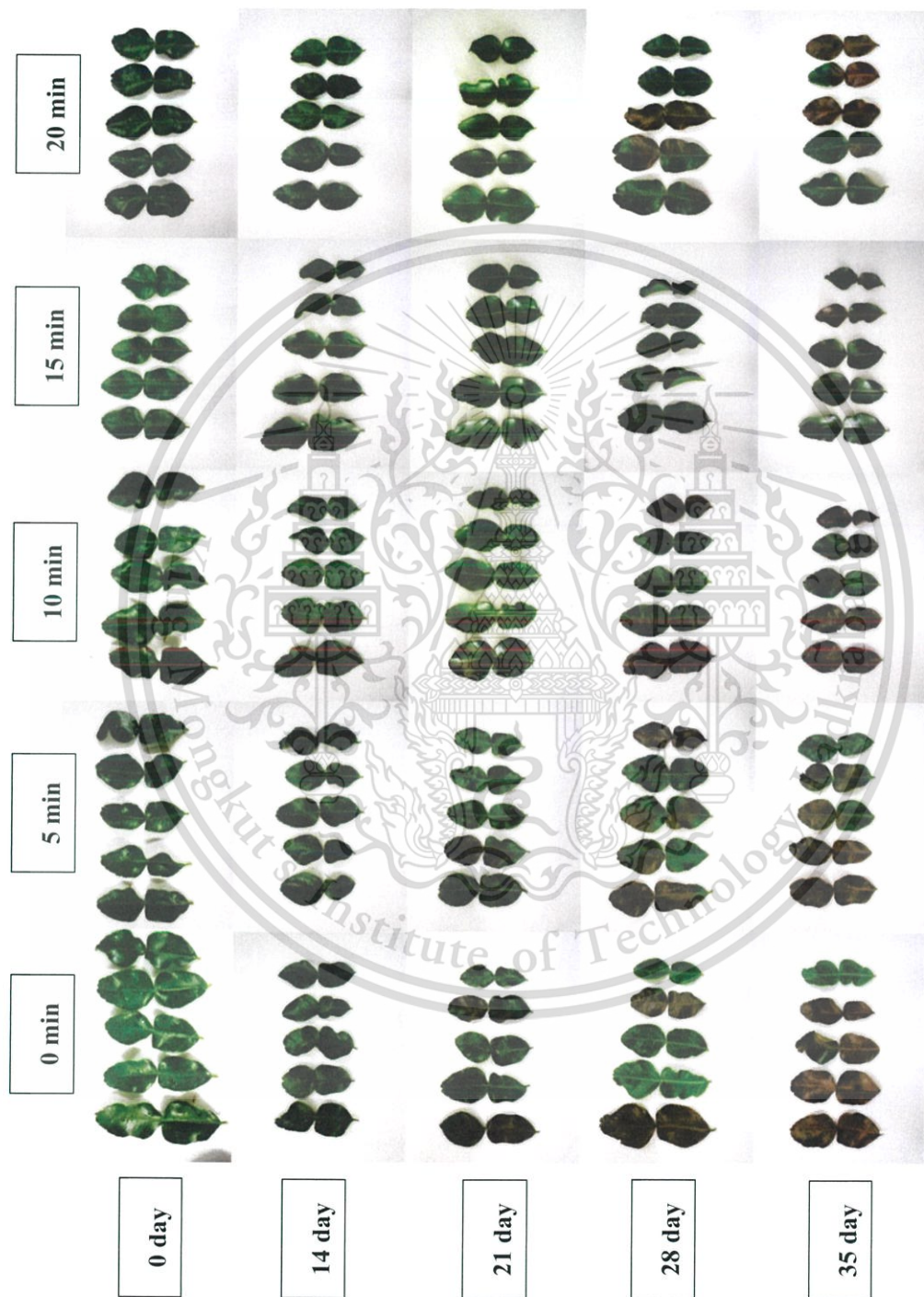


Figure 4.10 Visual quality of kaffir lime leaves treated with ambient vapor from RD6 GRFV during storage at 12°C for 35 days.

The shelf life, the time to reach the limit of marketability (score 3), of untreated kaffir lime leaves was 25.2 days (Table 4.4). Leaves treated with ambient RD6 GRFV vapor for 5 min (containing 0.0113 mmol L⁻¹ AA) did not differ in shelf life from untreated controls. In contrast, leaves treated with ambient RD6 GRFV vapor for 15-20 min (containing 0.0227-0.0455 mmol L⁻¹ AA) had significantly higher ($p \leq 0.05$) shelf life than untreated leaves. The highest shelf life, 35.4 days, was observed for leaves treated with ambient RD6 GRFV vapor for 15 min (containing 0.0341 ± 0.0001 mmol L⁻¹ AA). Similar extensions of shelf life occurred for the limit of edibility (score 2). Leaves treated with RD6 GRFV vapor for 15 min (containing 0.0341 ± 0.0001 mmol L⁻¹ AA) had the highest edibility shelf life at 40.2 days, while untreated leaves had an edibility shelf life of 30 days.

Table 4.4 Effects of RD6 GRFV vapor exposure on the shelf life of kaffir lime leaves stored at 12 °C

Exposure period (min)	RD6 GRFV vapor used ^a (g weight loss)	AA content in RD6 GRFV vapor phase ^b (mmol L ⁻¹)	Shelf life (days) [*]	
			Limit of marketability	Limit of edibility
0	0	0	25.2 ± 0.84 cd	30.0 ± 1.22 c
5	0.0128 ± 0.002	0.0113 ± 0.0001	25.0 ± 0.71d	30.4 ± 1.58 c
10	0.0256 ± 0.002	0.0227 ± 0.0001	27.2 ± 0.84 c	33.2 ± 1.92 bc
15	0.0384 ± 0.002	0.0341 ± 0.0001	35.4 ± 1.14 a	40.2 ± 1.79 a
20	0.0512 ± 0.002	0.0455 ± 0.0001	32.4 ± 1.67 b	35.4 ± 3.2 b

RD6 GRFV containing 8% (v/v) AA was used.

* Shelf life value is the mean ± one standard deviation of four replicates. Means with different letters in the same column are significantly different ($p \leq 0.05$) based on Tukey's test.

AA, acetic acid; RD6 GRFV, RD6 glutinous rice fermented vinegar.

^a The rate of RD6 GRFV vapor production was calculated from the rate of weight loss of the RD6 GRFV, 0.032 ± 0.002 g min⁻¹

^b AA content in GRVF vapor was calculated based on rate of GRVF vapor production and 8% (v/v) AA content.

4.4.1.2 Biochemical analysis of kaffir lime postharvest quality

(1) Photosynthetic pigment content

At the beginning of the experiment, chlorophyll a content was $23.05 \mu\text{g cm}^{-2}$. This value tended to decline in all treatments and control after 14 days. No significant difference was observed between treatments, but leaves treated with RD6 GRFV vapor for 10-20 min showed the smallest decrease in chlorophyll a, which was significant in comparison with the control. At the end of the experiment (day 35), chlorophyll a values were 15.14, 14.86, 16.08, 17.65 and $17.35 \mu\text{g cm}^{-2}$ for RD6 GRFV treatments for 0 (control), 5, 10, 15 and 20 min, respectively (Figure 4.11A).

Initial chlorophyll b content was $7.40 \mu\text{g cm}^{-2}$ and decreased in all treatments and control after 7 days of storage with no significant difference among treatments and the control. At the end of the experiment (day 35), chlorophyll b values were 5.18, 5.22, 5.25, 5.33 and $4.65 \mu\text{g cm}^{-2}$ for RD6 GRFV treatments for 0 (control), 5, 10, 15 and 20 min, respectively (Figure 4.11B).

At the beginning of the experiment, total chlorophyll content was $30.46 \mu\text{g cm}^{-2}$. Total chlorophyll content continuously decreased in all treatments after 7 days of storage time (Figure 4.11C). However, results showed the lowest total chlorophyll content in untreated leaves and RD6 GRFV treatment for 15 min (containing $0.0341 \text{ mmol L}^{-1}$ AA) significantly slowed the degradation of total chlorophyll. When comparing total chlorophyll of leaves, those treated with RD6 GRFV vapor for 10-20 min showed the smallest loss as of day 35. The difference among treatments was not significant, but the 15 min treatment was significantly different from the control. At the end of the experiment (day 35), total chlorophyll values were 20.32, 20.09, 21.34, 22.97 and $22.00 \mu\text{g cm}^{-2}$ for RD6 GRFV treatments for 0 (control), 5, 10, 15 and 20 min, respectively

Carotenoid content of kaffir lime leaves also decreased after 7 days of storage, with leaves treated for 15 min showing the smallest decrease. At the beginning of the experiment, the carotenoid content was $6.37 \mu\text{g cm}^{-2}$. At the end of the experiment (day 35), values were 3.96 and $4.45 \mu\text{g cm}^{-2}$ for leaves treated with RD6 GRFV for 0 (control) and 15 min (Figure 4.12).

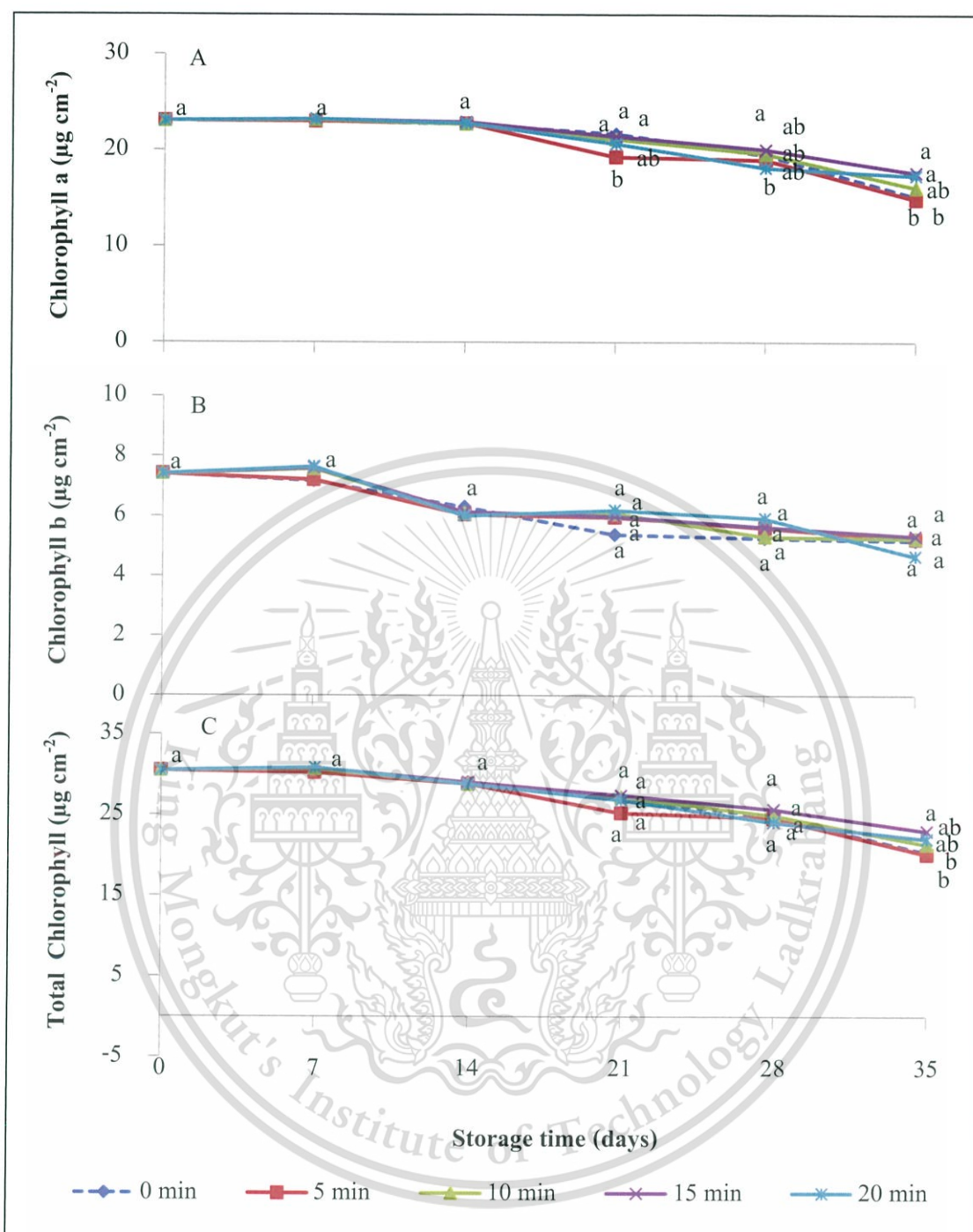


Figure 4.11 Photosynthetic pigment content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min); (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll. Values are the means of four replicates. The same letter indicates no significant difference between values ($p \geq 0.05$).

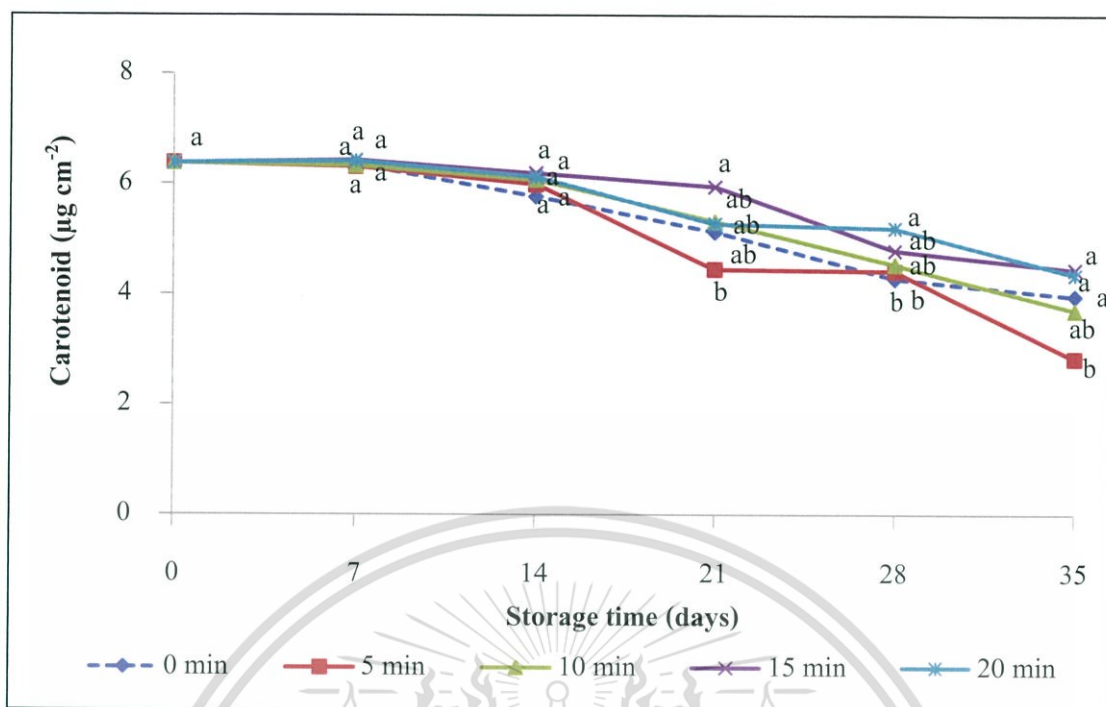


Figure 4.12 Carotenoid content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min). Values are the means of four replicates. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

(2) Membrane leakage

Electrical conductivity of a bathing medium after a period of incubation is used as an indicator of electrolyte leakage. Conductivity in deionized water was measured after floating for 1 h (Figure 4.13A), 2 h (Figure 4.13B) and 3 h (Figure 4.13C) of discs from kaffir lime leaves exposed to RD6 GRFV vapor for different times and then stored at 12 °C. Membrane leakage increased with both floating time and storage time. After 7 days of storage, leaves treated for 5-10 min (containing 0.0113-0.0227 mmol L⁻¹ AA) had increased membrane leakage and conductivity values higher than controls. Significant differences were observed between all treatments and controls.

At 35 days of storage, electrolyte leakage values after 1 h of floating (Figure 4.13A) were 1403.01, 1740.86, 1636.91, 1025.11 and 1130.04 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW); values after 2 h of floating (Figure 4.13B) were 1587.92, 2063.25, 1906.62, 1126.80 and 1249.90 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW) and values after 3 h of floating (Figure 4.13C) were 1822.10, 2188.18, 2050.80, 1216.11 and 1408.11 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW), respectively, for 0 (control), 5, 10, 15 and 20 min exposure. Over the

This material is reserved for educational use only, not allowed for commercial use.

entire storage period, leakage from leaves treated for 15-20 min (containing 0.0341-0.0455 mmol L⁻¹ AA) was lower than for the other treatments (Figure 4.13), indicating that greater membrane integrity was maintained overall.

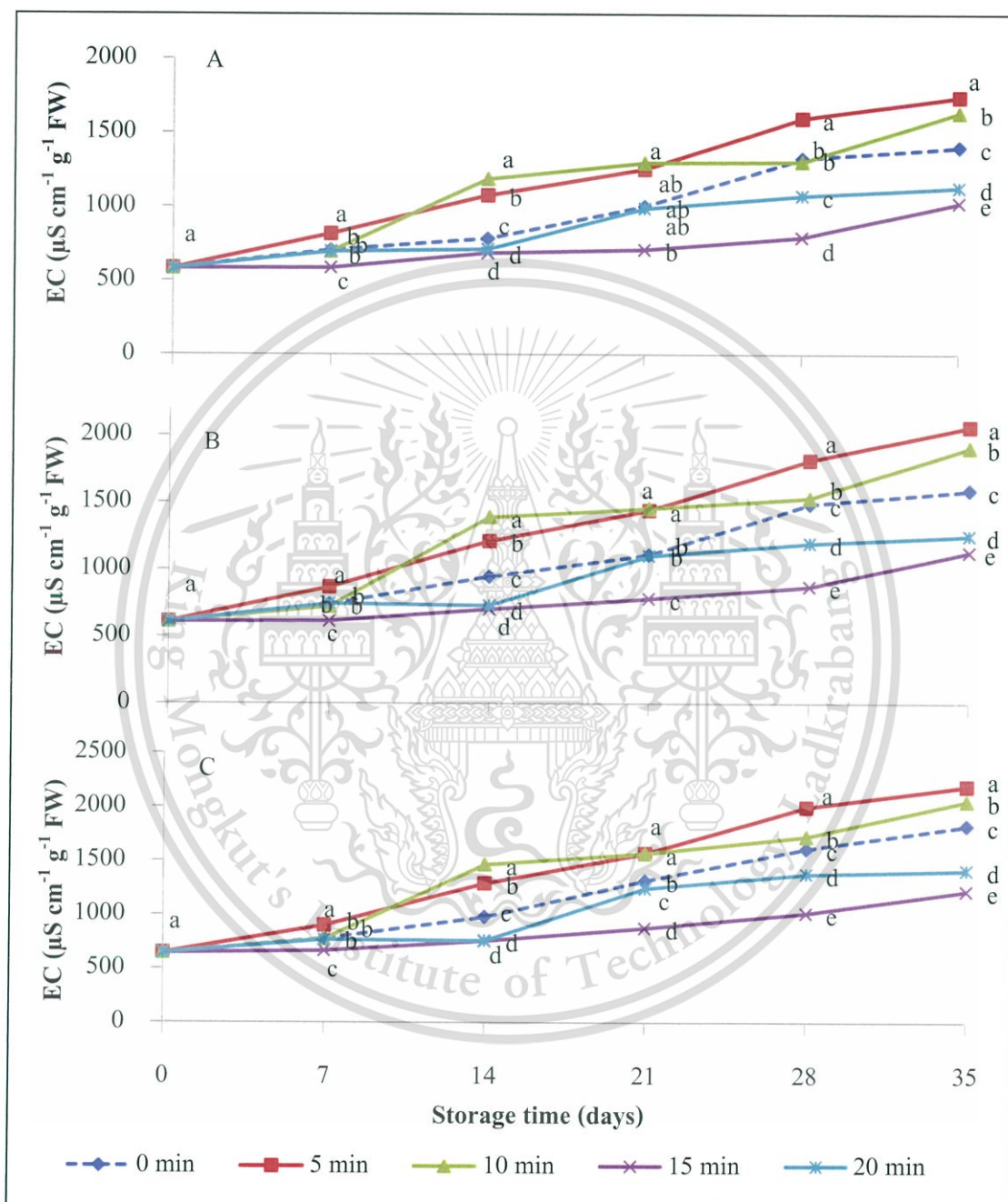


Figure 4.13 Electrical conductivity (EC) of a bathing medium after 1 h (A), 2 h (B) and 3 h (C) of floating of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min). Values are the means of four replicates. The same letter indicates there is no significant difference between the values ($p \geq 0.05$).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

(3) Lipid peroxidation (determination of malondialdehyde content)

Malondialdehyde concentration, a widely used indicator of lipid peroxidation, exhibited a pattern of increase during the whole storage period. Compared with the control, all RD6 GRFV vapor treatments significantly increased MDA content in leaves. At the end of the experiment, the control exhibited the highest lipid peroxidation while leaves treated for 15 min showed the lowest lipid peroxidation. Leaves had final MDA contents of 21.44 nmol g⁻¹ (FW) for the control and 17.11, 15.05, 14.63 and 14.93 nmol g⁻¹ (FW) for 5, 10, 15 and 20 min treatments, respectively (Figure 4.14). These results indicated that treatment with RD6 GRFV vapor for 15 min significantly reduced oxidation compared to the untreated control.

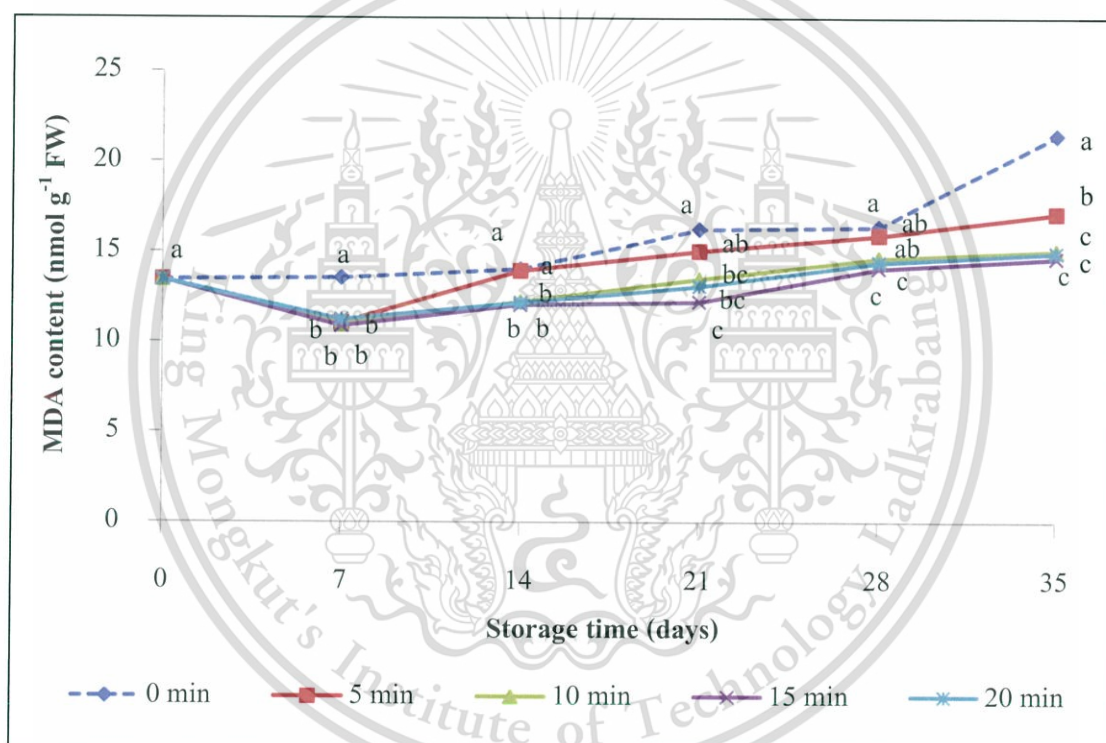


Figure 4.14 Lipid peroxidation expressed as the malondialdehyde (MDA) content of kaffir lime leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min). Values are the means of four replicates. The same letter indicates no significant differences between values ($p \geq 0.05$).

(4) DPPH radical scavenging activity

Antioxidant activity in treated and untreated kaffir lime leaves was determined using a DPPH scavenging assay and reported as the IC₅₀ value, where a low value indicates high

antioxidant activity (Figure 4.15). A similar pattern of DPPH radical scavenging ability was detected in control and treated samples, all of which exhibited decreased activity during storage. At the beginning of the experiment (day 0), the untreated extract of kaffir lime showed the highest antioxidant activity, with an IC_{50} of 3,263.9 ppm. After 7 days of storage, the DPPH radical scavenging abilities of treated leaf extracts were significantly higher than that of the control, and remained so until the end of storage. At the end of the experiment (day 35), leaves treated for 15 min (containing $0.0341 \text{ mmol L}^{-1}$ AA) showed the highest DPPH radical scavenging activity with an IC_{50} value of 6,964.8 ppm, while the control showed the lowest activity with an IC_{50} value of 9,744.2 ppm. IC_{50} values of other samples were 8,976.4, 8,772.8 and 8,368.6 ppm for 5, 10 and 20 min treatments, respectively (Figure 4.15).

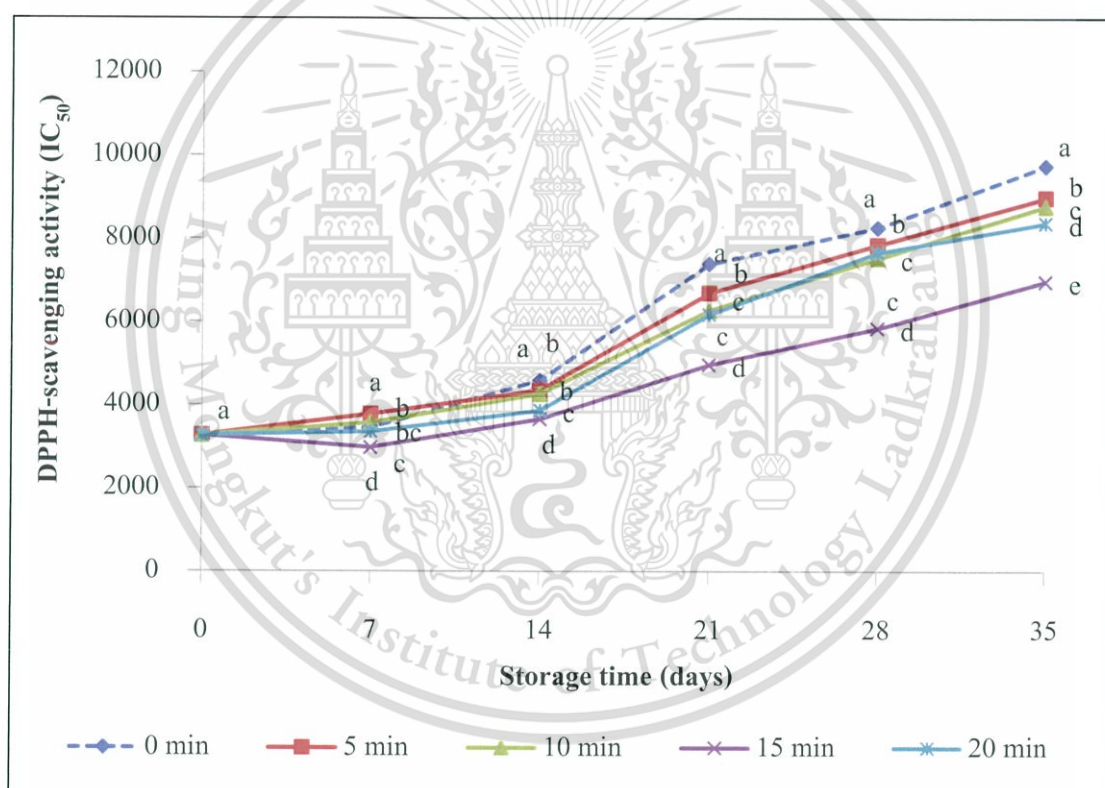


Figure 4.15 DPPH radical scavenging activity of kaffir lime leaves stored at 12°C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 5, 10, 15 and 20 min). Values are the means of four replicates. The same letter indicates no significant differences between values ($p \geq 0.05$).

4.4.2 Use of ambient RD6 GRFV vapor to reduce postharvest decay of sweet basil

4.4.2.1 Physical changes in treated sweet basil

(1) Changes in fresh weight

Fresh weight of sweet basil shoots is expressed as a percentage of that at the beginning of the experiment. All groups exhibited a pattern of decrease during the whole storage period (Figure 4.16). The weight loss of control leaves rapidly increased within the first 5 days of storage. After five days, the weight loss rates of all RD6 GRFV vapor treatments were lower than the control, and remained so until the end of storage. After 25 days, control leaf weight reached a minimum of 69.87%, while leaves treated with vapor for 2, 4, 6, 8 and 10 min (containing 0.0011-0.0057 mmol L⁻¹AA) reached values of 77.81, 79.23, 80.43, 81.72 and 89.66%, respectively. All treatments had significantly reduced weight loss compared with the control; leaves treated for 10 min lost the least.

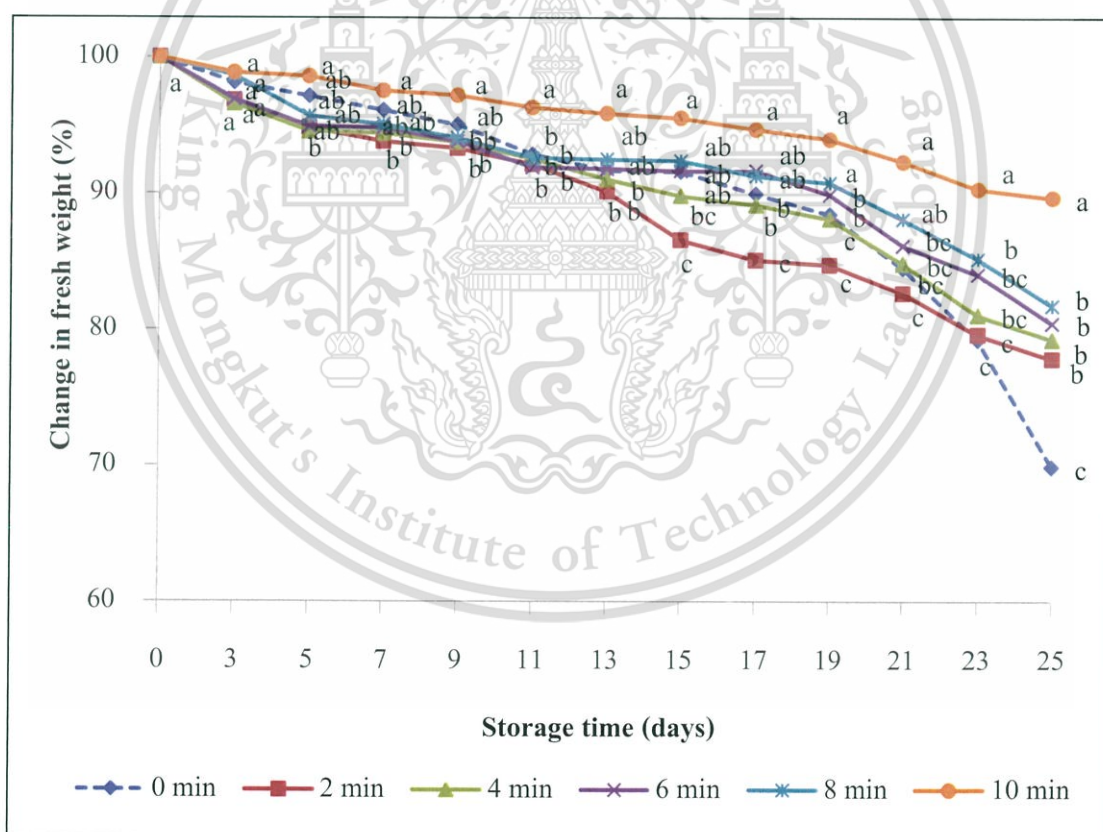


Figure 4.16 Change in fresh weight (%) of sweet basil leaves stored at 12 °C after treatment with ambient vapor from undiluted RD6 GRFV for periods ranging from 0 to 10 min. Values are the means of four replicates. The same letter indicates there is no significant difference between values ($p \geq 0.05$).

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

(2) Changes in leaf color during storage

Color values of sweet basil leaves were expressed as CIELAB color space units and the mean values for lightness (L^*), red-greenness (a^*) and blue-yellowness (b^*) parameters were calculated for each treatment.

L^* values of sweet basil treated with RD6 GRFV vapor for 2, 4, 6, 8 and 10 (containing 0.0011-0.0057 mmol L⁻¹ AA) all tended to decrease (increased darkness) during storage (Figure 4.17A). The conditions of leaves across all treatments were generally not significantly different from one another. At the end of storage (25 days), leaves treated for 8-10 min were significantly different from control and from leaves treated for 2 min. Final L^* values were 25.66, 28.26, 30.72, 34.35 and 36.29 for 2, 4, 6, 8 and 10 min of RD6 GRFV vapor treatment, respectively, while the control had a value of 24.37.

a^* values of leaves in all treatment groups increased (decreased darkness) during storage, and were not significantly different from one another (Figure 4.17B). At the end of storage (25 days), the control (-0.18) was not significantly different from leaves treated for 2-6 min, but was significantly different from leaves treated for 8-10 min. Final a^* values were -0.12, -0.67, -1.19, -2.29 and -2.20 for 2, 4, 6, 8 and 10 min of RD6 GRFV vapor treatment, respectively, while the control had a value of -0.18.

b^* values of leaves in all treatment groups tended to decrease (yellowish color) after 10 days and until the end of storage (Figure 4.17C). At 35 days of storage, the obtained b^* values were 1.18, 1.46, 1.70, 2.19 and 2.15 for 2, 4, 6, 8 and 10 min of RD6 GRFV vapor treatment, respectively. Sweet basil treated for 8 and 10 was significantly different from the control (1.21). Overall, the treated sweet basil samples presented lower L^* and b^* values (increased darkness and yellowish in color), while their a^* values were generally higher when compared to control.

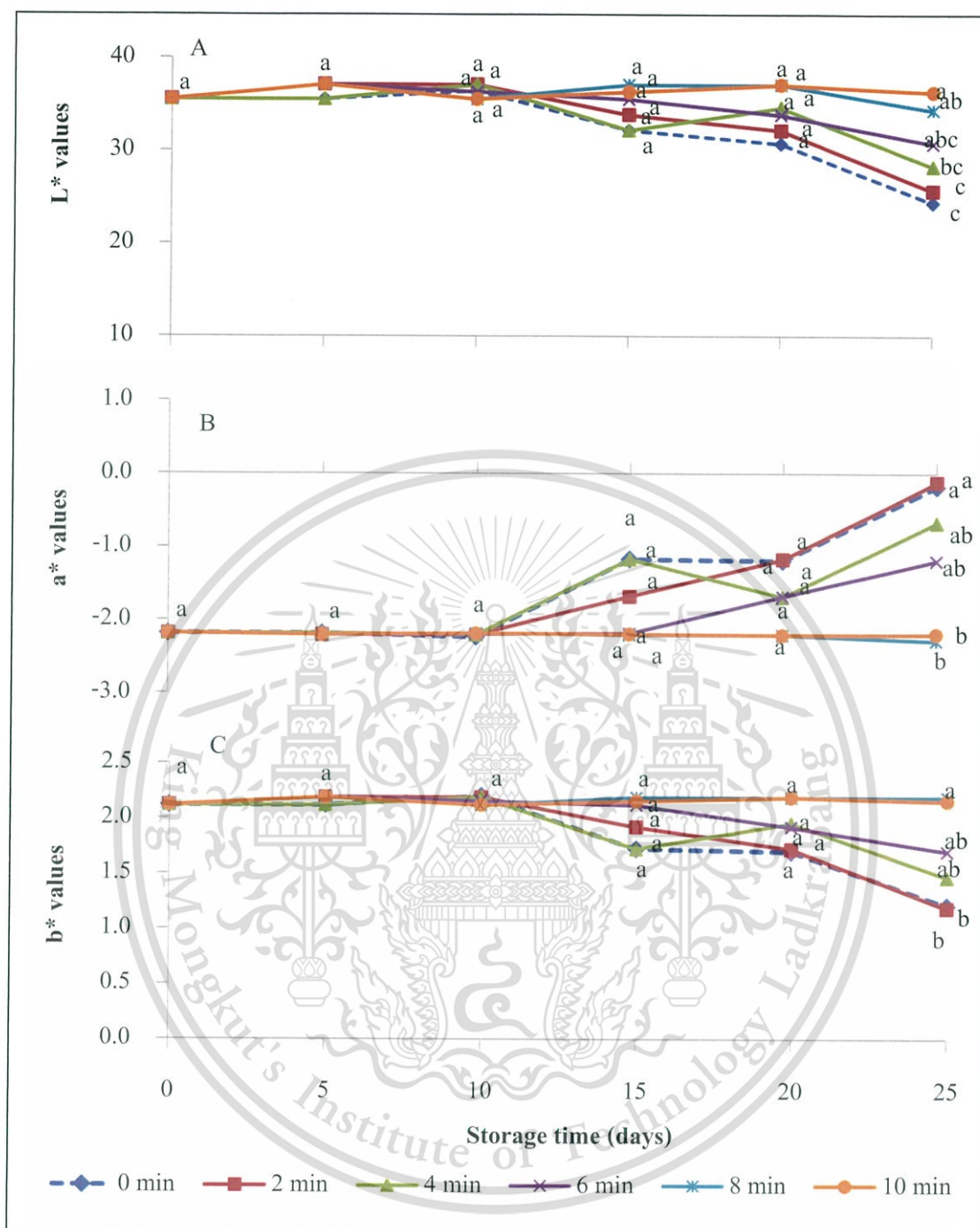


Figure 4.17 Changes in color of sweet basil leaves during storage at 12 °C after treatment with ambient vapor from diluted RD6 GRFV for periods ranging from 0 to 10 min, measured as (A) L* value, (B) a* value and (C) b* value. Values are the means of four replicates. The same letter indicates there is no significant difference between values ($p \geq 0.05$).

(3) Evaluation of visual quality and shelf-life

Visual quality of leaves was evaluated daily during storage. Quality scores decreased steadily with increasing storage time (Figure 4.18). For all treatments, scores did not begin to decrease until the fifth day of storage. After that point, untreated controls and leaves treated for 2-8 min all decreased more rapidly than leaves treated for 10 min. Leaves treated for 10 min had the highest visual appearance scores overall, until the end of storage. At day 20, leaves treated for 10 min were the only group to remain above the limit of marketability (score 3). Untreated controls and leaves treated for 2-8 min further decreased below the limit of edibility (score 2) after 27 days of storage. However, at the end of the storage period (limit of marketability), leaves treated with RD6 GRFV vapor for 10 min (containing 0.0113 ± 0.0001 mmol L⁻¹ AA) had visual quality scores higher (3 points) and untreated leaves had visual quality scores fell below the limit of edibility (1.6 points). RD6 GRFV vapor treated sweet basil leaves for 10 showed a slight black stain on leaf (Figure 4.19).

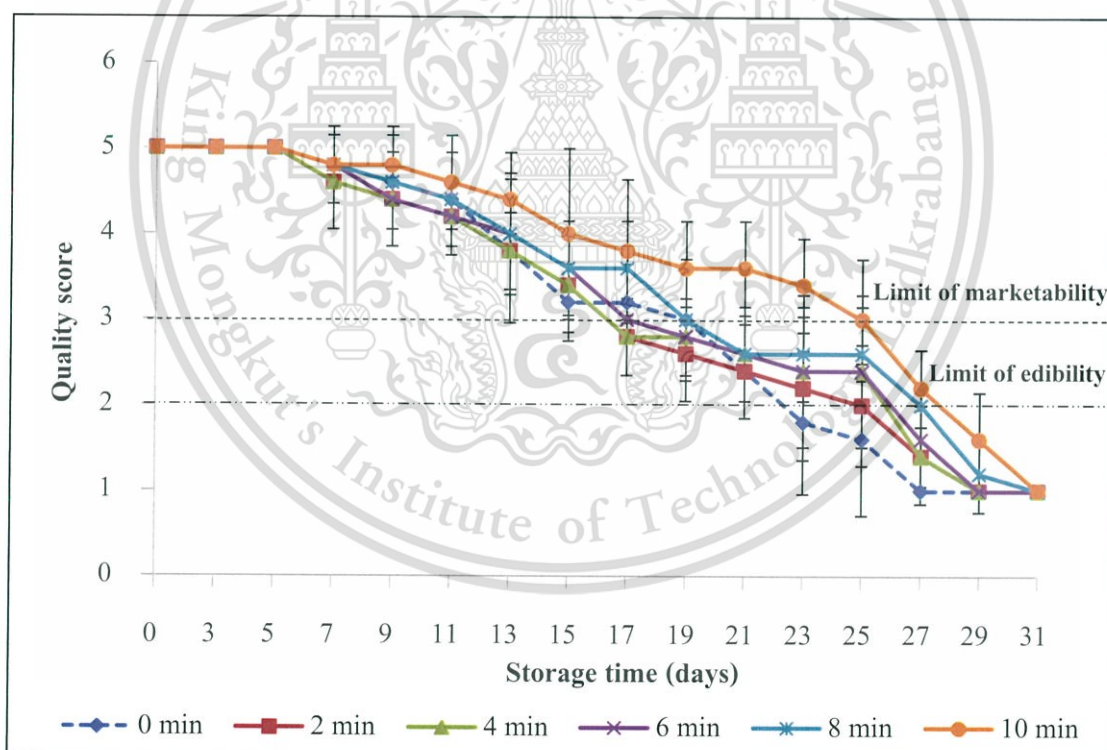


Figure 4.18 Visual quality score during storage at 12 °C of sweet basil leaves treated with ambient vapor from diluted RD6 GRFV. Values are each the mean of four replicates. Quality scores indicate degree of leaf darkening: 3 = black stains on 30% of the leaf area; 2 = black stains on 30-50% of the leaf area; and 1 = black stains on more than 50% of the leaf area.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

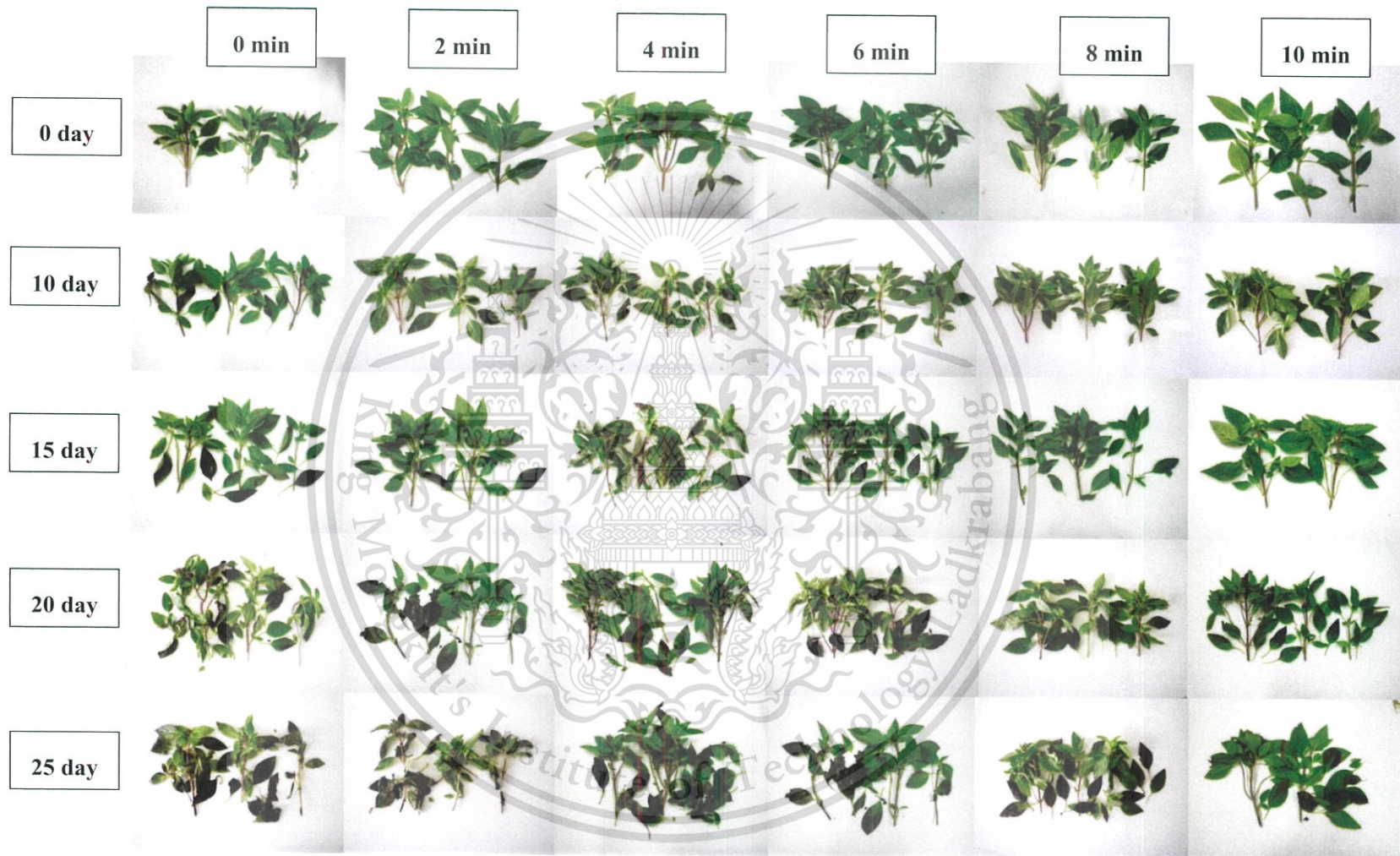


Figure 4.19 Visual quality of sweet basil leaves treated with ambient vapor from RD6 GRFV during storage at 12°C for 25 days.

The shelf life, the time to reach the limit of marketability (score 3), of untreated sweet basil leaves was 17.8 days (Table 4.5). Leaves treated with ambient RD6 GRFV vapor for 2-6 min (containing 0.0022-0.0066 mmol L⁻¹ AA) did not differ in shelf life from untreated controls. In contrast, leaves treated with ambient RD6 GRFV vapor for 8-10 min (containing 0.0088-0.0113 mmol L⁻¹ AA) had significantly higher ($p \leq 0.05$) shelf life than untreated leaves. The highest shelf life, 25.4 days, was observed for leaves treated with ambient RD6 GRFV vapor for 10 min (containing 0.0113 ± 0.0001 mmol L⁻¹ AA). Similar extensions of shelf life occurred for the limit of edibility (score 2). Leaves treated with RD6 GRFV vapor for 10 min (containing 0.0113 ± 0.0001 mmol L⁻¹ AA) had the highest edibility shelf life at 28 days, while untreated leaves had an edibility shelf life of 21 days.

Table 4.5 Effects of RD6 GRFV vapor exposure on the shelf life of sweet basil leaves stored at 12°C

Exposure period (min)	RD6 GRFV vapor used ^a (g weight loss)	AA content in RD6 GRFV phase ^b (mmol L ⁻¹)	Shelf life (days) [*]	
			Limit of marketability	Limit of edibility
0	0	0	17.8 ± 1.30 c	21.0 ± 1.41 d
2	0.0026 ± 0.002	0.0022 ± 0.0001	16.4 ± 0.89 c	24.0 ± 1.22 c
4	0.0051 ± 0.002	0.0044 ± 0.0001	16.2 ± 0.84 c	25.6 ± 0.55 bc
6	0.0077 ± 0.002	0.0066 ± 0.0001	17.0 ± 1.58 c	26.0 ± 0.71 b
8	0.0102 ± 0.002	0.0088 ± 0.0001	19.2 ± 0.84 b	26.8 ± 0.45 ab
10	0.0128 ± 0.002	0.0113 ± 0.0001	25.4 ± 0.89 a	28.0 ± 1.00 a

RD6 GRFV containing 4% (v/v) AA was used.

* Shelf life value is the mean ± one standard deviation of four replicates. Means with different letters in the same column are significantly different ($p \leq 0.05$) based on Tukey's test.

AA, acetic acid; RD6 GRFV, RD6 glutinous rice fermented vinegar.

^a The rate of RD6 GRFV vapor production was calculated from the rate of weight loss of the RD6 GRFV, 0.032 ± 0.002 g min⁻¹.

^b AA content in GRVF vapor used was calculated based on rate of GRVF vapor production and 4% (v/v) AA content.

4.4.2.2 Biochemical analysis of sweet basil postharvest quality

(1) Photosynthetic pigment content

At the beginning of the experiment, chlorophyll a content was $18.90 \mu\text{g cm}^{-2}$. Content continuously decreased as the storage period increased (Figure 4.20A). The results showed that the lowest total chlorophyll content was in the untreated control, while RD6 GRFV treatment for 8 and 10 min (containing 0.0088 - $0.0113 \text{ mmol L}^{-1}$ AA) significantly slowed the degradation of chlorophyll a. At the end of the storage period (day 25), chlorophyll a had decreased by $13.30 \mu\text{g cm}^{-2}$ in the controls, with decreases in the treated leaves being 13.05 , 13.63 , 14.46 , 16.72 and $16.91 \mu\text{g cm}^{-2}$ for 2, 4, 6, 8 and 10 min of RD6 GRFV treatment, respectively.

Chlorophyll b content showed a decrease during storage similar to that of chlorophyll a. At the beginning of experiment, the chlorophyll b content was $7.40 \mu\text{g cm}^{-2}$. At 25 days of storage, rapid decreases in chlorophyll b were observed in the untreated control as well as in leaves treated for 2, 4 and 6 min (0.0022 - $0.0034 \text{ mmol L}^{-1}$ AA), respectively. At the end of the experiment (day 25), values were 5.18 , 5.22 , 5.25 , 5.33 and $4.65 \mu\text{g cm}^{-2}$ for leaves treated for 0 (control), 5, 10, 15 and 20 min, respectively (Figure 4.20B).

At the beginning of the experiment, total chlorophyll content was $24.55 \mu\text{g cm}^{-2}$. Content decreased slightly in all treatments after storage (Figure 4.20C). Rapid decreases in total chlorophyll were observed in the untreated control as well as in leaves treated for 2, 4 and 6 min (equaling to 0.0022 , 0.0044 and $0.0066 \text{ mmol L}^{-1}$ AA), respectively. Treatment for 8 min (containing $0.0088 \text{ mmol L}^{-1}$ AA) and for 10 min (containing $0.0113 \text{ mmol L}^{-1}$ AA) was observed to significantly slow the degradation of total chlorophyll. At the end of the experiment (day 25), values were 17.06 , 20.98 and $21.08 \mu\text{g cm}^{-2}$ for RD6 GRFV treatments for 0 (control), 8 and 10 min, respectively. These results indicated chlorophylls a and b are both continuously degraded during storage, resulting in a continuous reduction a total chlorophyll (Figure 4.21C), and that degradation is significantly slowed by treatment with RD6 GRFV for 8 or 10 min.

At the beginning of the experiment, the carotenoid content was $5.07 \mu\text{g cm}^{-2}$. Leaves treated with RD6 GRFV showed a slight increase in carotenoid content after 5 days; however, the content in all treatments did not differ from that of the untreated control. At the end of the experiment (day 25), values were 5.88 , 5.88 , 5.74 , 5.66 , 5.50 and $5.29 \mu\text{g cm}^{-2}$ for leaves treated for 0 (control), 2, 4, 6, 8 and 10 min, respectively (Figure 4.21).

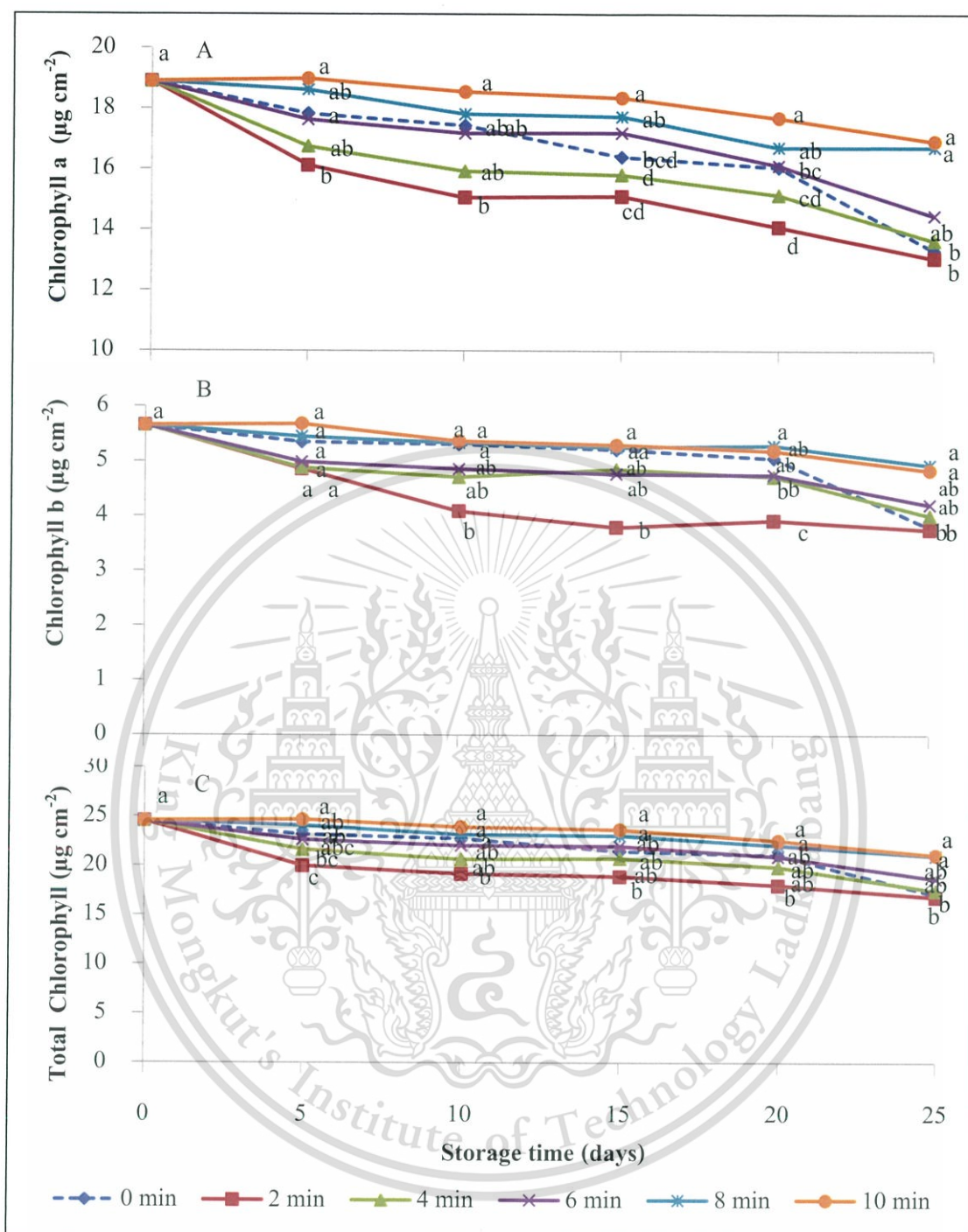


Figure 4.20 Photosynthetic pigment content of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 2, 4, 6, 8 and 10 min). (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll. Values are the means of four replicates. The same letter indicates there is no significant difference between values ($p \geq 0.05$).

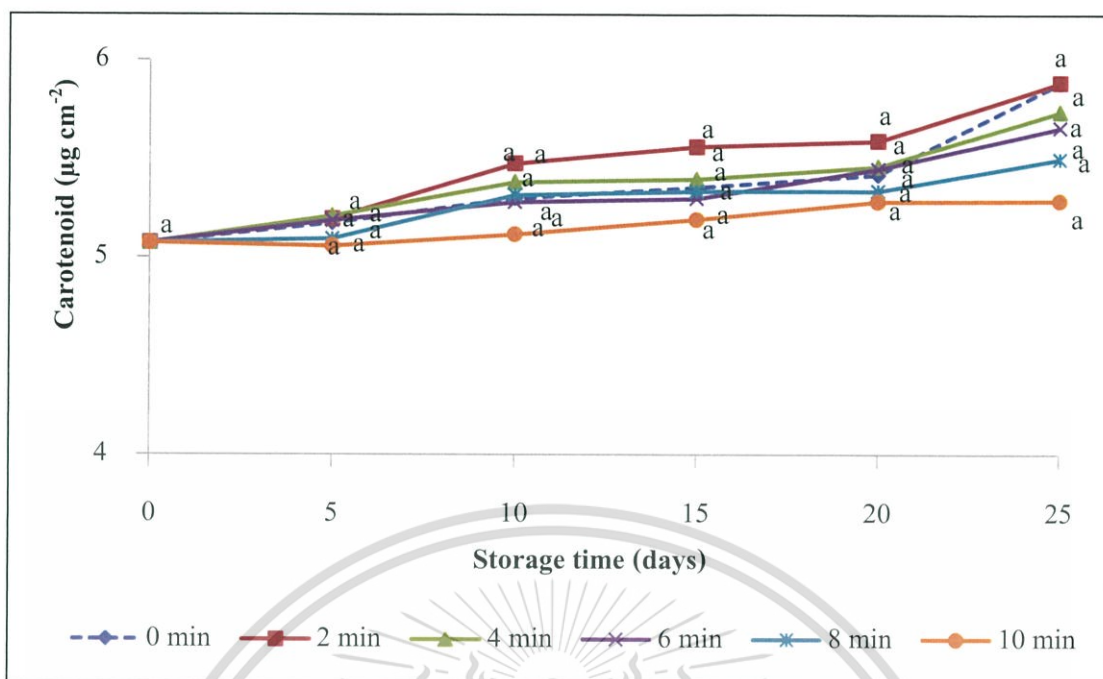


Figure 4.21 Carotenoid content of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (2, 4, 6, 8 and 10 min). Values are the means of four replicates. The same letter indicates there is no significant difference between values ($p \geq 0.05$).

(2) Membrane leakage

Electrical conductivity of a bathing medium after a period of incubation is used as an indicator of electrolyte leakage. Conductivity in deionized water was measured after floating for 1 h (Figure 4.22A), 2 h (Figure 4.22B), and 3 h (Figure 4.22C) of discs from sweet basil leaves exposed to RD6 GRFV vapor and then stored at 12 °C. Membrane leakage increased with both floating time and storage time. Leaves treated for 2-8 min (containing 0.0022-0.0088 mmol L⁻¹ AA) exhibited slightly increased membrane leakage. At 35 days of storage, leakage values after 1 h of floating (Figure 4.22A) were 638.59, 730.77, 722.06, 708.27, 661.38 and 619.25 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW); values after 2 h of floating (Figure 4.22B) were 679.99, 778.60, 745.79, 725.60, 708.08 and 669.17 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW) and values after 3 h of floating (Figure 4.22C) were 706.60, 816.67, 761.10, 754.53, 729.30 and 690.36 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (FW), respectively, for 0 (control), 2, 4, 6, 8 and 10 min exposures. Over the entire storage period, leakage from leaves treated for 10 min (containing 0.0113 mmol L⁻¹ AA) was slightly lower than that of other treatments and also significantly different from the control.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

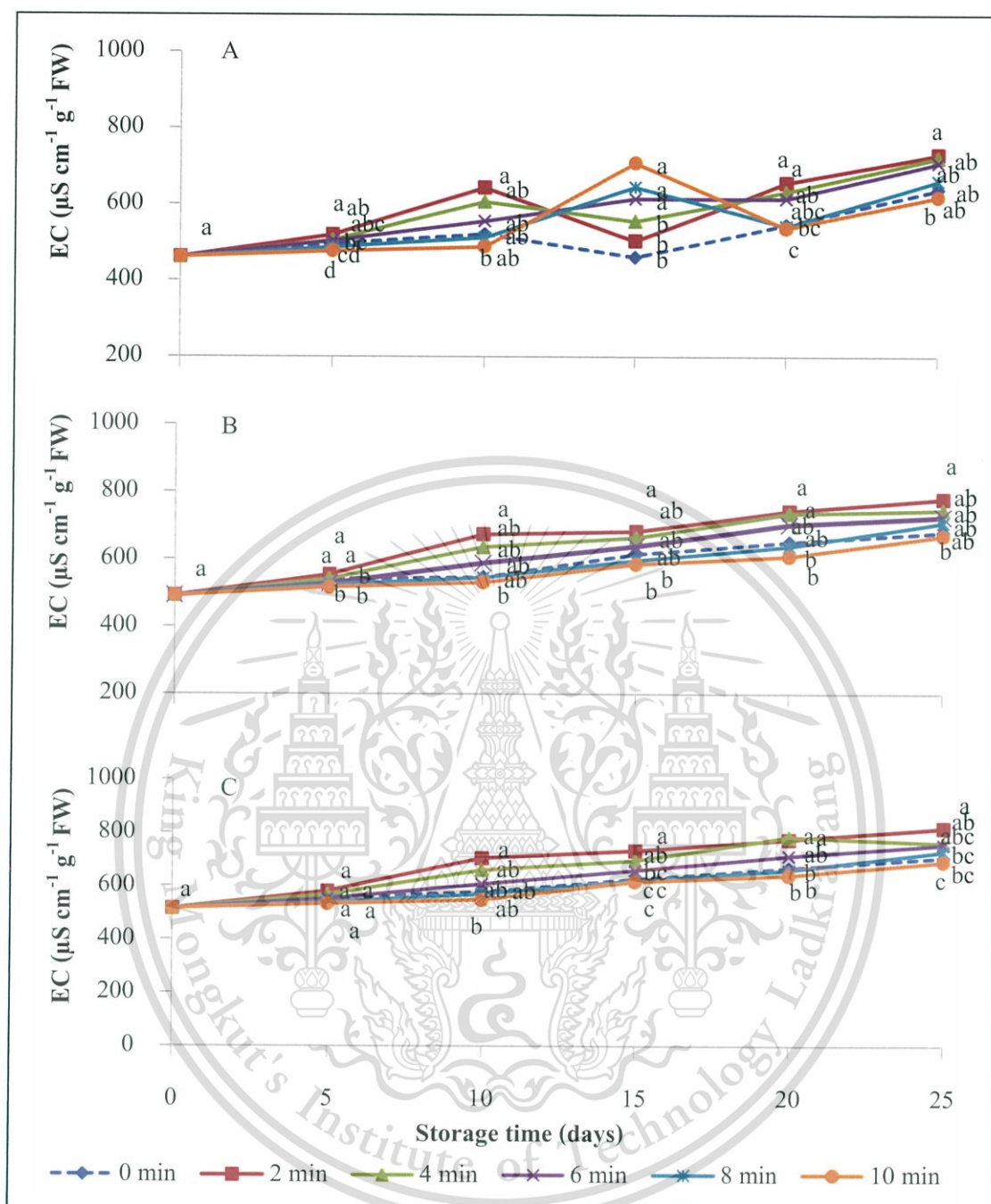


Figure 4.22 Electrical conductivity (EC) of a bathing medium after 1 h (A), 2 h (B), and 3 h (C) floating of sweet basil leaves stored at 12 °C after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 2, 4, 6, 8 and 10 min). Values are the means of four replicates. The same letter indicates there is no significant difference between values ($p \geq 0.05$).

(3) Lipid Peroxidation (determination of malondialdehyde content)

All samples showed increasing lipid peroxidation with storage time, as measured by increased MDA content. Leaves treated for 2-8 min showed slightly increased MDA content, but were not significantly higher than the control until the tenth day of storage. However, compared with the controls, the increase was significantly reduced ($p \leq 0.05$) for the 10 min treatment. At the end of the experiment (day 25), MDA content values were 5.35 nmol g^{-1} (FW) for the control and 5.12 nmol g^{-1} (FW) for 10 min treated samples (Figure 4.23). Samples treated for 2-4 min had MDA contents higher than other treatments and the control.

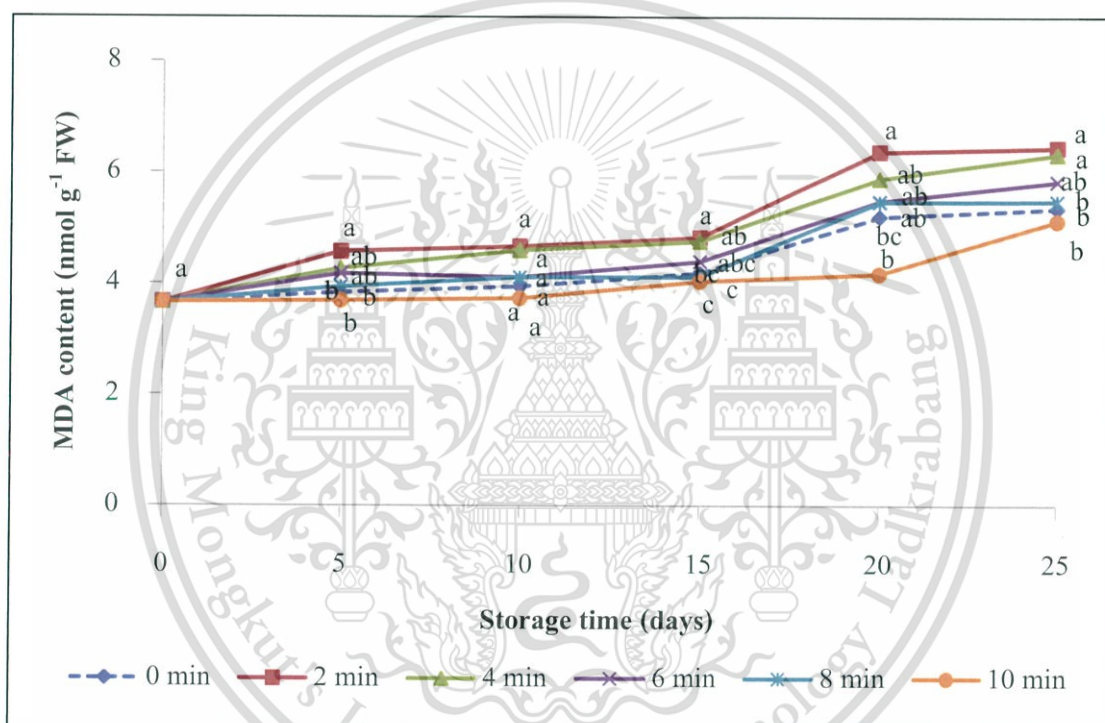


Figure 4.23 Lipid peroxidation expressed as the malondialdehyde (MDA) content of sweet basil leaves stored at $12 \text{ }^{\circ}\text{C}$ after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 2, 4, 6, 8 and 10 min). Values are the means of four replicates. The same letter indicates no significant differences between values ($p \geq 0.05$).

(4) DPPH radical scavenging activity

Antioxidant activity in treated and untreated sweet basil leaves was determined using a DPPH scavenging assay and reported as the IC_{50} value, where a low value indicates high antioxidant activity (Figure 4.24). A similar pattern of DPPH radical scavenging ability was detected in control and treated samples, all of which exhibited decrease during storage. At the

This material is reserved for educational use only, not allowed for commercial use.

beginning of the experiment (day 0), the untreated extract showed the highest antioxidant activity, with an IC_{50} of 1,409.27 ppm. After 15 days of storage, the DPPH radical scavenging ability of leaves treated for 10 min (containing $0.0113 \text{ mmol L}^{-1}$ AA) was significantly higher than that of the control. At the end of the experiment (day 25), leaves treated for 10 min showed the highest DPPH radical scavenging activity with an IC_{50} value of 1,807.38 ppm, while untreated leaves showed the lowest activity with an IC_{50} value of 2,370.04 ppm. Samples treated for 2, 4, 6 and 8 min showed IC_{50} values of 2,370.04, 2,371.81, 2,174.80 and 2,094.78 ppm, respectively (Figure 4.24).

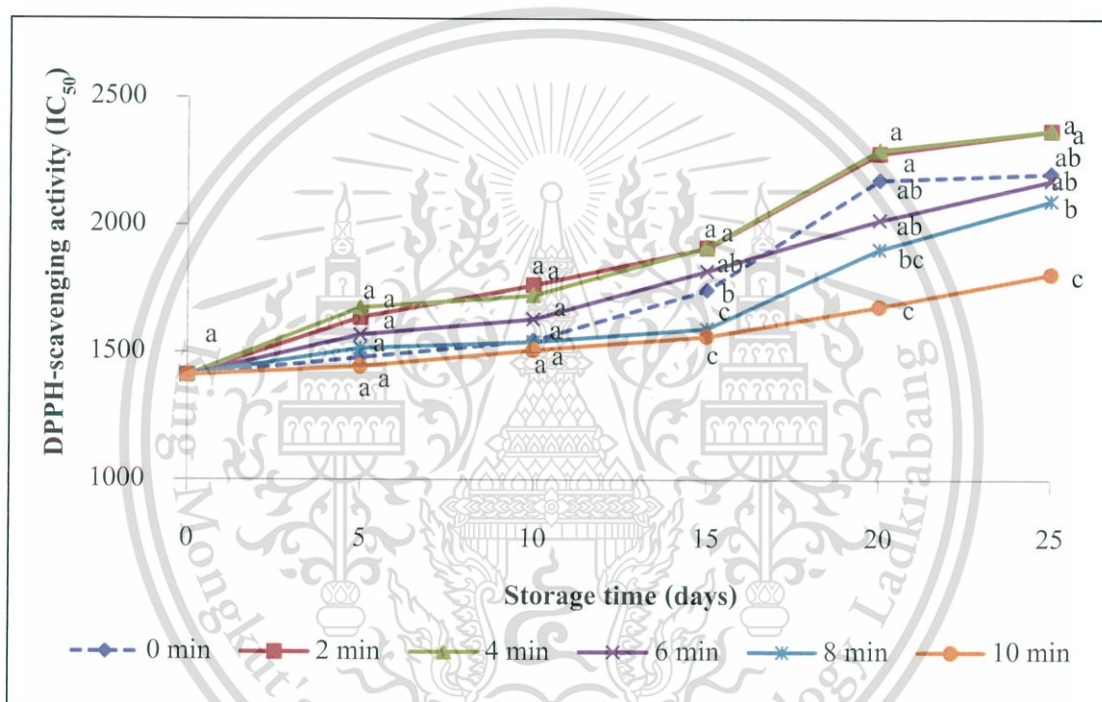


Figure 4.24 DPPH radical scavenging activity of sweet basil leaves stored at $12 \text{ }^{\circ}\text{C}$ after exposure to the ambient vapor above diluted RD6 GRFV for different times (0, 2, 4, 6, 8 and 10 min). Values are the means of four replicates. The same letter indicates no significant difference between values ($p \geq 0.05$).

CHAPTER 5

DISCUSSION

5.1 Experiment 1. Study of phenolic and browning compounds in RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

5.1.1 Phenolic compounds

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many phenolics have been shown to possess high antioxidant activities (Razali *et al.*, 2008). Of materials included in this study, RD6 GRFV was found to contain the highest quantity of phenolic acids. Total phenolic compounds of RD6 glutinous rice grain and steamed rice extracts were 0.055 and 0.012 mg GAE g⁻¹ DW, while values for rice wine and RD6 GRFV were 0.415 and 0.612 mg GAE mL⁻¹, respectively. A previous study on antioxidant activity of the raw, cooked and fermented Hom-Nil rice and black glutinous rice (*Oryza sativa*) extracts reported that total phenolic contents of black glutinous rice extracts were higher than those of Hom-Nil rice extracts (Sadabpod *et al.*, 2010). They found that raw black glutinous rice contained the highest quantity of phenolic acids (458.37 mg GAE g⁻¹), more than cooked black glutinous rice (452.75 mg GAE g⁻¹), fermented black glutinous rice (65.21 mg GAE g⁻¹), raw Hom Nil rice extract (272.07 mg GAE g⁻¹), cooked Hom Nil rice (235.42 mg GAE g⁻¹) and fermented Hom Nil rice extract (38.56 mg GAE g⁻¹). Muntana and Prasong (2010) also found that the total phenolic content of white, red and black rice bran extracts were in the range of 0.8931-0.9884, 1.0103-1.0494 and 1.0810-1.2239 mg gallic acid equivalent g⁻¹ (mg GAE g⁻¹), respectively. Another study found that the ethyl acetate extract of red fruit (*Pandanus conoideus* Lam) exhibited high total phenolic content (6.27 mg GAE g⁻¹ DW) (Rohman *et al.*, 2010). Lee *et al.* (2014) investigated the physicochemical properties and antioxidant activity of vinegar added with different levels (0, 1, 3, 5 and 7%) of *Akebia quinata* fruit during two-step fermentation. During acid fermentation, total acidities of vinegar increased. However, total polyphenol contents of vinegar added with 0-7% *Akebia quinata* fruit significantly increased and vinegar added with 7% *Akebia quinata* fruit showed the highest total polyphenol content (4.15 mg g⁻¹) after 13 days of fermentation.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

5.1.2 Browning compounds

Browning compounds are measured as a convenient index of the development of Maillard reaction products (MRPs), offering a visual estimate (absorbance at A_{420} nm). MRPs have been proven to be able to suppress oxidation in different foods like cereals, meat, juices and nuts (Kusznierewicz *et al.*, 2008; Açar *et al.*, 2009; Rufián-Henares and Delgado-Andrade, 2009; Serpen *et al.*, 2012) as well as in model systems (Wagner *et al.*, 2002). Results from this study showed that RD6 GRFV had the highest quantity of browning compounds compared to glutinous rice wine, steamed glutinous rice, or glutinous rice grain; this trend is the same as for total phenolic contents. The amount of browning compounds increased during fermentation; thus, vinegar fermentation of RD6 glutinous rice might have effects on the rate of Maillard reactions that produce MRPs. Aili *et al.* (2012) have investigated the total antioxidant capacity and browning compounds of tartary buckwheat vinegar and found that the amount of browning compounds increased during thermal processing; the greatest production took place on the fourth day. Xu *et al.* (2007) found that the antioxidant activity of Zhenjiang aromatic vinegar had a relationship with the absorbance at 420 nm.

5.2 Experiment 2. Comparison of antioxidant properties and DNA damage protective activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV

5.2.1 DPPH free radical scavenging activity

The DPPH radical scavenging assay was employed to evaluate the antioxidant properties of RD6 glutinous rice grain, steamed rice, RD6 glutinous rice wine and RD6 GRFV. The free radical scavenging activity of samples increased with increasing concentration. The IC_{50} values of glutinous rice grain, steamed glutinous rice, glutinous rice wine and GRFV were 6.58, 14.09, 16.10 and 4.33%, respectively. Lower IC_{50} indicates higher antioxidant capacity of the extract. Similarly, methanolic extracts of Thai rice cultivar 5718 (red color grain) showed the highest DPPH scavenging activity, with IC_{50} of $0.0057 \text{ mg mL}^{-1}$ (Muntana and Prasong, 2010). Jeong *et al.* (2011) reported the antioxidant activities of six Korean rice wines (Maesilju (MSJ), Kookhwaju-1 (KHJ-1), Kookhwaju-2 (KHJ-2), Gugijaju (GGJ), Sasamju (SSJ) and Sogokju (SGJ), and found that the wine with highest activity was KHJ-1 at 66%, followed by KHJ-2

(63%), SGJ (64%), MSJ (40%), GGJ (40%), and then SSJ with the lowest inhibition activity (35%).

5.2.2 Metal chelating activity

Ferrous ions can initiate lipid peroxidation by the Fenton's reaction, as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals (Halliwell, 1991). Metal chelating activity can reduce concentration of the catalyzing transition metal in lipid peroxidation. This study found that samples of RD6 glutinous rice grain, steamed rice, rice wine and RD6 GRFV all had no detectable ability to chelate Fe^{2+} . The obtained results indicated that their antioxidant properties might not be directly involved in ferrous ion chelating activity.

5.2.3 Reducing power

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Ebrahimzadeh *et al.*, 2010). Increasing absorbance at 700 nm indicates an increase in reductive ability. The reducing powers of RD6 glutinous rice wine and RD6 GRFV were 74.60 and 95.37%. The abilities of RD6 glutinous rice grain and steamed rice extracts to reduce ferrous ions were not detectable. It was found that the reducing power of test samples increased with increasing concentrations. As previous studies by Rao *et al.* (2010) and Arab *et al.* (2011) also reported that reducing power of rice bran extracts increased with increasing concentration. Gandhimathi and Bai (2013) reported that the reducing power of an ethanolic extract of *Randia dumetorum* leaves increased with increasing dosage. He *et al.* (2013) found that reducing capacity increased with the increase of North China rice wine.

5.2.4 Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems that have been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochstein and Atallah, 1988). This radical has the ability to react with nucleotides in DNA and cause strand breakage which contributes to carcinogenesis and mutagenesis; it can also cause oxidative damage to DNA, lipids and proteins (Kappus, 1991). The IC_{50} values for RD6 glutinous rice grain, steamed rice, rice wine and RD6 GRFV were found to be 6.77, 18.33, 4.30 and 4.11%, respectively, whereas positive standard manitol showed IC_{50} value of 0.4656%. Previous studies by Hong *et al.* (2009) also reported that takju (Korean rice wine) evidenced strong scavenging activity against hydroxyl, superoxide anion, and NO radicals. Kanouchi *et al.* (2016) reported that the brewed rice vinegar Kurozu had

This material is reserved for educational use only, not allowed for commercial use.

free radical scavenging activity against superoxide and hydroxyl radicals.

5.2.5 Inhibition of anti-lipid peroxidation

This study examined the abilities of RD6 glutinous rice grain, steamed glutinous rice, glutinous rice wine and GRFV to inhibit lipid peroxidation induced by ferrous sulphate in egg-yolk homogenates. The IC_{50} values were 6.28, 3.20, 8.76 and 8.37%, for RD6 glutinous rice grain, steamed glutinous rice, rice wine and RD6 GRFV, respectively, while that of standard vitamin C was 0.0624%. These results are similar to those reported by Koguchi *et al.* (2010) in that they observed high antioxidative activity in various rice wines made from black rice. Teramoto *et al.* (2011) reported that rice wine made from uncooked black rice grains had greater inhibition of lipid peroxidation than rice wine made from cooked black rice grains.

5.2.6 DNA damage protection induced by the Fenton's reaction

DNA damage protective activity was measured by conversion of plasmid DNA between forms. When electrophoresed on an agarose gel, the faster-moving band represents the native form of supercoiled circular DNA and the slower-moving band corresponds to the open circular form (Russo *et al.*, 2001). When pUC19 DNA was incubated with Fenton's reagent and various concentrations of sample, the amount of open circular form was slightly decreased and supercoiled form increased in all treatments. However, RD6 GRFV showed an amount of the supercoiled form that was nearly equal to the untreated control. These results indicated that RD6 GRFV has greater protective effects than wine at the same concentration. Kong *et al.* (2015) reported that fermented rice extract inhibited DNA damage from reactive oxygen species to a certain extent, whereas unfermented red brown rice showed only a weak inhibitory effect; however, at high concentrations the fermented extract might induce reductive damage to DNA.

Several *in vitro* methods for measuring antioxidant activity of food, beverages, biological samples and vinegar have been developed. Antioxidant activity was assayed using various methods such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenger (Diplock, 1997). Use of at least two methods with different approaches and mechanisms is recommended for precise characterization (Sakanaka and Ishihara, 2008). The aqueous extract of raw materials (grain and steamed glutinous rice) wine and RD6 GRFV used in this experiment showed antioxidant activity in six different *in vitro* assays. Raw materials (grain and steamed glutinous rice) tested in this experiment showed antioxidant activities in DPPH scavenger, hydroxyl radical scavenger and anti-lipid peroxidation along with DNA damage

This material is reserved for educational use only, not allowed for commercial use.

protective ability *in vitro* assay systems and their antioxidant properties may not be directly involved in ferrous ion chelating activity and reducing power. Rice wine and RD6 GRFV showed antioxidant activity in almost all of the different *in vitro* assay systems except that ferrous ion chelating activity was not detected in wine and RD6 GRFV. Among them, DPPH is a free radical that has been widely used to test free radical scavenging ability of various samples and it deals with peroxy radicals, the most abundant radicals in biological systems (Frankel, 1991; Yen *et al.*, 2002). The results showed RD6 GRFV showed free radical-scavengers, particularly of the peroxy radicals, which are the major propagators of the oxidation chain of fat, thereby breaking the chain reaction. Lipid peroxidation is one of the main causes of food spoilage during storage or processing because it produces rancid and unpleasant flavors and decreases safety and nutritional value (Lin and Liang, 2002; Wang *et al.*, 2002). In egg yolk homogenate, RD6 GRFV effectively inhibited the formation of MDA in a dose-dependent manner. This result showed that RD6 GRFV may be as useful in food processing as food preservative, helping to prevent the formation of unpleasant flavors and to increase shelf life. The hydroxyl radical, an oxygen radical, is the most reactive and severely damages biomolecules, e.g. carbohydrates, proteins, lipids and nucleic acids, and this damage causes aging, cancer, and several diseases (Suematsu *et al.*, 1977). In this study, the scavenging effect against hydroxyl radicals generated from the Fenton reaction was investigated using the 2-deoxyribose oxidation method and plasmid DNA nicking. The obtained results from both methods showed high scavenging activity indicating that it could be protect DNA damage and prevent some diseases involved in free radicals.

Above results revealed that RD6 GRFV is not only a source of natural antioxidant but also valued for its positive health benefits i.e. prevention of several diseases involved free radicals. Because of a great variety of bioactive components such as phenolic compounds and antioxidant substances in raw materials, together with browning compounds and antioxidation capacity may occur during making wine (alcoholic) and vinegar (alcoholic and acetic fermentations). In addition, RD6 GRFV is responsible for anti-lipid peroxidation and has potential use as food preservatives to prevent rancidity.

5.3 Experiment 3. Analysis of RD6 GRFV vapor by GC-MS.

The volatile compounds in RD6 GRFV vapor were analyzed by GC-MS. The major volatile compounds (>3%) in RD6 GRFV vapor were found to be ethyl acetate (77.78%), propane (12.70%), pentanal (5.49%), and acetic acid (3.82%). Most compounds were previously reported

This material is reserved for educational use only, not allowed for commercial use.

in rice, rice wine and rice vinegar. Pentanal, hexanal, acetic acid and propane were previously reported in rice (Yau and Liu, 1999; Lin *et al.*, 2010; Givianrad, 2012; Mathure *et al.*, 2014). Lin *et al.* (2010) reported that the most abundant volatile compounds in rice were pentanal and hexanal. Acetic acid, ethyl acetate, butan-1-ol, and hexanal in this study were previously identified as components of rice wine (Chuenchomrat *et al.*, 2008; Sirisantimethakom *et al.*, 2008; Chen *et al.*, 2013; Jung *et al.*, 2013; Spinosa *et al.*, 2015). In our study, acetic acid and ethyl acetate have been associated with volatile components of rice vinegar, and were previously reported in rice vinegar (Kim *et al.*, 2011; Spinosa *et al.*, 2015; Xing *et al.*, 2015). The volatile compounds detected in GRFV vapor can originate from the raw material (Zhang *et al.*, 2006) or be synthesized during the fermentation process (Soufleros *et al.*, 2004). Ethyl acetate is the most common ester produced in URV during acetification of upland rice wine. Acetic acid is a main product obtained from the acetification process by bacteria, *Acetobacter aceti*. RD6 GRFV was produced from RD6 glutinous rice wine. Vinegar is the product made from the conversion of ethyl alcohol to acetic acid by *Acetobacter* during the acetic fermentation process. Some of the detected volatile compounds of RD6 GRFV have evidenced a marked antimicrobial activity. Antimicrobial volatiles included the organic compounds, acetic acid (Sholberg *et al.*, 2004; Hassenberg *et al.*, 2010), and the aldehyde hexanal (Patrignani *et al.*, 2008). It seems probable that acetic acid and/or hexanal is the antimicrobial agent which may also play a role in the preservation of postharvest quality.

5.4 Experiment 4. Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime and sweet basil

5.4.1 Use of ambient RD6 GRFV vapor to reduce postharvest decay of kaffir lime

5.4.1.1 Physical changes in treated kaffir lime

Freshly harvested kaffir lime leaves were exposed to the ambient vapor of RD6 GRFV (containing 8% acetic acid) and stored in darkness at 12 °C. Kaffir lime leaf were fumigated with RD6 GRFV containing higher acetic acid concentration than that of sweet basil leaf because kaffir lime leaves are thicker and harder than sweet basil so it is more resistant to acetic acid than sweet basil leaf. Postharvest properties investigated include: shelf life, weight loss, visual quality, antioxidant activity, and the levels of certain biochemical compounds.

(1) Changes in fresh weight

As shown in Figure 9, the fresh weight loss of leaves packed in polyethylene bags and stored in darkness at 12 °C was significantly reduced by 15 min (containing 0.0341 mmol L⁻¹ AA) of exposure to RD6 GRFV vapor; the reduced rate of fresh weight loss increases shelf life through improved retention of visual quality. In previous studies, this vapor has been shown to extend postharvest life of apricot fruits (Sholberg *et al.*, 2000; Song *et al.*, 2010; Thavong *et al.*, 2010), flowers (Foukaraki *et al.*, 2009; In *et al.*, 2016), and vegetables (Thakur and Pandey, 2004; Tzortzakis and Economakis, 2007). Moreover, low storage temperature is a cause of decreased weight loss in Chinese cabbage (Daly and Tomkins, 1998) and other fresh vegetables (Niamthong *et al.*, 2007). The results suggested that treatment with GRFV vapor may reduce transpiration rate. The reduced rate of weight loss is an important quality attribute and is probably related to enhance storability potential through improved retention of visual quality.

(2) Changes in leaf color during storage

Color change is one of the main attributes, along with texture, that characterizes the freshness of most vegetables (Rico *et al.*, 2007) and may also be considered an index for estimating the first visible symptoms of senescence in leafy vegetables (Ferrante *et al.*, 2004). Fresh kaffir lime leaves have a dark green color with glossy sheen and change color during storage to dark brown. In the present study, lightness (L*) values of kaffir lime treated with RD6 GRFV vapor for 5, 10, 15 and 20 min (containing 0.0113-0.0455 mmol L⁻¹ AA) decreased (increased darkness) during storage. Greenness (a*) values in all treatment groups decreased during storage. Previous studies also observed decreasing lightness (L*) and greenness (a*) values during storage of holy basil (Niamthong *et al.*, 2007). The L*, a* and b* values in treated leaves were not significantly different from those of the control. In kaffir lime, the treated samples present lower L* and b* values (increased darkness and decrease yellowish in color), while a* value was generally higher (decreased greenness) when compared to control. Decrease in lightness and greenness may due to leaf containing chlorophyll pigment which makes leaves green, their destruction leads to degreening and associated carotenoid.

(3) Evaluation of visual quality and shelf-life

Visual appearance scores of kaffir lime decreased steadily with increasing storage time. Leaves treated with RD6 GRFV vapor for 15 min (containing 0.0341 mmol L⁻¹ AA) and stored in darkness at 12 °C had visual quality scores higher than those of other treatments. This 15 min treatment also recorded the highest shelf life at the limit of marketability of 35.4 days, This material is reserved for educational use only, not allowed for commercial use.

while untreated leaves had a shelf-life of 25.2 days. These results confirmed the application of fermented vinegar vapor to extend postharvest life of kaffir lime, as has been successfully used for coriander leaves (Krusong *et al.*, 2015b), strawberry (Krusong *et al.*, 2015a), and stone fruit (Sholberg *et al.*, 2000). Exposure to the vapor of GRFV (contain 8% acetic acid) for 15 min can be used to highest markedly extend the postharvest shelf life of kaffir lime at the limit of marketability by greenness maintenance and greater retardation of water loss (keep freshness) which eventually led to markedly extend the its postharvest shelf life at the limit of edibility.

5.4.1.2 Biochemical analysis of kaffir lime postharvest quality

(1) Photosynthetic pigment content

Changes in chlorophyll level are familiar indicators of leaf senescence and occur in many leafy vegetables after harvest. Here, the decline of chlorophyll and carotenoid content in kaffir lime leaves during storage was greatly delayed by treatment with RD6 GRFV vapor. Results showed the lowest total chlorophyll content in the untreated control, while leaves treated with RD6 GRFV for 15 min (containing $0.0341 \text{ mmol L}^{-1} \text{ AA}$) significantly slowed the degradation of total chlorophyll. The effects of diverse treatments on chlorophyll loss retardation have previously been reported, such as the use of ethanol vapor treatment on broccoli (Fukasawa *et al.*, 2010) and Thai lime fruit (Opio *et al.*, 2015), or 1-methylcyclopropene on rocket leaves (Koukounaras *et al.*, 2006) and coriander (Hassan and Mahfouz, 2012). It is clear that RD6 GRFV treatment helps to maintain chlorophyll levels during storage of kaffir lime. These results indicated that carotenoid content continuously decreased in all samples throughout the storage period. Similarly, carotenoid decomposition during storage was described in apple (Sharma and Sethi, 2000) and loquat fruits (Chen *et al.*, 2015).

(2) Membrane leakage

During senescence, vegetal tissues usually undergo deterioration and damage. One of the main symptoms is the loss of plasma membrane integrity, which leads to loss of cellular compartmentalization and the release of many solutes from the cytosol (Lemoine *et al.*, 2010). Measurement of electrolyte leakage is a good parameter to evaluate plasma membrane integrity. Membrane leakage from leaf discs floating on deionized water increased with floating time and storage time. Leaves treated with ambient RD6 GRFV vapor for 5-10 min (containing $0.0113\text{-}0.0227 \text{ mmol L}^{-1} \text{ AA}$) had increased membrane leakage, and conductivity values were higher after 7 days of storage than those of controls. However, for leaves treated with RD6 GRFV vapor for 15-20 min (containing $0.0341\text{-}0.0455 \text{ mmol L}^{-1} \text{ AA}$), leakage was lower over the entire storage

This material is reserved for educational use only, not allowed for commercial use.

period than in other treatments, indicating that higher membrane integrity was maintained. According to previous research, treatments may prevent and reduce electrolyte loss from cytosol, such as hot air treatment of broccoli (Lemoine *et al.*, 2009), tomato (Saltveit, 2005), strawberry (Vicente *et al.*, 2006), Bok Choy (Lu, 2007) and cotton (Kawakami *et al.*, 2007). It could be indicated that membrane leakage increases postharvest in many vegetable during senescence. Here, kaffir lime treated with vapor for 15-20 min showed significantly delayed rises in membrane leakage, indicating suppression of senescence likely through maintenance of membrane integrity.

(3) Lipid peroxidation (determination of malondialdehyde content)

Increases in membrane permeability have previously been reported to parallel increases in lipid peroxidation (Dan *et al.*, 1996; Jaleel *et al.*, 2007). Malondialdehyde (MDA) is an end product of lipid peroxidation and has been used as a direct indicator of membrane injury (Yuan *et al.*, 2010). It has been reported that membrane permeability and the level of MDA content both exhibited a pattern of increase during fruit and vegetable senescence (Yuan *et al.*, 2010, Dhindsa *et al.*, 1981). Kaffir lime leaves treated with RD6 GRFV vapor for 5-20 min (containing 0.0113-0.0455 mmol L⁻¹ AA) induced slightly increased MDA content, though values were lower than in the controls. In leaves treated with RD6 GRFV vapor, the lipid peroxidation was low (Figure 4.14) and was controlled by a complex array of antioxidant components within cells. Another postharvest treatment with ascorbic acid increased postharvest life and reduced lipid peroxidation of broccoli (Balouchi *et al.*, 2012). The RD6 GRFV vapor may have helped in maintenance of the membranous structure of cell organelles.

(4) DPPH radical scavenging activity

Senescence is associated with a defense system which includes antioxidant enzymes and antioxidants that scavenge reactive oxygen species (ROS) (Hounsome *et al.*, 2009). If production of ROS exceeds a cell's antioxidant defense capabilities, the result is cellular damage (senescence) and a reduction in the level of antioxidant compounds (Lemoine *et al.*, 2010). Radical scavenging capacity of the sample was tested using the stable free radical DPPH and measured in terms of hydrogen-donating or radical-scavenging ability (Prior *et al.*, 2005). A similar pattern of DPPH radical scavenging ability was detected in control and treated samples, which all exhibited decrease during storage, but the rates of decrease were significantly lower in treated leaves. Kaffir lime leaves treated for 15 min (containing 0.0341 mmol L⁻¹ AA) showed the highest DPPH radical scavenging activity, while the untreated control showed the lowest DPPH radical scavenging activity. Hence, RD6 GRFV treatment usefully mitigated postharvest

This material is reserved for educational use only, not allowed for commercial use.

antioxidant losses. Sun *et al.* (2012) also showed that 1-MCP treatment is a good practice for extending shelf life and reducing the loss of health-promoting compounds, particularly for antioxidants of Chinese kale and broccoli florets (Yuan *et al.*, 2010). Maximization of antioxidant potential is important not only to protect the tissues against ROS and thus delay senescence, but also to preserve the food nutritional quality. Here, RD6 GRFV treatment of kaffir lime maximizes the level of antioxidant capacity.

5.4.2 Use of ambient RD6 GRFV vapor to reduce postharvest decay of sweet basil

5.4.2.1 Physical changes in treated sweet basil

(1) Changes in fresh weight

Water loss is a common factor adversely affecting the postharvest shelf life of most fresh produce, including fruits (Nunes and Emond, 2007b; Fattahi *et al.*, 2010), flowers (Seglie *et al.*, 2011; Mangave *et al.*, 2013), and vegetables (Nunes and Emond, 2007a; Berry *et al.*, 2010). Results of the present study indicated that fresh weight loss during storage is reduced by 10 min of exposure to RD6 GRFV vapor; the reduced rate increases shelf life through improved retention of visual quality. Leaves treated with RD6 GRFV for 10 min (containing 0.0113 mmol L⁻¹ AA) had significantly reduced weight loss compared with controls, and showed the highest fresh weight of shoots. Abd Elwahab and Rashid (2013) previously showed that treatment with acetic acid vapor reduced the extent of decay and maintained quality of table grapes. In addition, hot water treatments delayed ripening rate by reducing fruit softening and weight loss (Mama *et al.*, 2016).

(2) Changes in leaf color during storage

L* and b* values of sweet basil treated with RD6 GRFV vapor for 2, 4 and 6 min tended to decrease (increased darkness) during storage. Values in leaves treated for 8 or 10 min were significantly different from the control. The treated samples for 8 or 10 min presented higher L* and b* values carotenoid was relatively stable during the storage. Carotenoids are pigment responsible for the yellow color of leaf and *protect chlorophyll* molecules from photo-oxidation and other components from oxidative damage. The while a* values of both treatment were generally lower when compared to control indicated indicating a retardation of chlorophyll degradation. In a previous study, postharvest treatment helped delay or slow color changes in broccoli (Fukasawa *et al.*, 2010), sweet cherries (Bai *et al.*, 2011), table grapes (Candir *et al.*, 2012), tomatoes (Khairi *et al.*, 2015), and cucumbers (Omoba and Onyekwere, 2016).

(3) Evaluation of visual quality and shelf-life

Freshly harvested sweet basil leaves were exposed to the ambient vapor of RD6 GRFV (diluted to contain 4% acetic acid). As shown in Figure 19, the shelf life of shoots packed in polyethylene bags and stored in darkness at 12 °C was significantly increased by vapor treatment. Sweet basil leaves were considered to have reached the limit of marketability when they showed black stains on 30% of the leaf area, noticeable loss of turgidity and possible slight loss of green color. Edibility was limited by development of black stains 30-50% of the leaf area, severe loss of turgidity and wilting of leaf blades. As shown in Figure 19 and Table 5, leaves treated with ambient GRFV vapor for 10 min containing $0.011 \pm 0.0001 \text{ mmol L}^{-1}$ AA recorded the highest shelf life of 25.4 days at the limit of marketability and 28.0 days at the limit of edibility. In previous studies, repeated treatments with ethanol vapor increased the shelf life of oriental sweet melons (Jin *et al.*, 2013). In previous studies, this vapor has been shown to extend postharvest life of strawberry (Krusong *et al.*, 2015a) and of coriander leaves (Krusong *et al.*, 2015b).

5.4.2.2 Biochemical analysis of sweet basil postharvest quality

(1) Photosynthetic pigment content

Leaf content of chlorophyll a is known to continuously decrease as the storage period increases. Here, the decline of chlorophyll in sweet basil leaves during storage was greatly delayed by treatment with RD6 GRFV vapor. The results of this study show untreated controls have the lowest total chlorophyll content, while treatment with RD6 GRFV for 8 or 10 min (containing 0.0088-0.0113 mmol L^{-1} AA) significantly slows the degradation of chlorophyll a. Chlorophyll b content showed decreased loss similar to that of chlorophyll a, while the carotenoid content of leaves in all treatments did not differ from the untreated control. Total chlorophyll content decreased slightly in all treatments after storage. Rapid decreases in total chlorophyll were observed in the untreated control as well as in leaves treated for 2, 4 or 6 min. Clearly, treatment with RD6 GRFV vapor for 8 or 10 min significantly slows the degradation of both chlorophyll a and b, and thus of total chlorophyll. A vapor treatment effect on chlorophyll loss has previously been reported in rocket (Koukounaras *et al.*, 2006), spinach leaves (Grozeff *et al.*, 2010), coriander (Hassan and Mahfouz, 2012), and Chinese kale (Sun *et al.*, 2012). The use of low intensity light pulses has also been shown to reduce chlorophyll loss and delay postharvest senescence of *Ocimum basilicum* leaves (Costa *et al.*, 2013). It is clear that RD6 GRFV treatment helps to maintain chlorophyll levels during storage in sweet basil. Carotenoid content in all

This material is reserved for educational use only, not allowed for commercial use.

GRFV vapor treatments as well as untreated leaf tended to increase as the storage time increased. The total carotenoids content was not affected by GRFV vapor for sweet basil refrigerated for 25 days but tended to increase as the storage time increased. Salunkhe *et al.* (1991) states that carotenoid degradation during storage is low for intact living tissues and that in fact postharvest carotene production can occur in some products. Howard *et al.* (1999) studied changes in β -carotene on a wet weight basis of broccoli, carrots, and green beans during refrigerated storage for two consecutive harvest years. They reported a 10% increase in β -carotene in both years for carrots refrigerated for 14 days. Sudhakar and Maini (1994) studied the stability of carotenoids in mango pulp with various additives and found that ascorbic acid and antioxidants help protect the carotenoids from degradation.

(2) Membrane leakage

Electrical conductivity of a bathing medium is used as an indicator of electrolyte leakage (increased permeability of cell membranes). Conductivities were measured after 1 h, 2 h and 3 h of floating on deionized water of sweet basil leaves exposed for different times to the RD6 GRFV vapor and subsequently stored at 12 °C. Membrane leakage increased with floating time and storage time. Leaves treated RD6 GRFV vapor for 2-8 min (containing 0.0022-0.0088 mmol L⁻¹ AA) exhibited slightly increased membrane leakage. However, leaves treated for 10 min (containing 0.0113 mmol L⁻¹ AA) had slightly lower leakage than in the other treatments over the entire storage period, and was significantly different from the control. Previous studies showed that 1-MCP treated leaves in spinach (Grozoff *et al.*, 2010) and sponge gourd treated with salicylic acid (Cong *et al.*, 2017) had lower solute leakage after storage. Together, these results suggest that RD6 GRFV vapor could decrease damage to cell membranes and therefore preserve the cell membrane integrity of sweet basil.

(3) Lipid peroxidation (determination of malondialdehyde content)

Malondialdehyde content, produced from the peroxidation of membrane lipids, is often used as an indicator of oxidative stress and damage (Ohkawa *et al.*, 1979). The control and RD6 GRFV vapor treated samples all showed increasing lipid peroxidation with storage time, as measured by their increasing MDA contents. Here, high lipid peroxidation was accompanied by high membrane leakage and shortened shelf lives in untreated leaves and in leaves treated for 2, 4 or 6 min. Increases in membrane permeability have previously been reported to parallel increases in lipid peroxidation (Dan *et al.*, 1996). However, compared with the controls, the increase in leaf MDA content was reduced by the 10 min GRFV treatment (containing 0.011 mmol L⁻¹ AA). In

the GRFV vapor-treated leaves, the lipid peroxidation was low and was controlled by a complex array of antioxidant components within cells. The 10 min GRFV vapor may have helped the maintenance of the membrane structure of cell organelles.

(4) DPPH radical scavenging activity

Antioxidant activity in treated and untreated leaves was determined using a DPPH scavenging assay. Here, antioxidant activity is reported as the IC_{50} value, where a low value indicates high antioxidant activity. A similar decrease in DPPH radical scavenging ability during storage was detected in both control and treated samples; however, the rates of decrease were significantly lower in vapor treated leaves. Hence, GRFV treatment usefully mitigated postharvest antioxidant losses. Maximization of antioxidant potential is important not only to protect the tissues against ROS and thus delay senescence, but also to preserve the food nutritional quality. Here, RD6 GRFV vapor treatment of sweet basil maximizes the antioxidant capacity. The DPPH radical scavenging ability of sweet basil treated for 10 min was significantly higher than that of the control. Previous studies showed that repeated treatment affects the antioxidant potential of tomatoes (Soto-Zamora *et al.*, 2005; Toor and Savage, 2006; Yahia *et al.*, 2007), strawberries (Erkan *et al.*, 2008), apples (Lu *et al.*, 2012), coriander (Hassan and Mahfouz, 2012), and peach fruit (Liu *et al.*, 2015). Many reports have indicated the value of fermented vinegar for preserving fresh produce from microbial spoilage after harvest (Sholberg *et al.*, 2000; Tzortzakis, 2010; Krusong *et al.*, 2015a). This is the first report of the effects of RD6 GRFV vapor on the postharvest physiology of kaffir lime and sweet basil, specifically slowing fresh weight loss and chlorophyll degradation and helping to maintain antioxidant capacity.

The vapor-phase RD6 GRFV contained organic acid, ester, aldehyde, and alcohol volatile compounds, of which ethyl acetate was the most abundant. The content of these volatile compounds may also play a role in the preservation of postharvest quality of kaffir lime and sweet basil. Similarly, treatments with acetic acid vapors reduced decay of apples (Sholberg *et al.*, 2001) and table grapes (Venditti *et al.*, 2012) during storage. In the RD6 GRFV vapor-treated leaves, could be used to markedly extend the postharvest storage life of kaffir lime and sweet basil by helping to reduce rate of weight loss and may have helped the maintenance of the membrane structure of cell organelles and reduced membrane components damage. In addition, RD6 GRFV treatment usefully mitigated postharvest antioxidant losses, which may protect tissue against oxidative damage. This vapor contained these volatile compounds helped reduce of postharvest losses and quality deterioration including extended the postharvest life of kaffir lime and sweet basil.

This material is reserved for educational use only, not allowed for commercial use.

CHAPTER 6

CONCLUSION AND SUGGESTION

6.1 Conclusion

The results of this work suggest that raw material (RD6 glutinous rice grain and steamed glutinous rice RD6), RD6 glutinous rice wine and RD6 GRFV have found to contain of total phenols and browning compound, which may have been increased during the fermentation process.

The antioxidant and DNA damage protective activity of RD6 glutinous rice grain, steamed RD6 glutinous rice, RD6 glutinous rice wine and RD6 GRFV were evaluated using the DPPH radical scavenging assay, metal chelating activity, reducing power ability, hydroxyl radical scavenging activities, inhibition of anti-lipid peroxidation and DNA damage protection induced by Fenton's reaction. The antioxidant activity was found that raw material (RD6 glutinous rice grain and steamed glutinous rice RD6), RD6 glutinous rice wine and RD6 GRFV has the DPPH scavenging activity, hydroxyl radical scavenging activities including inhibition of anti-lipid peroxidation and found that all the tested samples did not eliminate radicals metal chelating. The reducing powers was found that RD6 glutinous rice wine and RD6 GRFV but the abilities of RD6 glutinous rice grain and steamed rice extracts to reduce ferrous ions were not detectable. In addition, all tested sample was effectiveness in preventive DNA damage protection.

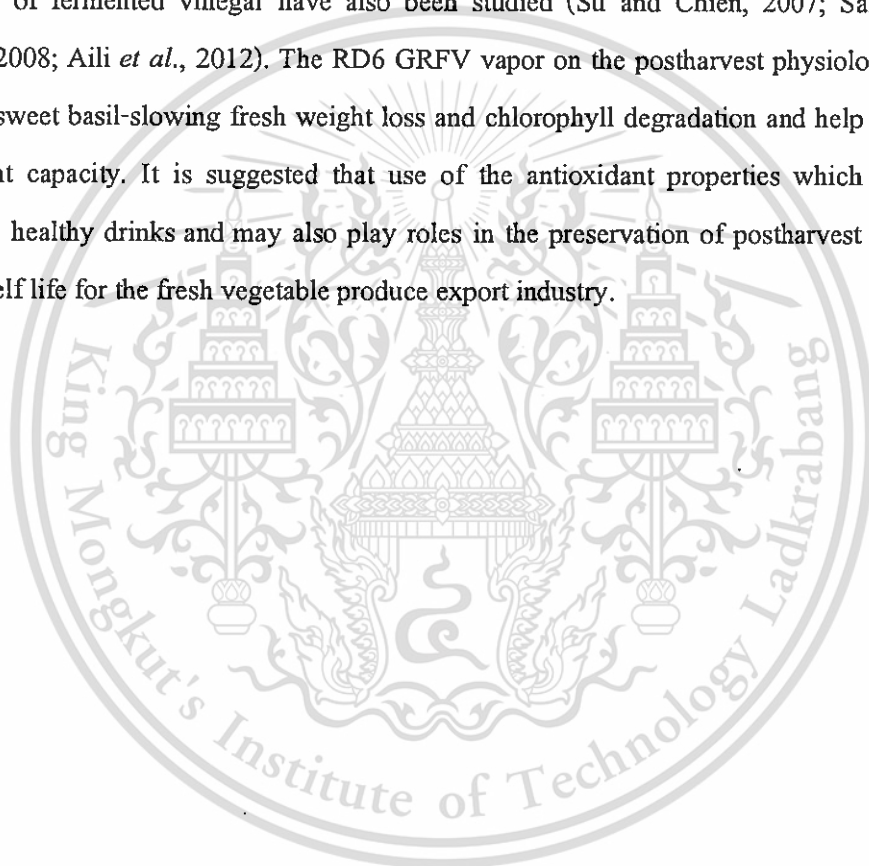
The GC-MS results found that there were a number of other significant volatiles in URFV vapor, including ethyl acetate, propane, pentanal and acetic acid.

The exposure to the vapor of RD6 GRFV (contain 8% acetic acid) for 15 min can be used to markedly extend the postharvest storage life of kaffir lime by helping to maintain freshness (reduced water loss), membrane leakage (maintain membrane integrity), lipid peroxidation (reduced membrane components damage) and better retention of antioxidants (reduced senescence, better retention of nutritional value). Meanwhile, greenness and color values were unchanged by RD6 GRFV but they may have slightly helped in change of leaf color. The effect of RD6 GRFV (contain 4% acetic acid) for 10 min can be used to markedly extend the postharvest storage life of sweet basil by helping to maintain greenness (higher chlorophyll content) and freshness (reduced water loss) and better retention of antioxidants (reduced senescence, better

retention of nutritional value). Meanwhile, membrane leakage and lipid peroxidation were unchanged by RD6 GRFV but they may have slightly helped in maintaining membrane integrity.

6.2 Suggestion

In this work, antioxidant properties that either from the raw material from which the vinegar is produced (in this case, RD6 glutinous rice) or from the conditions under which the fermentation process is carried out, affect the levels of antioxidant substances which may also play roles in the preservation of antimicrobial properties and postharvest quality. The antioxidant properties of fermented vinegar have also been studied (Su and Chien, 2007; Sakanaka and Ishihara, 2008; Aili *et al.*, 2012). The RD6 GRFV vapor on the postharvest physiology of kaffir lime and sweet basil-slowing fresh weight loss and chlorophyll degradation and help to maintain antioxidant capacity. It is suggested that use of the antioxidant properties which to develop affordable healthy drinks and may also play roles in the preservation of postharvest quality and extend shelf life for the fresh vegetable produce export industry.



REFERENCES

- Abd Elwahab, S.M. and Rashid, I.A.S. 2013. "Effect of Acetic Acid Fumigation and Waxing on Decay, Storability and Marketability of Mandarin Fruits." **Journal of Applied Sciences Research** 9(3) : 2146-2155.
- Açar, O.C., Gökmen, V., Pellegrini, N. and Fogliano, V. 2009. "Direct Evaluation of the Total Antioxidant Capacity of Raw and Roasted Pulses, Nuts and Seeds." **European Food Research and Technology** 229(6) : 961-996.
- Ageeva, N.M., Markosov, V.A., Muzychenko, G.F., Bessonov, V.V. and Khanferyan, R.A. 2015. "Antioxidant and Antiradical Properties of Red Grape Wines." **Voprosy Pitaniia** 84(2) : 63-67.
- Aghdam, M.S., Motallebiazar, A., Mostofi, Y., Moghaddam, J.F. and Ghasemnezhad, M. 2011. "Methyl Salicylate Affects the Quality of Hayward Kiwifruits during Storage at Low Temperature." **Journal of Agricultural Science** 3(2) : 149-156.
- Aili, W., Huanlu, S., Changzhong, R. and Zaigui, L. 2012. "Key Aroma Compounds in Shanxi Aged Tartary Buckwheat Vinegar and Changes during Its Thermal Processing." **Flavour and Fragrance Journal** 27(1) : 47-53.
- Anderson, R.J., Bower, J.P. and Bertling, I. 2011. "Effect of Light and Packaging on Shelf-Life of Fresh-Cut Sweet Basil (*Ocimum basilicum*) and Rosemary (*Rosmarinus officinalis*)." **Acta Horticulturae** 911(67) : 573-578.
- Arab, F., Alemzadeh, I. and Maghsoudi, V. 2011. "Determination of Antioxidant Component and Activity of Rice Bran Extract." **Scientia Iranica** 18(6) : 1402-1406.
- Asoda, T., Terai, H., Kato, M. and Suzuki, Y. 2009. "Effects of Postharvest Ethanol Vapor Treatment on Ethylene Responsiveness in Broccoli." **Postharvest Biology and Technology** 52 : 216-220.
- Bai, J., Plotto, A., Spotts, R. and Rattanapanone, N. 2011. "Ethanol Vapor and Saprophytic Yeast Treatments Reduce Decay and Maintain Quality of Intact and Fresh-Cut Sweet Cherries." **Postharvest Biology and Technology** 62(2) : 204-212.
- Bakir, S., Toydemir, G., Boyacioglu, D., Beekwilder, J. and Capanoglu, E. 2016. "Fruit Antioxidants during Vinegar Processing: Changes in Content and *In Vitro* Bio-Accessibility." **International Journal of Molecular Sciences** 17(10) : 1-12.

- Balouchi, Z., Peyvast, G.A., Ghasemnezhad, M. and Dadi, M. 2012. "Effects of Ascorbic Acid in Delaying Florets Senescence of Broccoli during Post-Harvest Storage." **South Western Journal of Horticulture Biology and Environment** 3(2) : 167-183.
- Barbosa, C.K.R., Fonseca, M.C.M., Silva, T.P., Finger, F.L., Casali, V.W.D. and Cecon, P.R. 2016. "Effect of Hydrocooling Packaging and Cold Storage on the Postharvest Quality of Peppermint (*Mentha piperita* L.)." **Revista Brasileira de Plantas Mediciniais** 18(1) : 248-255.
- Belović, M., Kevrešan, Ž., Pestorić, M. and Mastilović, J. 2015. "The Influence of Hot Air Treatment and UV Irradiation on the Quality of Two Tomato Varieties after Storage." **Food Packaging and Shelf Life** 5 : 63-67.
- Berry, A.D., Sargent, S.A. and Huber, D.J. 2010. "Effect of Postharvest Application of 1-MCP on Basil Shoot Quality during Storage at Chilling Temperature Respiration." **Florida State Horticultural Society** 123 : 264-268.
- Brenneisen, P., Steinbrenner, H. and Sies, H. 2005. "Selenium, Oxidative Stress, and Health Aspects." **Molecular Aspects of Medicine** 26 : 256-267.
- Candir, E., Ozdemir, A.E., Kamiloglu, O., Soyulu, E.M., Dilbaz, R. and Ustun, D. 2012. "Modified Atmosphere Packaging and Ethanol Vapor to Control Decay of 'Red Globe' Table Grapes during Storage." **Postharvest Biology and Technology** 63(1) : 98-106.
- Cantin, C.M., Holcroft, D. and Crisosto, C.H. 2011. "Postharvest Application of 1-Methylcyclopropene (1-MCP) Extends Shelf Life of Kiwifruit." **Acta Horticulturae** 913 : 621-626.
- Cantwell, M.I. and Reid, M.S. 2002. "Postharvest Handling Systems." **Fresh Herbs** In A.A. Kader, eds. *Postharvest Technology of Horticultural Crops*. 3rd ed. University of California Agricultural and National Resource Publ., California. 327-331.
- Chen, S., Xu, Y. and Qian, M.C. 2013. "Aroma Characterization of Chinese Rice Wine by Gas Chromatography-Olfactometry, Chemical Quantitative Analysis, and Aroma Reconstitution." **Journal of Agricultural and Food Chemistry** 61(47) : 11295-11302.
- Chen, Y., Lin, S., Xu, L. and Wu, J. 2015. "Effects of Postharvest Storage Temperatures on Carotenoid Content of Loquat Fruits." **Acta Horticulturae** 1092 : 159-165.
- Chuenchomrat, P., Assavanig, A. and Lertsiri, S. 2008. "Volatile Flavor Compounds Analysis of Solid State Fermented Thai Rice Wine (Ou)." **ScienceAsia** 34 : 199-206.

- Comis, D. 2002. **Spray Weeds with Vinegar? USDA- ARS news and Information.** [Online]. Available : <http://www.ars.usda.gov/is/pr/2002/020515.htm>.
- Cong, H., Jin-hua, Z., Qing, W., Hai-zhou, D. and Li-pu, G. 2017. "Salicylic Acid Alleviates Postharvest Chilling Injury of Sponge Gourd (*Luffa cylindrica*)." **Journal of Integrative Agriculture** 16(03) : 735-741.
- Costa, L., Montano, Y.M., Carrión, C., Rolny, N. and Guiameta, J.J. 2013. Application of Low Intensity Light Pulses to Delay Postharvest Senescence of *Ocimum basilicum* Leaves. **Postharvest Biology and Technology** 86 : 181-191.
- Crisco Company. 2005. **1 Strawberry Lane Orrville, Ohio 44667.** [Online]. Available : http://www.crisco.com/basics/all_about/vinegar.asp.
- D'Angelo, M.A., Raices, M., Panowski, S.H. and Hetzer, M.W. 2009. "Age-Dependent Deterioration of Nuclear Pore Complexes Causes a Loss of Nuclear Integrity in Postmitotic Cells." **Cell** 136 : 284-295.
- Daly, P. and Tomkins, B. 1998. "Developing a Sustainable Asian Vegetable Industry in East Gippsland." Final Report to the **Rural Industries Research and Development Corporation Project DAV-70a.**
- Dan, K., Nagata, M. and Yamashita, I. 1996. "Changes in Lipid Peroxidation and Antioxidants Content in Cotyledons of Japanese Radish." **Journal of the Japanese Society for Horticultural Science** 65(3) : 603-608.
- D'Aquino, S., Mistriotis, A., Briassoulis, D., Di Lorenzo, M.L., Malinconico, M. and Palma, A. 2016. "Influence of Modified Atmosphere Packaging on Postharvest Quality of Cherry Tomatoes Held at 20°C." **Postharvest Biology and Technology** 115 : 103-112.
- Dehpour, A.A., Ebrahimzadeh, M.A., Nabavi, S.F. and Nabavi, S.M. 2009. "Antioxidant Activity of Methanol Extract of *Ferula assafoetida* and Its Essential Oil Composition." **Grasas Aceites** 60(4) : 405-412.
- Dhindsa, R.S., Dhindsa, P.P. and Thorpe, T.A. 1981. "Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide." **Experimental Botany** 32(126) : 93-101.

- Dinis, T.C.P., Madeira, V.M.C. and Almeida, M.L.M. 1994. "Action of Phenolic Derivates (Acetoaminophen, Salicylate and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers." **Archives of Biochemistry and Biophysics** 315 : 161-169.
- Diplock, A.T. 1997. "Will the Good Fairies Please Prove us that Vitamin E Lessens Human Degenerative Disease?." **Free Radical Research** 27 : 511-532.
- Ebrahimzadeh, M.A., Nabavi, S.F., Nabavi, S.M. and Eslami, B. 2010. "Antihypoxic and Antioxidant Activity of *Hibiscus esculentus* Seeds." **Grasas Aceites** 61 : 30-36.
- Ebrahimzadeh, M.A., Pourmorad, F. and Bekhradnia, A.R. 2008. "Iron Chelating Activity, Phenol and Flavonoid Content of Some Medicinal Plants from Iran. **African Journal of Biotechnology** 7(18) : 3188-3192.
- Elizabeth, K. and Rao, M.N.A. 1990. "Oxygen Radical Scavenging Activity of Curcumin." **International Journal of Pharmaceutical Compounding** 58 : 237-240.
- Ella, L., Zion, A., Nehemia, A. and Ammon, L. 2003. "Effect of the Ethylene Action Inhibitor 1-Methylcyclopropene on Parsley Leaf Senescence and Ethylene Biosynthesis." **Postharvest Biology and Technology** 30 : 67-74.
- Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y. and Ohta, M. 1998. "Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including *Escherichia Coli* O157:H7." **Journal of Food Protection** 61(8) : 953-959.
- Erkan, M., Wang, S.Y. and Wang, C.Y. 2008. Effect of UV Treatment on Antioxidant Capacity, Antioxidant Enzyme Activity and Decay in Strawberry Fruit. **Postharvest Biology and Technology** 48(2) : 163-171.
- Fagundes, C., Moraes, K., Pérez-Gago, M.B., Palou, L., Maraschin, M. and Monteiro, A.R. 2015. Effect of Active Modified Atmosphere and Cold Storage on the Postharvest Quality of Cherry Tomatoes. **Postharvest Biology and Technology** 109 : 73-81.
- Farahnaky, A. and Afshari-Jouybari, H. 2011. Physicochemical Changes in Mazafati Date Fruits Incubated in Hot Acetic Acid for Accelerated Ripening to Prevent Diseases and Decay. **Scientia Horticulturae** 127(3) : 313-317.
- Fattahi, J., Fifaii, R. and Babri, M. 2010. Postharvest Quality of Kiwifruit (*Actinidia deliciosa* cv. Hayward) Affected by Pre-Storage Application of Salicylic Acid. **South-Western Journal of Horticulture, Biology and Environment** 1(2) : 175-186.

- Feng, B., Chen, Y. and Zhang, X.D. 2002. "Effect of Water Vapor Treatment on Apatite Formation on Precalcified Titanium and Bond Strength of Coatings to Substrates." **Journal of Biomedical Materials Research** 59(1) : 12-17.
- Ferrante, A., Incrocci, L., Maggini, R., Serra, G. and Tognoni, F. 2004. "Color Changes of Fresh-Cut Leafy Vegetables during Storage." **Journal of Food, Agriculture and Environment** 2(3&4) : 40-44.
- Fortin, H., Vigora, C., Lohezic-Le, F., Robina, V., Le Bosse, B., Boustiea, J. and Arnoros, M. 2002. "In Vitro Antiviral Activity of Thirty-Six Plants from La Reunion Island." **Fitoterapia** 3 : 346-350.
- Foukaraki, S.G., Terry, L.A., Pompodakis, N.E., Papadimitriou, M.D. and Lydakakis, D.E. 2009. "Effect of Methyl Jasmonate Vapor Treatment and Sucrose Solutions on Vase Life and Non-Structural Carbohydrate Concentration in Petals of Cut 'First Red' Roses." **Acta Horticulturae** 847 : 179-184.
- Frankel, E.N. 1991. "Recent Advances in Lipid Oxidation." **Journal of the Science of Food and Agriculture** 54 : 495-511.
- Fратиани, F., Cefola, M., Pace, B., Cozzolino, R., Giulio, B.D., Cozzolino, A., d'Acierno, A., Coppola, R., Logrieco, A.F. and Nazzaro, F. 2017. "Changes in Visual Quality, Physiological and Biochemical Parameters Assessed during the Postharvest Storage at Chilling or Non-Chilling Temperatures of Three Sweet Basil (*Ocimum basilicum* L.) Cultivars." **Food Chemistry** 229(15) : 752-760.
- Fukasawa, A., Suzuki, Y., Terai, H. and Yamauchi, N. 2010. "Effects of Postharvest Ethanol Vapor Treatment on Activities and Gene Expression of Chlorophyll Catabolic Enzymes in Broccoli Florets." **Postharvest Biology and Technology** 55 : 97-102.
- Gandhimathi, S. and Bai, G.V.S. 2013. "In Vitro Antioxidant Activity of *Randia Dumetorum* Lam Leaf Extract." **International Journal of Herbal Medicine** 1(4) : 107-111.
- Gharezi, M., Joshi, N. and Sadeghian, E. 2012. "Effect of Post Harvest Treatment on Stored Cherry Tomatoes." **Journal of Nutrition and Food Science** 2(8) : 1-10.
- Givianrad, M.H. 2012. "Characterization and Assessment of Flavor Compounds and Some Allergens in Three Iranian Rice Cultivars during Gelatinization Process by HS-SPME/GC-MS." **Journal of Chemistry** 9(2) : 716-728.

- Goto, S., Kogure, K., Abe, K., Kimata, Y., Kitahama, K., Yamashita, E. and Terada, H. 2001. "Efficient Radical Trapping at the Surface and Inside the Phospholipid Membrane is Responsible for Highly Potent Antiperoxidative Activity of the Carotenoid Astaxanthin." **Biochimica et Biophysica Acta-Biomembranes** 1512(2) : 251-258.
- Goulson, M.J. and Warthesen, J.J. 1999. "Stability and Antioxidant Activity of Beta Carotene in Conventional and High Oleic Canola Oil." **Journal of Food Science** 64(6) : 996-999.
- Grozeff, G., Micieli, M., Gomeza, F., Fernandez, L., Guimeta, J., Chavesb, A. and Bar-toli, C., 2010. "1-Methyl Cyclopropene Extends Postharvest Life of Spinach Leaves." **Postharvest Biology and Technology** 55 : 182-185.
- Halliwell, B. 1991. "Reactive Oxygen Species in Living Systems: Source, Biochemistry, and Role in Human Disease." **The American Journal of Medicine** 91 : 14-22.
- Halliwell, B. 1999. "Antioxidant Defence Mechanisms: from the Beginning to the End (of the beginning)." **Free Radical Research** 31 : 261-272.
- Halliwell, B. and Gutteridge, J.M.C. 1981. "Formation of Thiobarbituric Acid Reactive Substances from Deoxyribose in the Presence of Iron Salts: The Role of Superoxide and Hydroxyl Radicals." **FEBS Letters** 128 : 347-352.
- Halliwell, B., Gutteridge, J.M.C. and Aruoma, O.I. 1987. "The Deoxyribose Method: A Simple "Test-Tube" Assay for Determination of Rate Constants for Reactions of Hydroxyl Radicals." **Analytical Biochemistry** 165 : 215-219.
- Hansakul, P., Srisawat, U., Itharat, A. and Lerdvuthisophon, N. 2011. "Phenolic and Flavonoid Contents of Thai Rice Extracts and their Correlation with Antioxidant Activities using Chemical and Cell Assays. **Journal of the Medical Association of Thailand** 94(7) : 122-130.
- Hassan, F.A.S. and Mahfouz, S.A. 2010. "Effect of 1-Methylcyclopropene (1-MCP) Treatment on Sweet Basil Leaf Senescence and Ethylene Production during Shelf-Life." **Postharvest Biology and Technology** 55 : 61-65.
- Hassan, F.A.S. and Mahfouz, S.A. 2012. "Effect of 1-Methylcyclopropene (1-MCP) on the Postharvest Senescence of Coriander Leaves during Storage and Its Relation to Antioxidant Enzyme Activity." **Scientia Horticulturae** 141 : 69-75.

- Hassenberg, K., Geyer, M. and Herplich, W.B. 2010. "Effect of Acetic Acid Vapor on the Natural Microflora and *Botrytis cinerea* of Strawberries." **European Journal of Horticultural Science** 75(4) : 141-146.
- He, S., Mao, X., Liu, P., Lin, H., Du, Z., Lv, N., Han, J. and Qiu, C. 2013. "Research into the Functional Components and Antioxidant Activities of North China Rice Wine (Ji Mo Lao Jiu)." **Food Science and Nutrition** 1(4) : 307-314.
- Heath, R.L. and Packer, L. 1968. "Photooxidation in Isolated Chloroplast. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation." **Archives of Biochemistry and Biophysics** 125 : 189-198.
- Hochstein, P. and Atallah, A.S. 1988 . "The Nature of Oxidants and Antioxidant Systems in the Inhibition of Mutation and Cancer." **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis** 202(2) : 363-375.
- Hong, Y.H., Bae, S.H., Jung, E.Y., Son, H.S., Shin, K.S., Kwon, K.H. and Suh, H.J. 2009. "Radical Scavenging Activities of Korean Traditional Rice Wine, Takju." **Journal of Food Science and Nutrition** 14(2) : 109-115.
- Hou, W.C., Lee, M.H., Chen, H.J., Liang, W.L., Han, C.H., Liu, Y.W. and Lin, Y.H. 2001. "Antioxidant Activities of Dioscorin, the Storage Protein of Yam (*Dioscorea batatas* Decne)." **Journal of Agricultural and Food Chemistry** 49 : 4956-4960.
- Hounsome, N., Hounsome, B., Tomos, D. and Edwards-Jones, G. 2009. "Changes in Antioxidant Compounds in White Cabbage during Winter Storage." **Postharvest Biology and Technology** 52 : 173-179.
- Howard, L.A., Wong, A.D., Perry, A.K. and Klein, B.P. 1999 "β-Carotene and Ascorbic Acid Retention in Fresh and Processed Vegetables." **Journal of Food Science** 64 : 929-936.
- In, B.C., Lee, J.H., Lee, A.K. and Lim, H.L. 2016. "Conditions during Export Affect the Potential Vase Life of Cut Roses (*Rosa hybrida* L.)." **Horticulture, Environment, and Biotechnology** 57(5) : 504-510.
- Jacobi, K.K., Macrae, E.A. and Hetherington, S.E. 2001. "Loss of Heat Tolerance in 'Kensington' Mango Fruit Following Heat Treatments." **Postharvest Biology and Technology** 21 : 321-330.

- Jagtap, U.B. and Bapat, V.A. 2014. "Phenolic Composition and Antioxidant Capacity of Wine Prepared from Custard Apple (*Annona squamosa* L.) Fruits." **Journal of Food Processing and Preservation** 39 : 175-182.
- Jagtap, U.B., Waghmare, S.R., Lokhande, V.H., Suprasanna, P. and Bapat, V.A. 2011. "Preparation and Evaluation of Antioxidant Capacity of Jackfruit (*Artocarpus heterophyllus* Lam.) Wine and Its Protective Role against Radiation Induced DNA Damage." **Industrial Crops and Products** 34(3) : 1595-1601.
- Jaleel, C.A., Gopi, R. and Panneerselvam, R. 2007. "Alterations in Lipid Peroxidation, Electrolyte Leakage and Proline Metabolism in *Catharanthus Roseus* under Treatment with Triadimefon, a Systemic Fungicide." **Comptes Rendus Biologies** 330 : 905-912.
- Janero, D.R. 1990. "Malondialdehyde and Thiobarbituric Acidreactivity as Diagnostic Indices of Lipid Peroxidation and Peroxidative Tissue Injury." **Free Radical Biology and Medicine** 9(6) : 515-540.
- Jansom, C., Moolkam, S., Jansom, V., Skulkhu, E. and Bhamarapratana, K. 2010. "Antioxidant Activity and Chemical Constituents in Purple Glutinous Rice Thailand Local Genotypes." **Thammasat Medical Journal** 10(2) : 136-143.
- Jeong, J.W., Nam, P.W., Lee, S.J. and Lee, K.G. 2011. "Antioxidant Activities of Korean Rice Wine Concentrates." **Journal of Agricultural and Food Chemistry** 59(13) : 7039-7044.
- Jin, Y.Z., Lv, D.Q., Liu, W.W., Qi, H.Y. and Bai, X.H. 2013. "Ethanol Vapor Treatment Maintains Postharvest Storage Quality and Inhibits Internal Ethylene Biosynthesis during Storage of Oriental Sweet Melons." **Postharvest Biology and Technology** 86 : 372-380.
- Jirapong, N., Sriraveeroj, C. and Wongs-Aree, C. 2010. "Abscission of Sweet Basil Leaves Induced by Ethylene Under Modified Atmosphere Packaging." **Acta Horticulturae** 875 : 431-434.
- Jun, H., Song, G.S., Yang, E.I., Youn, Y. and Kim, Y.S. 2012. "Antioxidant Activities and Phenolic Compounds of Pigmented Rice Bran Extracts." **Journal of Food Science** 77(7) : 759-764.
- Jung, H., Lee, S.J., Lim, J.H., Kim, B.K. and Park, K.J. 2013. "Chemical and Sensory Profiles of Makgeolli, Korean Commercial Rice Wine, from Descriptive, Chemical, and Volatile Compound Analyses." **Food Chemistry** 152 : 624-632.

- Kaewsuksaeng, S., Tatmala, N., Srilaong, V. and Pongprasert, N. 2015. "Postharvest Heat Treatment Delays Chlorophyll Degradation and Maintains Quality in Thai Lime (*Citrus aurantifolia* Swingle cv. Paan) Fruit." **Postharvest Biology and Technology** 100 : 1-7.
- Kanouchi, H., Kakimoto, T., Nakano, H., Suzuki, M., Nakai, Y., Shiozaki, K., Akikoka, K., Otomaru, K., Nagano, M. and Matsumoto, M. 2016. "The Brewed Rice Vinegar Kurozu Increases HSPA1A Expression and Ameliorates Cognitive Dysfunction in Aged P8 Mice." **PLoS ONE** (3) : 1-12.
- Kappus, H. 1991. "Lipid-Peroxidation Mechanism and Biological Relevance." **Free Radicals and Food Additives**, Taylor and Francis, New York, USA. 59-74.
- Katzer, G. 2002. Lime (*Citrus aurantifolia* (Christm. et Panz.) Swingle). Gernet Katzer's Spice Pages. [Online] Available : http://www-ang.kfunigraz.ac.at/~katzer/engl/Citr_aur.html.
- Kawakami, E.M., Oosterhuis, D.M. and Snider, J.L. 2010. "1-Methylcyclopropene Effects on the Physiology and Yield of Field-Grown Cotton." **Journal of Cotton Science** 14 : 233-239.
- Kehrer, C.L. 1921. "The Chemistry of Vinegar." **Journal of Food Product and The American Vinegar Industry** 1 : 5-20.
- Khairi, A.N., Falah, M.A.F., Suyantohadi, A., Takahashi, N. and Nishina, H. 2015. "Effect of Storage Temperatures on Color of Tomato Fruit (*Solanum lycopersicum* Mill.) Cultivated under Moderate Water Stress Treatment." **Italian Oral Surgery** 3 : 178-183.
- Kiers, C.T., de Boer, J.L., Olthof, R. and Spek, A.L. 1976. "The Crystal Structure of a 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Modification." **Acta Crystallographica Section B: Structural Science, Crystal Engineering and Materials** 32(8) : 2297-2305.
- Kim, G.R., Yoon, S.R., Lee, S.W., Jeong, M.S., Kwak, J.Y., Jeong, Y.J., Yeo, S.H. and Kwon, J.H. 2011. "Analysis of the Free Amino Acids and Volatile-Flavor Compounds in the Commercial Brown-Rice Vinegar Prepared via Static Acetic-Acid Fermentation." **Korean Journal of Food Preservation** 18(5) : 803-810.
- Kim, S., Park, J. and Hwang, I.K. 2002. Quality Attributes of Various Varieties of Korean Red Pepper Powders (*Capsicum annuum* L.) and Color Stability during Sunlight Exposure. **Journal of Food Science** 67 : 2957-2961.

- Kim, S.H., Cho, H.K. and Shin, H.S. 2012. "Physicochemical Properties and Antioxidant Activities of Commercial Vinegar Drinks in Korea." **Food Science and Biotechnology** 21(6) : 1729-1734.
- Kitts, D.D., Wijewickreme, A.N. and Hu, C. 2000. "Antioxidant Properties of a North American Ginseng Extract." **Molecular and Cellular Biochemistry** 203 : 1-10.
- Koguchi, M., Saigusa, N. and Teramoto, Y. 2010. "Antioxidative Activity of Alcoholic Beverages Made from Purple Rice (*Oryza sativa* var. *Indica* cv. *Shium*)." **Food Science and Technology Research** 16 : 157-162.
- Kojo, S. 2004. "Vitamin C: Basic Metabolism and Its Function as an Index of Oxidative Stress." **Current Medicinal Chemistry** 11 : 1041-1064.
- Kong, E.L., Lee, B.K., Michelle, Ginjom, I. and Nissom, P.M. 2015. "DNA Damage Inhibitory Effect and Phytochemicals of Fermented Red Brown Rice Extract." **Asian Pacific Journal of Tropical Disease** 5(9) : 732-736.
- Koukounaras, A., Siomos, A.S. and Sfakiotakis, E. 2006. "1-Methylcyclopropene Prevents Ethylene Induced Yellowing of Rocket Leaves." **Postharvest Biology and Technology** 41 : 109-111.
- Krusong, W., Dansai, P. and Itharat, A. 2012. "Combination Impact of Turmeric Extract and Fermented Vinegar on Eeduction of Inoculated *Salmonella* Typhimurium on Fresh Lettuce." **KMITL Science and Technology** 12 : 77-84.
- Krusong, W., Jindaprasert, A., Laosinwattana, C. and Teerarak, M. 2015a. "Baby Corn Fermented Vinegar and Its Vapor Control Postharvest Decay in Strawberries." **New Zealand Journal of Crop and Horticultural Science** 43(3) : 193-203.
- Krusong, W., Pornpukdeewatana, S. and Teerarak, M. 2016. "Susceptibility of *Klebsiella pneumoniae* on Coriander Leaves to Liquid- and Vapor-Phase Ethanol." **FEMS Microbiology Letters** 363(9) : 1-7.
- Krusong, W., Teerarak, M. and Laosinwattana, C. 2015b. "Liquid and Vapor-Phase Vinegar Reduced *Klebsiella Pneumoniae* on Fresh Coriander." **Food Control** 50 : 502-508.
- Kusznierewicz, B., S'miechowska, A., Bartoszek, A. and Namiesnik, J. 2008. "The Effect of Heating and Fermenting on Antioxidant Properties of White Cabbage." **Food Chemistry** 108(3) : 853-861.

- Lagopodi, A.L., Cetiz K., Koukounaras, A. and Sfakiotakis, E.M. 2009. "Acetic Acid, Ethanol and Steam Effects on the Growth of *Botrytis cinerea* *In Vitro* and Combination of Steam and Modified Atmosphere Packaging to Control Decay in Kiwifruit." **Journal of Phytopathology** 157 : 79-84.
- Lange, D.L. and Cameron, A.C. 1998. "Controlled-Atmosphere Storage of Sweet Basil." **HortScience** 33(4) : 741-743.
- Lee, E.K., Kwon, W.Y., Lee, J.W., Yoon, J.A., Chung, K.H., Song, B.C. and An, J.H. 2014. "Quality Characteristics and Antioxidant Activity of Vinegar Supplemented Added with *Akebia quinata* Fruit during Fermentation." **Journal of the Korean Society of Food Science and Nutrition** 43(8) : 1217-1227.
- Lee, J.C., Kim, H.R., Kim, T. and Jang, Y.S. 2002. "Antioxidant Property of an Ethanol Extract of the Stem of *Opuntia ficus-indica* var. *Saboten*." **Journal of Agricultural and Food Chemistry** 50 : 6490-6496.
- Lemoine, M.L., Civello, P., Chaves, A. and Martinez, G.A. 2009. "Hot Air Treatment Delays Senescence and Maintains Quality of Fresh-Cut Broccoli Florets during Refrigerated Storage." **Food Science and Technology** 42 : 1076-1081.
- Lemoine, M.L., Civello, P.M., Chaves, A.R. and Martinez, G.A. 2010. "Influence of a Combined Hot Air and UV-C Treatment on Quality Parameters of Fresh-Cut Broccoli Florets at 0 °C." **Food Science and Technology** 45 : 1212-1218.
- Lichtenthaler, H.K. 1987. "Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes." **Methods in Enzymology** 148 : 350-382.
- Lin, C.C. and Liang, J.H. 2002. "Effect of Antioxidants on the Oxidative Stability of Chicken Breast Meat in a Dispersion System." **Journal of Food Science** 67 : 530-533.
- Lin, J.Y., Fan, W., Gao, Y.N., Wu, S.F. and Wang, S.X. 2010. "Study on Volatile Compounds in Rice by HS-SPME and GC-MS." **Julius-Kühn-Archiv** 425 : 125-134.
- Linster, C.L. and Van Schaftingen, E. 2007. Vitamin C Biosynthesis, Recycling and Degradation in Mammals. **The FEBS Journal** 274 : 1-22.
- Liu, H., Cao, J. and Jiang, W. 2015. "Changes in Phenolics and Antioxidant Property of Peach Fruit during Ripening and Responses to 1-Methylcyclopropene." **Postharvest Biology and Technology** 108 : 111-118.

- Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. "Free Radicals, Antioxidants and Functional Foods: Impact on Human Health." **Pharmacognosy Reviews** 4(8) : 118-126.
- Lowe, N.M., Frase, W.D. and Jackson, M.J. 2002. "Is There a Potential Therapeutic Value of Copper and Zinc for Osteoporosis?." **Proceedings of the Nutrition Society** 61(2) : 181-185.
- Lu, S. 2007. "Effect of Packaging on Shelf-Life of Minimally Processed Bok Choy (*Brassica chinensis* L.)." **LWT - Food Science and Technology** 40(3) : 460-464.
- Lu, X.G., Ma, Y.P. and Liu, X.H. 2012. "Effects of Maturity and 1-MCP Treatment on Postharvest Quality and Antioxidant Properties of 'Fuji' Apples during Long-term Cold Storage." **Horticulture, Environment and Biotechnology** 53(5) : 378-386.
- Machado, F.L. de C., Costa, J.M.C. and Batista, E.N. 2012. "Application of Carnauba-Based Wax Maintains Postharvest Quality of "Ortanique" Tangor." **Ciência e Tecnologia de Alimentos** 32(2) : 261-266.
- Mahmood, R., Soheila, M. and Saeid, A. 2008. "Radical Scavenging and Reducing Power of *Salvia mirzayanii* Subfractions." **Molecules** 13 : 2804-2813.
- Mama, S., Yemer, J., Woelore, W. and Sodo, W. 2016. "Effect of Hot Water Treatments on Shelf Life of Tomato (*Lycopersicon esculentum* Mill)." **Journal of Natural Sciences Research** 6(17) : 69-77.
- Mangave, B.D., Singh, A. and Mahatma, M.K. 2013. "Effects of Different Plant Growth Regulators and Chemicals Spray on Postharvest Physiology and Vase Life of *Heliconia inflorescence* cv. Golden Torch." **Plant Growth Regulation** 69 : 259-264.
- Mari, M., Bautista-Baños, S. and Sivakumar, D. 2016. "Decay Control in the Postharvest System: Role of Microbial and Plant Volatile Organic Compounds." **Postharvest Biology and Technology** 122 : 70-81.
- Mathure, S.V., Jawali, N., Thengane, R.J. and Nadaf, A.B. 2014. "Comparative Quantitative Analysis of Headspace Volatiles and Their Association with BADH2 Marker in Non-Basmati Scented, Basmati and Non-Scented Rice (*Oryza sativa* L.) Cultivars of India." **Food Chemistry** 142 : 383-391.
- Medina, E., Romero, C., Brenes, M. and De Castro, A. 2007. "Antimicrobial Activity of Olive Oil, Vinegar, and Various Beverages against Foodborne Pathogens." **Journal of Food Protection** 70(5) : 1194-1199.

- Meir, S., Ronen, R., Lurie, S. and Hadas, S. 1997. "Assessment of Chilling Injury during Storage: Chlorophyll Fluorescence Characteristics of Chilling-Susceptible and Triazole-Induced Chilling Tolerant Basil Leaves." **Postharvest Biology and Technology** 10 : 213-220.
- Ministry of Public Health. 2000. "Notification of the Ministry of Public Health (No. 204) B.E. 2543 (2000) Re: Vinegar. Published in the Government Gazette 118, Special Part 6 Ngor, dated 24th January, 2001.
- Mori, T., Terai, H., Yamauchi, N. and Suzuki, Y. 2009. "Effects of Postharvest Ethanol Vapor Treatment on the Ascorbate Glutathione Cycle in Broccoli Florets." **Postharvest Biology and Technology** 52 : 134-136.
- Muntana, N. and Prasong, S. 2010. "Study on Total Phenolic Contents and Their Antioxidant Activities of Thai White, Red and Black Rice Bran Extracts." **Pakistan Journal of Biological Sciences** 13(4) : 170-174.
- Muthal, A.P., Rojatkhar, S.R. and Bodhankar, S.L. 2015. "*In-vitro* Free Radicals Scavenging and Antioxidant Activity of Rice Bran Extract." **Pharmacologia** 6(8) : 377-385.
- Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F. and Jafari, M. 2008. "Free Radical Scavenging Activity and Antioxidant Capacity of *Eryngium caucasicum* Trautv and *Froripia subpinata*." **Pharmacologyonline** 3 : 19-25.
- Ng, D.S.H., Rose, L.C., Suhaimi, H., Mohamad, H., Rozaini, M.Z.H. and Taib, M. 2011. "Preliminary Evaluation on the Antibacterial Activities of *Citrus hystrix* oil Emulsions Stabilized by Tween 80 and Span." **International Journal of Pharmacy and Pharmaceutical Sciences** 80(3) : 2-4.
- Ngamdee, P., Wichai, U. and Jiamyangyuen, S. 2016. "Correlation between Phytochemical and Mineral Contents and Antioxidant Activity of Black Glutinous Rice Bran, and Its Potential Chemopreventive Property." **Food Technology and Biotechnology** 54(3) : 282-289.
- Niamthong, T., Sittipod, S. and Chonhenchob, V. 2007. "Development of Holy Basil Storage Using Low Temperatures and Modified Atmosphere Packaging." **Kasetsart Journal (Natural Science)** 293 : 286-293.
- Nissen, S.B., Tjonneland, A., Stripp, C., Olsen, A., Christensen, J., Overvad, K., Dragsted, L.O. and Thomson, B. 2003. "Intake of Vitamin A, C and E from Diets and Supplements and Breast Cancer in Post-Menopausal Women." **Cancer Causes Control** 149(8) : 695-704.

- Nunes, C.N. and Emond, J.P. 2007a. "Relationship between Weight Loss and Visual Quality of Fruits and Vegetables." **Proceedings of the Florida State Horticultural Society** 120 : 235-245.
- Nunes, C.N., Emond, J.P., Brecht, J.K., Dea, S. and Proulx, E. 2007b. "Quality Curves for Mango Fruit (cv. Tommy Atkins and Palmer) Stored at Chilling and Non Chilling Temperatures." **Journal of Food Quality** 30 : 104-120.
- Nurhani, K. 2013. "Extraction of *Citrus hystrix* D.C. (Kaffir Lime) Essential Oil Using Automated Steam Distillation Process: Analysis of Volatile Compounds." **Malaysian Journal of Analytical Sciences** 17(3) : 359-369.
- Ohkawa, H., Ohishi, N. and Yagi, K. 1979. "Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction." **Analytical Biochemistry** 95 : 351-358.
- Oliveira, M., Abadias, M., Usall, J., Torres, R., Teixidó, N. and Viñas, I. 2015. "Application of Modified Atmosphere Packaging as a Safety Approach to Fresh-Cut Fruits and Vegetables-A review." **Trends in Food Science and Technology** 46 : 13-26.
- Omoba, O.S. and Onyekwere, U. 2016. "Postharvest Physicochemical Properties of Cucumber Fruits (*Cucumis sativus* L.) Treated with Chitosan-Lemon Grass Extracts under Different Storage Durations." **African Journal of Biotechnology** 15(50) : 2758-2766.
- Opio, P., Pongphen, J., Pongprasert, N., Wongs-Aree, C., Suzuki, Y. and Srilaong, V. 2015. "Postharvest Ethanol Vapor Treatment Delays Chlorophyll Degradation and Maintains Quality of Thai Lime (*Citrus aurantifolia* Swingle cv. Paan) Fruit." **Agricultural Sciences Journal** 46(3 Suppl.) : 173-176.
- Ortiz, J., Marín-Arroyo, M.R., Noriega-Domínguez, M.J., Navarro, M. and Arozarena, I. 2013. "Color, Phenolics, and Antioxidant Activity of Blackberry (*Rubus glaucus* Benth.), Blueberry (*Vaccinium floribundum* Kunth.), and Apple Wines from Ecuador." **Journal of Food Science** 78 : C985-C993.
- Oyaizu, M. 1986. "Studies on Products of Browning Reactions: Antioxidant Activities of Products of Browning Reaction Prepared from Glucosamine." **Journal of Nutrition** 44 : 307-315.
- Palombini, V.S., Maruyama, S.A., Claus, T., Carbonera, F., Souza, N.E.D., Visentainer, J.V., Teresinha, S., Gomes, M. and Matsushita, M. 2013. "Evaluation of Antioxidant Potential of Brazilian Rice Cultivars." **Food Science and Technology** 33(4) : 699-704.

- Pandey, N., Chaurasia, J.K., Tiwari, O.P. and Tripathi, Y.B. 2007. "Antioxidant Properties of Different Fractions of Tubers from *Pueraria tuberosa* Linn." **Food Chemistry** 105(1) : 219-222.
- Patrignani, F., Iucci, L., Belletti, N., Gardini, F., Guerzoni, M.E. and Lanciotti, R. 2008. "Effects of Sub-Lethal Concentrations of Hexanal and 2-(E)-Hexenal on Membrane Fatty Acid Composition and Volatile Compounds of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Escherichia coli*." **International Journal of Food Microbiology** 123 : 1-8.
- Peltier, K. 2016. "Vinegar for Green Cleaning." [Online] Available : <https://www.thespruce.com/vinegar-definition-green-cleaning-uses-1707034>
- Pengkumsri, N., Chaiyasut, C., Saenjum, C., Sirilun, S., Peerajan, S., Suwannalert, P., Sirisattha, S. and Sivamaruthi, B.S. 2015. "Physicochemical and Antioxidative Properties of Black, Brown and Red Rice Varieties of Northern Thailand." **Food Science and Technology** 35(2) : 331-338.
- Pepler, H.J. and Beaman, R.G. 1967. "Microbial Technology." In: **Yeoman**. Chapter 13 Vinegar Fermentation. 1st ed. Illinois: Reinhold Publishing Corporation. 344-359.
- Perera Niranjala, O.D.A. and Karunaratne, A. "Response of Bananas to Postharvest Acid Treatments." **The Journal of Horticultural Science and Biotechnology** 76(1) : 70-76.
- Pérez, A.G., Sanz, C., Ríos, J.J., Olías, R. and Olía, J.M. 1999. "Effects of Ozone Treatment on Postharvest Strawberry Quality." **Journal of Agricultural and Food Chemistry** 47(4) : 1652-1656.
- Pinsirodom, P., Rungcharoen, J. and Liumminful, A. 2010. "Quality of Commercial Wine Vinegars Evaluated on the Basis of Total Polyphenol Content and Antioxidant Properties." **Asian Journal of Food and Agro-Industry** 3(4) : 389-397.
- Prior, R.L., Wu, X. and Schaich, K. 2005. "Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements." **Journal of Agricultural and Food Chemistry** 53 : 4290-4302.
- Pryor, W.A. 2000. "Vitamin E and Heart Disease: Basic Science to Clinical Intervention Trials." **Free Radical Biology and Medicine** 28 : 141-164.

- Radi, M., Jouybari, H.A., Mesbahi, G., Farahnaky, A. and Amiri, S. 2010. "Effect of Hot Acetic Acid Solutions on Postharvest Decay caused by *Penicillium expansum* on Red Delicious apples." **Scientia Horticulturae** 126 : 421-425.
- Rahim, A., Arjuna, A., Pakki, E., Syaiful, S.A., Rewa, A.M., Alam, G. and Murdifin, M. 2016. "Antioxidant and HPTLC Study of Black Glutinous Rice Extract from South Sulawesi Indonesia." **International Journal of Pharmacognosy and Phytochemical Research** 8(5) : 771-776.
- Rao, B.S., Shanbhoge, R., Rao, B.N., Adiga, S.K., Upadhy, D., Aithal, B.K. and Kumar, M.R.S. 2009. "Alcoholic Extract of *Cymbopogon citrates* against Radiation-Induced DNA Damage on V79 Cells and Free Radical Scavenging Ability against Radicals Generated *In Vitro*." **Human and Experimental Toxicology** 28(4) : 195-202.
- Rao, S.V.C., Reddy, S.G., Babu, P.P. and Reddy, A.R. 2010. "The Antioxidant and Antiproliferative Activities of Methanolic Extracts from Njavara Rice Bran. **BMC Complementary and Alternative Medicine** 10 : 2-9.
- Ratanachinakorn, B., Songchan, K., Kwanhong, P., Srihanyarat, S., Thitiprasert, V. and Lairungreang, C. 2007. "Effect of Temperature on Storage Life of Fresh-cut Fruits and Vegetables." **Postharvest and Processing Research and Development Division** 165-174.
- Razali, N., Razab, R., Mat Junit, S. and Abdul Aziz, A. 2008. "Radical Scavenging and Reducing Properties of Extracts of Cashew Shoots (*Anacardium occidentale*)." **Food Chemistry** 111 : 38-44.
- Razmkhab, S., Lopez-Toledano, A., Ortega, J.M., Mayen, M., Merida, J. and Medina, M. 2002. "Adsorption of Phenolic Compounds and Browning Products in White Wines by Yeasts and Their Cell Walls." **Journal of Agricultural and Food Chemistry** 50(25) : 7432-7437.
- Rico, D., Martín-Diana, A.B., Barat, J.B. and Barry-Ryan, C. 2007. "Extending and Measuring the Quality of Fresh-Cut fruit and Vegetables; a Review." **Trends in Food Science and Technology** 18(7) : 373-386.
- Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. and Glover, W. 1999. "Phenolic Compounds and Their Role in Oxidative Processes in Fruits." **Food Chemistry** 66(4) : 401-436.

- Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R. and Mulatsih, W. 2010. "Antioxidant Activity, Total Phenolic, and Total Flavaonoid of Extracts and Fractions of Red Fruit (*Pandanus conoideus* Lam)." **International Food Research Journal** 17(1) : 97-106.
- Rufián-Henares, J. and Delgado-Andrade, C. 2009. "Effect of Digestive Process on Maillard Reaction Indexes and Antioxidante Properties of Breakfast Cereals." **Food Research International** 42(3) : 394-400.
- Russo, A., Izzo, A.A., Cardile, V., Borrelli, F. and Vanella, A. 2001. "Indian Medicinal Plants as Antiradicals and DNA Cleavage Protectors." **Phytomedicine** 8 : 125-132.
- Sadabpod, K., Kangsadalampai, K. and Tongyonk, L. 2010. "Antioxidant Activity and Antimutagenicity of Hom Nil Rice and Black Glutinous Rice." **Journal of Health Research** 24(2) : 49-54.
- Saha, M.R., Alam, M.A., Akter, R. and Jahangir, R. 2008. "In Vitro Free Radical Scavenging Activity of *Ixora coccinea* L." **Bangladesh Journal of Pharmacology** 3(2) : 90-96.
- Sakanaka, S. and Ishihara, Y. 2008. "Comparison of Antioxidant Properties of Persimmon Vinegar and Some Other Commercial Vinegars in Radical-Scavenging Assays and on Lipid oxidation in Tuna Homogenates." **Food Chemistry** 107(2) : 739-744.
- Saltveit, M.E. 2005. "Aminoethoxyvinylglycine (AVG) Reduces Ethylene and Protein Biosynthesis in Excised Discs of Mature-Green Tomato Pericarp Tissue" **Postharvest Biology and Technology** 35(2) : 183-190.
- Salunkhe, D.K., Bolin, H.R. and Reddy, N.R. 1991. "Chemical Composition and Nutritional Quality, in Storage, Processing, and Nutritional Quality of Fruits and Vegetables." **Processed Fruits and Vegetables**, CRC Press, Boca Raton, FL, 2 : 115-145.
- Salvador, A., Abad, I., Amal, L. and Martínez-Jávega, J.M. 2006. "Effects of Ozone in Postharvest Quality of Persimmon." **Journal of Food Science** 71 : 443-446.
- Seglie, L., Martina, K., Devecchi, M., Roggero, C., Trottac, F. and Scariot, V. 2011. "The Effects of 1-MCP in Cyclodextrin-Based Nanosponges to Improve the Vase Life of *Dianthus caryophyllus* Cut Flowers." **Postharvest Biology and Technology** 59 : 200-205.
- Serpen, A., Gokmen, V. and Fogliano, V. 2012. "Total Antioxidant Capacities of Raw and Cooked Meats." **Meat Science** 90 : 60-65.

- Shao, X. and Tu, k. 2014. "Hot Air Treatment Improved the Chilling Resistance of Loquat Fruit Under Cold Storage." **Journal of Food Processing and Preservation** 38(2) : 694-703.
- Sharma, K.D. and Sethi, V. 2000. "Studies on the Storage of Apple from Three Different Locations." **Beverage Food World** 4 : 28-37.
- Shazia, Q., Mohammad, Z.H., Rahman, T. and Shekhar, H.U. 2012a. "Correlation of Oxidative Stress with Serum Trace Element Levels and Antioxidant Enzyme Status in Beta Thalassemia Major Patients: A Review of the Literature." **Anemia** 1-7.
- Shazia, T., Sania, A., Sadaf, N.A., Hafiz, B.A. and Naeem, A. 2012b. "Physicochemical Characterization and Frying Quality of Canola and Sunflower Oil Samples." **Journal of The Chemical Society of Pakistan** 34 : 513-517.
- Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. "Antioxidative Properties of Xanthan on the Autoxidation of Soybean oil in Cyclodextrin Emulsion." **Journal of Agricultural and Food Chemistry** 40 : 945-948.
- Sholberg, P., Haag, P., Hocking, R. and Bedford, K. 2000. "The Use of Vinegar Vapor to Reduce Postharvest Decay of Harvested Fruit." **HortScience** 35 : 898-903.
- Sholberg, P.L., Cliff, M. and Moyls, A.L. 2001. "Fumigation with Acetic Acid Vapor to Control Decay of Stored Apples." **Fruits** 56(5) : 355-366.
- Sholberg, P.L., Shephard, T., Randall, P. and Moyls, L. 2004. "Use of Measured Concentrations of Acetic Acid Vapor to Control Postharvest Decay in d'Anjou Pears." **Postharvest Biology and Technology** 32 : 89-98.
- Sies, H. 1997. "Oxidative Stress: Oxidants and Antioxidants." **Experimental Physiology** 82(2) : 291-295.
- Sies, H. and Stahl, W. 1995. "Vitamins E and C, Beta-Carotene, and Other Carotenoids as Antioxidants." **The American Journal of Clinical Nutrition** 62(6 Suppl.) : 1315S-1321S.
- Simon, J. and Morales, M. 1999. "Basil: a Source of Aroma Compounds and a Popular Culinary and Ornamental Herb." In: J. Janick (ed.), **Perspectives on New Crops and New Uses**, ASHS Press, Alexandria, VA. 16 : 499-505.
- Sirisantimethakom, L., Laopaiboon, L., Danvirutai, P. and Laopaiboon, P. 2008. "Volatile Compounds of a Traditional Thai Rice Wine." **Biotechnology** 7 : 505-513.

- Soares, J.R., Dinis, T.C.P., Cunha, A.P. and Almeida, L.M. 1997. "Antioxidant Activities of Some Extracts of *Thymus zygis*." **Free Radical Research** 26 : 469-478.
- Soler-Rivas, C., Espin, J.C. and Wichers, H.J. 2000. "An Easy and Fast Test to Compare Total Free Radical Scavenger Capacity of Food Stuffs." **Phytochemical Analysis** 11 : 330-338.
- Song, W., Derito, C.M., Liu, M.K., He, X.J., Dong, M. and Liu, R.H. 2010. "Cellular Antioxidant Activity of Common Vegetables." **Journal of Agricultural and Food Chemistry** 58(11) : 6621-6629.
- Soonthornvipat, T. 2012. "Effect of Modified Atmosphere Packaging and Temperature for Quality of Bergamot Leaves." Practice in Faculty of Agricultural Technology, Chiang Mai Rajabhat University. Thailand, 2012.
- Soto-Zamora, G., Yahia, E.M., Brecht, J.K. and Gardea, A. 2005. "Effects of Postharvest Hot Air Treatments on the Quality and Antioxidant Levels of Tomato Fruit." **LWT- Food Science and Technology** 8 : 113-116.
- Soufleros, E.H., Mygdalia, A.S. and Natskoulis, P. 2004. "Characterisation and Safety Evaluation of the Traditional Greek Fruit Distillate "Mouro" by Flavour Compounds and Mineral Analysis." **Food Chemistry** 86 : 624-36.
- Spinosa, W.A., Júnior, V. dos S., Galvan, D., Fiorio, J.L. and Gomez, R.J.H.C. 2015. "Vinegar Rice (*Oryza sativa* L.) Produced by a Submerged Fermentation Process from Alcoholic Fermented Rice." **Food Science and Technology (Campinas)** 35(1) : 196-201.
- Sripum, C., Kukreja, R. K., Charoenkiatkul, S., Kriengsinyos, W. and Suttisansanee, U. 2016. "The Effect of Storage Conditions on Antioxidant Activities and Total Phenolic Contents of Parboiled Germinated Brown Rice (Khao Dok Mali 105)." **International Food Research Journal** 23(4) : 1827-1831.
- Su, M. and Chien, P. 2007. "Antioxidant Activity, Anthocyanins, and Phenolics of Rabbiteye Blueberry (*Vaccinium ashei*) Fluid Products as Affected by Fermentation." **Food Chemistry** 104 : 182-187.
- Subash, S. and Subramanian, P. 2009. "Morin a Flavonoid Exerts Antioxidant Potential in Chronic Hyperammonemic Rats: A Biochemical and Histopathological Study." **Molecular and Cellular Biochemistry** 327 : 153-161.

- Sudhakar, D.V. and Maini, S.B. 1994. "Stability of Carotenoids during Storage of Mango Pulp." **Journal of Food Science and Technology** 31 : 228-230.
- Suematsu, T., Kamada, T., Abe, H., Kikuchi, S. and Yagi, K. 1977. "Serum Lipoperoxide Level in Patients Suffering from Liver Diseases." **Clinica Chimica Acta** 79 : 267-270.
- Sun, B., Yan, H., Liu, N., Wei, J. and Wang, Q. 2012. "Effect of 1-MCP Treatment on Postharvest Quality Characters, Antioxidants and Glucosinolates of Chinese Kale." **Food Chemistry** 131(2) : 519-526.
- Tagliacruzchi, D., Verzelloni, E. and Conte, A. 2008. "Antioxidant Properties of Traditional Balsamic Vinegar and Boiled Must Model Systems." **European Food Research and Technology** 227 : 835-843.
- Tanaka, J., Nakanishi, T., Ogawa, K., Tsuruma, K., Shimazawa, M., Shimoda, H. and Hara, H. 2011. "Purple Rice Extract and Anthocyanidins of the Constituents Protect against Light-Induced Retinal Damage *In Vitro* and *In Vivo*." **Journal of Agricultural and Food Chemistry** 59 : 528-536.
- Tananuwong, K. and Tewaruth, W. 2010. "Extraction and Application of Antioxidants from Black Glutinous Rice." **LWT - Food Science and Technology** 43(3) : 476-481.
- Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U. and Techadamrongsin, Y. 2001. Repellency of Volatile Oils from Plants against Three Mosquito Vectors. **Journal of Vector Ecology** 26(1) : 76-82.
- Teramoto, Y., Koguchi, M., Wongwicharn, A. and Saigusa, N. 2011. "Production and Antioxidative Activity of Alcoholic Beverages Made from Thai Ou Yeast and Black Rice (*Oryza sativa* var. *Indica* cv. *Shiun*)." **African Journal of Biotechnology** 10(52) : 10706-10711.
- Teruaki, F. 1981. "Magnetic Interaction in Solvent-Free DPPH and DPPH--Solvent Complexes." **Bulletin of the Chemical Society of Japan** 54(10) : 3110-3116.
- Thakur, A.K. and Pandey, M. 2004. "Improvement of Postharvest Quality of Tomato Fruits by Ethanol Vapour Treatment." **Journal of Applied Horticulture** 6(2) : 39-42.
- Thavong, P., Archbold, D.D., Pankasemsuk, T. and Koslanund, R. 2010. "Postharvest Use of Hexanal Vapor and Heat Treatment on Longan Fruit Decay and Consumer Acceptance." **International Journal of Food Science and Technology** 45(11) : 2313-2320.

- Toor, R.K. and Savage, G.P. 2006. "Changes in Major Antioxidant Components of Tomatoes during Postharvest Storage." **Food Chemistry** 99(4) : 724-727.
- Turkseven, S., Kruger, A., Mingone, C.J., Kaminski, P., Inaba, M., Rodella, L.F., Ikehara, S., Wolin, M.S. and Abraham, N.G. 2005. "Antioxidant Mechanism of Heme Oxygenase-1 Involves an Increase in Superoxide Dismutase and Catalase in Experimental Diabetes." **American Journal of Physiology - Heart and Circulatory Physiology** 289 : 701-707.
- Tzortzakis, N., Chrysargyris, A., Sivakumar, D. and Loulak, K. 2016. "Vapor or Dipping Applications of Methyl Jasmonate, Vinegar and Sage Oil for Pepper Fruit Sanitation towards Grey Mould." **Postharvest Biology and Technology** Volume 118 : 120-127.
- Tzortzakis, N.G. 2010. "Ethanol, Vinegar and *Origanum vulgare* Oil Vapor Suppress the Development of Anthracnose Rot in Tomato Fruit." **International Journal of Food Microbiology** 142 : 14-18.
- Tzortzakis, N.G. and Economakis, C.D. 2007. "Maintaining Postharvest Quality of the Tomato." **Journal of Food Quality** 30 : 567-580.
- Tzortzakis, N.G., Tzanakaki, K. and Economakis, C.D. 2011. "Effect of Origanum Oil and Vinegar on the Maintenance of Postharvest Quality of Tomato." **Food and Nutrition Sciences** 2(9) : 974-982.
- Vaughan, J.G. and Geissler, C.A. 1997. "The New Oxford Book of Food Plants: a Guide to the Fruit, Vegetables, Herbs and Spices of the World." **Oxford University Press**. Oxford, England. 239 pp.
- Venditti, T., Dore, A., Molinu, M.G. and D'Hallewin, G. 2012. "Effect of Acetic Acid Repeated Treatments on Postharvest Quality of "Taloppo" Table Grape." **Communications in Agricultural and Applied Biological Sciences** 77(3) : 219-224.
- Venditti, T., Dorea, A., Molinua, M.G., Agabbio, M. and D'hallewina, G. 2009. "Combined Effect of Curing Followed by Acetic Acid Vapor Treatments Improves Postharvest Control of *Penicillium digitatum* on Mandarins." **Postharvest Biology and Technology** 54 : 111-114.
- Venditti, T., Ladu, G., Cubaiu, L., Myronycheva, O. and D'hallewin G. 2017. "Repeated Treatments with Acetic Acid Vapors during Storage Preserve Table Grapes Fruit Quality." **Postharvest Biology and Technology** 125 : 91-98.

- Vicente, A.R., Mart'inez, G.A., Chaves, A.R. and Civello, P.M. 2006. "Effect of Heat Treatment on Strawberry Fruit Damage and Oxidative Metabolism during Storage." **Postharvest Biology and Technology** 40 : 116-122.
- von Sonntag, C. 1987. **The Chemical Basis of Radiation Biology**. Taylor and Francis, London. 515 p.
- Wagner, K.H., Derkits, S., Herr, M., Schuh, W. and Elmadfa, I. 2002. "Antioxidative Potential of Melanoidins Isolated from a Roasted Glucose-Glycine Model." **Food Chemistry** 78 : 375-382.
- Walter, M., Marchesan, E., Massoni, P.F.S., da Silva, L.P., Sartori, G.M.S. and Ferreira, R.B. 2013. "Antioxidant Properties of Rice Grains with Light Brown, Red and Black Pericarp Colors and the Effect of Processing." **Food Research International** 50(2) : 698-703.
- Wang, B., Pace, R.D., Dessai, A.P., Bovel-Benjamin, A. and Philips, B. 2002. "Modified Extraction Method for Determining 2-Thiobarbituric Acid Values in Meat with Increased Specificity and Simplicity." **Journal of Food Science** 67 : 2833-2836.
- Woolf, A.B. and Laing, W.A. 1996. "Avocado Fruit Skin Fluorescence Following Hot Water Treatments and Pretreatments." **Journal of the American Society for Horticultural Science** 121(1) : 147-151.
- Wszelaki, A.L. and Mitcham, E.J. 2003. "Effect of Combinations of Hot Water Dips, Biological Control and Controlled Atmospheres for Control of Gray Mold on Harvested Strawberries." **Postharvest Biology and Technology** 27 : 255-264.
- Xing, J., Sun, H.M., Li, Z.Y. and Qin, X.M. 2015. "Comparison of Volatile Components Between Raw and Vinegar Baked Radix Bupleuri by GC-MS Based Metabolic Fingerprinting Approach." **Evidence-Based Complementary and Alternative Medicine** 1-7.
- Xing, Z., Wang, Y., Feng, Z. and Tan, Q. 2008. "Effect of Different Packaging Films on Postharvest Quality and Selected Enzyme Activities of *Hypsizygus marmoreus* Mushrooms." **Journal of Agricultural and Food Chemistry** 56(24) : 11838-11844.
- Xu, Q., Tao, W. and Ao, Z. 2007. "Antioxidant Activity of Vinegar Melanoidins." **Food Chemistry** 102 : 841-849.
- Yahia, E.M., Soto-Zamora, G., Brecht, J.K. and Gardea, A. 2007. "Postharvest Hot Air Treatment Effects on the Antioxidant System in Stored Mature-Green Tomatoes." **Postharvest Biology and Technology** 44 : 107-115.

- Yamashita, F., Miglioranza, L.H.D.S., Miranda, L.D.A. and Souza, C.M.D.A.E. 2002. "Effects of Packaging and Temperature on Postharvest of Atemoya." **Revista Brasileira de Fruticultura** 24 : 658-660.
- Yanisch-Perron, C., Vieira, J. and Messing, J. 1985. "Improved M13 Phage Cloning Vectors and Host Strains: Nucleotide Sequences of the M13mp18 and pUC19 Vectors." **Gene** 33 : 103-119.
- Yau, N.J.N. and Liu, T.T. 1999. "Instrumental and Sensory Analysis of Volatile Aroma of Cooked Rice." **Journal of Sensory Studies** 14 : 209-233.
- Yen, G.C., Chang, Y.C. and Chen, J.P. 2002. "Antioxidant Activity of Mycelia from *Aspergillus candidus*." **Journal of Food Science** 67 : 567-572.
- Yuan, G., Sun, B., Yuan, J. and Wang, Q. 2010. "Effect of 1-Methylcyclopropene on Shelf Life, Visual Quality, Antioxidant Enzymes and Health-Promoting Compounds in Broccoli Florets." **Food Chemistry** 118 : 774-781.
- Zhang, Q., Zhang, S., Xie, C., Zeng, D., Fan, C., Li, D. and Bai, Z. 2006. "Characterization of Chinese Vinegars by Electronic Nose." **Sensors and Actuators B Chemical** 119(2) : 538-546.
- Zhou, T., Xu, S., Sun, D.W. and Wang, Z. 2002. "Effects of Heat Treatment on Postharvest Quality of Peaches." **Journal of Food Engineering** 54 : 17-22.
- Zubair, M., Anwar, F. and Shahid S.A. 2012. "Effect of Extraction Solvents on Phenolics and Antioxidant Activity of Selected Varieties of Pakistani Rice (*Oryza sativa* L.)." **International Journal of Agriculture and Biology** 14 : 935-940.

AUTHOR BIOGRAPHY

- NAME : Miss Kanokporn Changsawake
- DATE OF BIRTH : 28 October 1986
- ADDRESS : 29 M. 5 Klongsibsong Nongjok Bangkok 10530, Thailand.
- EDUCATION :
- 2005 : Nawaminthrachinuthit Suankularb Wittayalai Pathumthani School,
Lamlukka, Pathum Thani, Thailand
- 2009 : B.Sc. (Environmental Horticulture management)
King Mongkut's Institute of Technology Ladkrabang, Bangkok,
Thailand
- 2011 : M.Sc. (Horticulture)
King Mongkut's Institute of Technology Ladkrabang, Bangkok,
Thailand
- PUBLICATIONS :
- 2013 : **Changsawake, K., Laosinwattana, C. and Teerarak, M.** 2013. Residual Effects of Synthetic Alachlor Herbicide and Its Cytogenetic on Root Tip Cell of *Allium Cepa* L., pp. 660-662. *In* Proceeding of 24th Asian-Pacific Weed Science Society Conference, 22-25 October 2013, Bandung, Indonesia.
- Teerarak, M., Changsawake, K., Huypao, J., Wichittrakarn, P., Charoenying, P., Chumsawas, N. and Laosinwattana, C. 2013. Herbicidal Activity of Porganic™, Phytotoxic Effects and Its Physiological Mechanisms on Bioassay Plants, pp. 383-390. *In* Proceeding of 24th Asian-Pacific Weed Science Society Conference, 22-25 October 2013, Bandung, Indonesia.
- 2014 : **Changsawake, K., Krusong, W., Laosinwattana, C. and Teerarak, M.** 2014. Antioxidant Properties and DNA Damage Protective Potential of RD6 Glutinous Rice, pp. 39-46. *In* Proceeding of The 12th International Symposium on Biocontrol and Biotechnology, 11-13 December 2014, Chumphon, Thailand.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

2015

Changsawake, K., Pilasombut, K., Laosinwattana, C. and Teerarak, M. 2015. Antioxidant Properties of Flower Extract of *Etlingera elatior* (Jack) R.M. Smith, pp.73-76. *In* Proceeding of 2nd International Symposium on Agricultural Technology, 1-3 July 2015, Pattaya, Thailand.

Kongkarn, N., Tangwatcharin, P., Teerarak, M., Changsawake, K. and Pilasombut, K. 2015. Antibacterial Activity of Roselle (*Hibiscus sabdariffa* Linn.) Flower Extract, pp.301-304. *In* Proceeding of 2nd International Symposium on Agricultural Technology, 1-3 July 2015, Pattaya, Thailand.

Changsawake, K., Krusong, W., Laosinwattana, C. and Teerarak, M. 2015. Evaluation of Hydroxyl Radical Scavenging, Anti-Lipid Peroxidation Abilities and Total Phenolic Content of RD6 Glutinous Rice Grain, pp. 73-79. *In* Proceeding of International Symposium on Engineering and Natural Sciences. 12-14 August 2015, Beijing, China.

Teerarak, M., Changsawake, K., Kongkarn, N., Laosinwattana, C. and Pilasombut, K. 2015. Evaluation of Antioxidant Properties of Ethanol Extract from Dried Bael Fruit and Its Antibacterial Activity of Spoiling and Pathogen Food-Related Bacteria, pp. 94-104. *In* Proceeding of International Symposium on Engineering and Natural Sciences. 12-14 August 2015, Beijing, China.

Changsawake, K., Krusong, W., Laosinwattana, C. and Teerarak, M. 2015. Use of Ambient Upland Rice Fermented Vinegar Vapor to Extend Shelf Life of Sweet Basil (*Ocimum basilicum* L.). *Journal of Agricultural Technology*. 11(8): 2249-2256.

2016

Changsawake, K., Krusong, W., Laosinwattana, C. and Teerarak, M. 2016. Extending the Shelf-Life of Kaffir Lime (*Citrus hystrix* DC.) with Use of Ambient Glutinous Rice Vinegar Vapor, pp. 70-76. *In* Proceeding of International Forum-Agriculture, Biology, and Life Science. 5-7 August 2016, Kurume, Japan.

This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.

2017

Changsawake, K., Krusong, W., Laosinwattana, C. and Teerarak, M.
2017. Retarding Changes of Postharvest Qualities of Sweet Basil
(*Ocimum basilicum* Linn.) by Vapor-Phase Vinegar. *Journal of Herbs,
Spices and Medicinal Plants*, 1-15.



This material is reserved for educational use only, not allowed for commercial use.

Forbidden to modify the content, and cite the document when use.