

**ANTHRACNOSE INCIDENCE, BIOCHEMICAL CHANGES, POSTHARVEST
QUALITY AND GAS EXCHANGE OF CHITOSAN-COATED PAPAYA**

By

ASGAR ALI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of
Philosophy**

May 2006

DEDICATION

**To my father Professor Dr. Abdul, Shakoor Warsi without his support
and inspiration this goal could have not been achieved.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ANTHRACNOSE INCIDENCE, BIOCHEMICAL CHANGES, POSTHARVEST QUALITY AND GAS EXCHANGE OF CHITOSAN-COATED PAPAYA

By

Asgar Ali

May 2006

Chairman: Associate Professor Mahmud Tengku Muda Mohamed, PhD

Faculty : Agriculture

A study was conducted to investigate the effect of chitosan -1. on growth of *Colletotrichum gloeosporioides*, the causal organism of anthracnose; 2. mechanisms involved in controlling anthracnose disease; 3. biochemical changes; 4. physico-chemical quality characteristics and extension of storage life; 5. fruit surface study using SEM and 6. gaseous exchange of papaya fruit during storage at 12 ± 1 °C. Data were analyzed using analysis of variance and differences between treatments mean were determined by LSD. The results revealed that mycelial growth of *C. gloeosporioides* was reduced significantly ($P \leq 0.05$) at all concentrations as compared to the control. The maximum percent inhibition in mycelium growth (100%) was obtained at 2% chitosan. Similarly, conidial germination inhibition was 100% for 2.0% and 80.4% for 1.5% chitosan coating. Microscopic studies revealed that when chitosan solution was brought in direct contact with conidia there were marked deformities accompanied with shrunken conidia cellular damage and finally death of the cells at 1.5 and 2%

coatings. Chitosan (1.5%) was found highly effective in reducing of anthracnose disease upto 93.0% during five weeks storage. The reduction was found to be 85.4% during four days of ripening after five weeks of storage. There was no added advantage of 2.0% chitosan on disease reduction during storage. Marked effect on reducing disease demonstrated the fungicidal effect of chitosan. In addition to its direct microbial activity, the study strongly suggested that chitosan induces a series of defense reactions through production of inducible compounds such as phenols, peroxidase, chitinase and β -1, 3-glucanase in papaya fruits with 1.5% chitosan showing greatest activities. 1.5% chitosan coating showed maximum beneficial effect in reducing weight loss, maintaining firmness, delaying changes in peel colour and the slowing changes in soluble solids concentration (SSC) after five weeks of storage. Non-coated and 0.5% coated fruits gave significantly higher SSC compared to fruits coated with higher percentages of chitosan (1, 1.5 and 2%). The titratable acidity declined throughout the storage period with slower rate in coated fruits.

Overall sensory assessment of quality after ripening showed fruits were significantly better in quality when coated with 1.5% chitosan which were assigned higher sensory score than 1% chitosan coated fruits. Two percent coated fruits were rated as zero because of their inability to ripen.

Scanning Electron Microscopy (SEM) showed that there were no deep cracks on the surface of 1.5% chitosan coated fruits whereas in non-coated fruit cracks were found on the surface after four weeks of storage.

Chitosan coatings significantly reduced respiration rate and ethylene evolution. The coating also reduced oxygen and increased carbon dioxide level inside the fruits, thus created modified atmosphere within fruits. Modification of atmosphere was inversely proportional to the concentration applied. The two percent chitosan extremely modified the atmosphere which might be the reason for the fruits being unable to ripen when transferred to ambient temperature. Treatment with 1.5% chitosan seems to produce ideal atmosphere for maintenance of quality of papaya during storage.

The results from all experiments carried out in the study showed that 1.5% chitosan coating reduced the anthracnose disease by 93.0% and extended postharvest life upto five weeks while maintaining acceptable quality. One percent resulted in poorer quality fruits as compared to 1.5% coated fruits. Two percent chitosan seems non-physiological for Eksotika papaya-II in term of maintaining quality. As a non-toxic, biodegradable byproduct from sea food, chitosan has the potential to become a natural preservative for protecting papaya fruits, thus assisting the goal of sustainable agriculture. Extension of storage from upto five weeks would facilitate the export of fruits to long distance markets by sea and thereby cost of export would be reducing making the fruits more competitive in the world market.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**INSIDEN ANTRAKNOSE, PERUBAHAN BIOKEM, KUALITI LEPAS TUAI
DAN PERTUKARAN GAS DARI PEMBERIAN CHITOSAN PADA BETIK**

Oleh

ASGAR ALI

May 2006

Pegerusi : Profesor Madya Mahmud Tengku Muda Mohamed, PhD

Fakulti : Pertanian

Suatu kajian telah dijalankan terhadap penggunaan salutan kitosan untuk pengekalan kualiti pascatuai betik Eksotika II semasa penyimpanan. Objektif kajian adalah menilai keberkesanan kitosan didalam mengawal antraknos buah betik diperingkat pascatuai. Data telah dianalisa menggunakan analisis varian dan perbezaan antara min di dalam rawatan telah ditentukan oleh LSD. Keputusan menunjukkan pertumbuhan misilia *C. gloesporioides* telah berkurangan secara signifikan ($P \leq 0.05$) pada semua kepekatan berbanding dengan kawalan. Perencatan yang maksimum (100%) diperolehi dengan penggunaan 2% kitosan. Perencatan percambahan konidia juga adalah sama iaitu 100% dengan penggunaan 2% kitosan, diikuti oleh 80.4% untuk 1.5%, 65% untuk 1% dan 57% untuk 0.5% penyalutan kitosan. Kajian mikroskop menunjukkan larutan kitosan dirawat secara terus kepada konidia telah menyebabkan konidia berubah bentuk diikuti dengan pengecutan konidia akibat

kerosakan sel dan berakhir dengan kematian sel pada kepekatan 1.5 dan 2% penyalutan. Kitosan (1.5%) adalah paling berkesan dalam mengurangkan penyakit anthracnose sehingga 93.0% semasa lima minggu penyimpanan. Selepas penyimpanan selama lima minggu apabila buah yang dirawat diletakkan dalam persekitaran. Pada kawalan penyakit semasa penyimpanan ambient, rawatan 1.5% kitosan berjaya mengawal penyakit sehingga 85%. Tiada perubahan pada penurunan penyakit semasa penyimpanan dengan penggunaan 2.0% kitosan. Penurunan penyakit menunjukkan kesan kitosan sebagai racun kulat. Selain dari pada aktiviti mikrob secara terus, kajian mencadangkan kitosan mengaktifkan reaksi pertahanan secara bersiri melalui penghasilan bahan seperti fenol, peroxidase, kitin dan β -1, 3-glucanase dalam buah betik dimana 1.5% kitosan menunjukkan aktiviti yang paling sesuai. Penyalutan 1.5 % kitosan menunjukkan kesan yang paling maksimum dalam mengurangkan kehilangan berat, mengawal ketegaran, melambatkan perubahan warna kulit dan perubahan dalam kepekatan pepejal larut selepas lima minggu penyimpanan. Betik yang tidak disaluti dan yang disaluti dengan 0.5% mempunyai SSC yang tinggi berbanding betik yang disaluti dengan peratus kitosan yang tinggi. Asid tertitrat telah menurun masa penyimpanan dengan penurunan yang perlahan dalam buah yang disaluti. SSC dan TA memberikan rasa enak kepada buah betik. Keseluruhan ujian sensori terhadap kualiti telah membuktikan perbezaan yang ketara pada buah yang disaluti dengan 1.5% di mana telah memperolehi 3.99 mata penilaian diikuti dengan 3.14 bagi buah yang disalut dengan 1.0% dalam skala 0-5. Buah yang disaluti dengan 2.0% mempunyai kadar paling rendah dengan tidak memperolehi

sebaran mata penilaian. Buah ini tidak disukai oleh panel penilai. Ini terjadi kerana 2.0% menyebabkan buah tidak dapat diranumkan.

Penyalutan kitosan mengurangkan kadar respirasi secara signifikan evolusi etilena, oksigen dalaman dan menaikkan karbon dioksida dalaman terubahsuai didalam buah seterusnya menghasilkan perubahan atmosfera. Kadar perubahan atmosfera adalah bertentangan secara langsung dengan kepekatan yang telah digunakan. Dua peratus kitosan telah mengubah atmosfera dalaman buah secara keterlaluan. Ini mungkin menjadi penyebab kepada buah tidak masak bila dipindahkan kepada suhu persekitaran. Rawatan dengan 1.5% kitosan dapat menghasilkan atmosfera yang ideal untuk mengekalkan kualiti betik semasa penyimpanan. Semua keputusan menunjukkan salutan dengan kitosan 1.5% mengurangkan penyakit antraknos kepada 93% dan dapat disimpan selama lima minggu dengan pengekalan kualiti yang baik. Rawatan 1% kitosan tidak menunjukkan keputusan yang lebih baik dari pada rawatan 1.5%. Sebagai bahan tidak toksik dan boleh reput secara semulajadi serta dihasilkan daripada makanan laut, kitosan mempunyai potensi untuk menjadi bahan pengawet semulajadi bagi mengawal kualiti buah betik. Ini merupakan hasrat pertanian lestari. Penambahan masa penyimpanan daripada tiga kepada lima minggu telah memberi kemudahan untuk mengeksport betik ke pasaran yang lebih jauh melalui laut yang dapat mengurangkan kos pengangkutan menyebabkan buah ini menjadi lebih kompetitif di pasaran dunia.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Associate Professor Dr. Mahmud, Tengku Muda Mohamed, the chairman of my supervisory committee for his dedicated efforts and scholarly guidance during the entire period of research and preparation of thesis. His countless patience, encouragement, and generosity are commendable. I am also indebted to Associate Professor Dr. Zaki Abd. Rahman and Associate Professor Dr. Kamaruzaman Sijam, the members of supervisory committee for their assistance at the various stages of my research.

I am certainly grateful to Assoc. Prof. Dr. Siti Hajar Ahmad for her valuable discussion and constructive criticism during my experiments. My appreciation and honest thanks are due to all staff members of postharvest, pathology and chemistry laboratories, for their willing assistance and help during my studies.

Completing this research work owe the members of my family particularly my father, parents in law, wife, and my little master Hamzah an incalculable debt for their love, care, sacrifices, spiritual support through their prayers and patience , which made life easy throughout my studies.

Most profound thanks go to Malaysian government represented by Universiti Putra Malaysia, for giving me Graduate Research Assistantship (GRA) throughout my study.

Best of all, all praises and thanks be to Allah the most beneficent and merciful, the creator of this magnificent universe and its constituents for the immeasurable guidance, good health, and direction. His Name shall ever be praised and glorified.

I certify that an Examination Committee has met on 15th May 2006 to conduct the final examination of Asgar Ali on his Doctor of Philosophy thesis entitled "Anthracnose Incidence, Biochemical Changes, Postharvest Quality and Gas Exchange of Chitosan-Coated Papaya" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Mohd. Fauzi Ramlan, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Russly Abd. Rahman, PhD

Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Siti Hajar Ahmad, PhD

Associate professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Saichol Ketsa, PhD

Professor
Faculty of Agriculture
Kasetsart University Thailand
(External Examiner)



HASANAH MOHD. GHAZALI, PhD
Professor / Deputy Dean
School of Graduate Studies
University Putra Malaysia

Date: 11 JUL 2006

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

Mahmud Tengku Muda Mohamed, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Kamaruzaman Sijam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohammad Zaki Ab. Rahman, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ASGAR ALI

Date:

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xvii
LIST OF PLATES	xx
CHAPTER	
1 INTRODUCTION	1.1
2 LITERATURE REVIEW	2.1
2.1 Papaya	2.1
2.1.1 Anthracnose	2.3
2.2 Physiological Basis for the CA/MA Storage of Fruits and Vegetables	2.6
2.3 Physico-chemical Characteristics and Barrier Properties of Edible Coating	2.11
2.4 Chitosan	2.20
2.4.1 Fungicidal Activity of Chitosan	2.25
2.4.2 Mechanism of Action	2.32
2.4.3 Effect of Chitosan Coating on the Postharvest Quality of Fresh Produce	2.39
2.5 Quality of Fresh Produce	2.41
2.6 Scanning Electron Microscopy of Fruit Surface	2.48
3 GENERAL MATERIALS AND METHODS	3.1
3.1 Fruit	3.1
3.2 Chitosan Coating Solutions	3.1
3.3 Postharvest Treatments	3.2
4 EFFECT OF CHITOSAN ON ANTHRACNOSE DISEASE CAUSED BY (<i>Colletotrichum gloeosporioides</i>) ON PAPAYA DURING STORAGE	
4.1 Introduction	4.1
4.2 Materials and Methods	4.4
4.2.1 Isolation of Fungi Associated with Postharvest Rot of Papaya	4.4

4.2.2	<i>In vitro</i> Study on Effect of Chitosan on Growth of <i>C. gloeosporioides</i>	4.6
4.2.3	Disease Incidence and Severity on Papaya Fruits	4.9
4.3	Results and Discussion	4.11
4.3.1	Isolation of fungi Associated with Postharvest Fruit Rot	4.11
4.3.2	Effect of Chitosan on <i>In vitro</i> Growth of <i>C. gloeosporioides</i>	4.14
4.3.3	Effect of Chitosan on Anthracnose Disease of Papaya	4.22

5 EFFICACY OF CHITOSAN ON THE PRODUCTION OF INDUCIBLE COMPOUNDS IN PAPAYA FRUITS AS INDICATOR OF THE RESISTANCE MECHANISM

5.1	Introduction	5.1
5.2	Materials and Methods	5.4
5.2.1	Effect of chitosan on Disease Development	5.5
5.2.2	Determination of Total Phenolic Compounds	5.5
5.2.3	Enzyme Extraction and Assay of Peroxidase	5.5
5.2.4	Enzyme Extraction and Assay of Chitinase Activity	5.7
5.2.5	Extraction and Assay of β -1,3 Glucanase Activity	5.8
5.3	Results and Discussion	5.9
5.3.1	Disease Development	5.9
5.3.2	Induced Compounds as an Indicator of Anthracnose Resistance in Papaya Fruits	5.10

6 EFFECTS OF CHITOSAN COATING ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF PAPAYA FRUITS DURING STORAGE

6.1	Introduction	6.1
6.2	Materials and Methods	6.4
6.2.1	Plant Materials	6.4
6.2.2	Determination of Physical Quality	6.5
6.2.3	Determination of Chemical Quality	6.7
6.2.4	Scanning Electron Microscopy (SEM) of Chitosan Coated Papaya Pericarp	6.9
6.2.5	Sensory Assessment of Ripe Fruits	6.10
6.3	Results and Discussion	6.12
6.3.1	Physical Quality	6.12
6.3.2	Chemical Quality	6.30
6.3.3	Scanning Electron Micrograph Coated Papaya	6.43
6.3.4	Quality of Fruits after Ripening	6.45
6.3.5	Sensory Evaluation	6.49

7	EFFECT OF CHITOSAN COATING ON GAS EXCHANGE OF PAPAYA FRUITS		
7.1	Introduction		7.1
7.2	Materials and Methods		7.4
7.2.1	Fruits and Treatments		7.4
7.2.2	Determination of Respiration and Ethylene Production in Papaya Fruits		7.4
7.2.3	Determination of Internal Gas Composition of Papaya Fruits		7.5
7.3	Results and Discussion		7.7
7.3.1	Respiration and Ethylene Production Rate		7.7
7.3.2	Internal gas Compositions		7.16
8	GENERAL DISCUSSION AND CONCLUSION		8.1
	REFERENCES		R1
	BIODATA OF THE AUTHOR		B1

LIST OF TABLES

Table	Page
4.1. Fungi isolated from rotting papaya (<i>Carica papaya</i>) fruits.	4.11
4.2. Rates of disease development caused by the major fungi isolated from papaya fruits incubated at $28 \pm 2^\circ \text{C}$.	4.12
4.3. Effect of different concentrations of chitosan on anthracnose disease incidence (DI %) and severity after 5 weeks of storage at 12°C with additional four days at ambient temperature.	4.24
6.1 Hedonic scale for the evaluation of sensory assessment of ripe papaya fruits.	6.10
6.2 Effect of different concentrations of chitosan coating on the physical quality of ripe Eksotika II papaya after 5 weeks of storage when the fruits were allowed to ripen at ambient temperature ($28 \pm 2^\circ \text{C}$).	6.46
6.3 Effect of different concentrations of chitosan coating on the biochemical quality of ripe Eksotika II papaya.	6.49
6.4 Effect of different concentrations of chitosan on the sensory traits	6.50

LIST OF FIGURES

Figure		Page
2.1	Schematic diagram showing preparation of chitin and chitosan.	2.23
4.1	Effect of different concentrations of chitosan on mycelial growth inhibition of <i>C. gloeosporioides</i> after seven days' incubation.	4.15
4.2	Effect of different concentrations of chitosan on inhibition of conidial germination of <i>C. gloeosporioides</i> .	4.17
4.3	Effect of different concentrations of chitosan on anthracnose incidence caused by <i>C. gloeosporioides</i> on papaya fruits during storage.	4.23
4.4	Effect of chitosan concentration on the severity of anthracnose in papaya fruits during five weeks' of cold storage	4.26.
5.1	Effect of different concentrations of chitosan on anthracnose incidence (%) in papaya fruits.	5.10
5.2	Total phenols in papaya fruits treated with different concentrations of chitosan and inoculated with <i>C. gloeosporioides</i> .	5.11
5.3	Total peroxidase (PO) activity in papaya fruits treated with different concentrations of chitosan and challenge inoculated with <i>C. gloeosporioides</i> .	5.14
5.4	Chitinase activity in papaya fruits treated with different concentrations of chitosan and challenge inoculated with <i>C. gloeosporioides</i> .	5.17
5.5	Glucanase activity in papaya fruits treated with different concentrations of chitosan and challenge inoculated with <i>C. gloeosporioides</i> .	5.18
6.1	Effect of different concentrations of chitosan coatings on the weight loss of papaya fruits during storage of five weeks.	6.13

6.2	Effect of different concentrations of chitosan on the firmness of papaya fruits during storage for five weeks.	6.19
6.3	Development of L* of peel in Eksotika-II papaya coated with different concentrations of chitosan over five weeks of storage at $12 \pm 1^{\circ}\text{C}$.	6.25
6.4	Development of C* of peel in Eksotika-II papaya coated with different concentrations of chitosan over five weeks of storage at $12 \pm 1^{\circ}\text{C}$.	6.26
6.5	Development of h ⁰ of peel in Eksotika-II papaya coated with different concentrations of chitosan over five weeks of storage at $12 \pm 1^{\circ}\text{C}$.	6.27
6.6	Effect of different concentrations of chitosan coatings on the soluble solids concentrations of papaya fruits during storage of five weeks.	6.31
6.7	Effect of different concentrations of chitosan on titratable acidity of papaya during storage for five weeks.	6.35
6.8	Effect of different concentrations of chitosan on the pH of papaya during storage for five weeks.	6.38
6.9	Effect of different concentrations of chitosan coating on the ascorbic acid content of papaya during storage for five weeks.	6.40
7.1	Effect of different concentrations of chitosan on respiration rate of papaya fruits during storage at $12 \pm 1^{\circ}\text{C}$ plus two days ambient temperature. Arrow indicating fruits transferred to ambient temperature after five weeks of cold storage.	7.8
7.2	Effect of different concentrations of chitosan on ethylene evolution from papaya fruits during storage at $12 \pm 1^{\circ}\text{C}$ plus two days at ambient temperature. Arrow indicating fruits transferred to ambient temperature after five weeks of cold storage.	7.10
7.3	Effect of different concentrations of chitosan on internal CO ₂ (%) in papaya fruits during storage at 12°C plus two days ambient temperature. Arrow indicating fruits transferred to ambient temperature after five weeks of cold storage.	7.17
7.4	Effect of different concentrations of chitosan on percentage of internal O ₂ in papaya fruits during storage at 12°C plus two days at ambient temperature. Arrow indicating fruits transferred to ambient temperature after five weeks of cold storage.	7.18

LIST OF PLATES

Plates	Page
4.1 Effect of different concentrations of chitosan on mycelial growth of <i>C. gloeosporioides</i> isolated from papaya fruits after seven days of incubation at $28 \pm 2^{\circ}$ C. A = Control; B = 0.25; C = 0.5; D = 0.75; E = 1; F = 1.25; G = 1.5; H = 1.75; I = 2 % chitosan in 0.5 % acetic acid.	4.16
4.2 Effect of different concentrations of chitosan on conidial germination of <i>C. gloeosporioides</i> after 7 hours' incubation. (a) 0.5%, (b) 1 % (c) 1.5% (d) 2% chitosan solution (e) Control (deionized water) and (f) ungerminated healthy conidia. gt: germ tube; arrow showing shriveled and shrunken conidia.	4.18
6.1 Effect of chitosan coating on the papaya pericarp. (A) Film of chitosan; (B) Pericarp of chitosan-coated fruit; (C) pericarp without chitosan coating. The arrow points to a deep crack in the surface.	6.44