CHITOSAN-COATED FOOD PRESERVING FILM SURFACE MODIFIED BY USING DBD PLASMA TECHNIQUE FOR ANTIMICROBIAL PROPERTY IMPROVEMENT



Siriporn Theapsak

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science The Petroleum and Petrochemical College, Chulalongkorn University in Academic Partnership with The University of Michigan, The University of Oklahoma, and Case Western Reserve University 2010

530020

Thesis Title:	Chitosan-Coated Food Preserving Film Surface Modified by
	Using DBD Plasma Technique for Antimicrobial Property
	Improvement
By:	Siriporn Theapsak
Program:	Polymer Science
Thesis Advisors:	Assoc. Prof. Ratana Rujiravanit
	Prof. Seiichi Tokura

Accepted by the Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Master of Science.

. Dean

(Asst. Prof. Pomthong Malakul)

Thesis Committee:

Ratana Ruji manit

(Assoc. Prof. Ratana Rujiravanit)

(Prof. Seiichi Tokura)

(Assoc. Prof. Sumaeth Chavadej)

Panza Norsponit

(Dr. Panya Wongpanit)

ABSTRACT

5172033063: Polymer Science Program
Siriporn Theapsak: Chitosan-Coated Food Preserving Film Surface
Modified by Using DBD Plasma Technique for Antimicrobial
Property Improvement.
Thesis Advisors: Assoc. Prof. Ratana Rujiravanit and Prof. Seiichi
Tokura 80 pp.

Keywords: Dielectric Barrier Discharge/ Hydrophilicity/ Chitosan/ Surface Modified/ Antimicrobial agent.

In order to improve the antimicrobial activity of food preserving films, the surfaces of four polymeric films (PVC, PE, PP, and PLA) were modified by dielectric barrier discharge (DBD) plasma before the chitosan coating to augment the interaction between the polymeric films and chitosan. The plasma-treated polymeric film samples were further immersed in a chitosan acetate solution with different concentrations of chitosan. The degree of deacetylation and the viscosity-average molecular weight of chitosan were 97 % and 807 kDa, respectively. The optimum plasma treatment time was 10 s as determined from contact angle measurements. The effect of the plasma treatment on the surface roughness was determined by atomic force microscopy (AFM). It was found that the surface roughness of all the plasmatreated films increased as compared to the plasma-untreated films. From the spectra of the X-ray photoelectron spectroscopy (XPS), the increments of polar functional groups, such as $O=C-O^{-1}$ and $C-O^{-1}$, on the plasma-treated polymeric films were observed. It might be postulated that the DBD plasma enhanced the hydrophilicity of the polymeric films. The effects of chitosan concentration and washing cycles on the amount of coated chitosan on the polymeric films were investigated by the Kjaldahl method. The chitosan-coated polymeric films exhibited excellent antimicrobial activity against Escherichia coli and Staphylococcus aureus.

บทคัดย่อ

ศิริภรณ์ เทพศักดิ์ : การเคลือบไคโตซานบนพื้นผิวแผ่นฟิล์มถนอมอาหารที่ถูกคัดแปลง ด้วยเทคนิคพลาสมาแบบไคอิเล็กทริคแบริเออร์ดิสซาร์งเพื่อเพิ่มคุณสมบัติการด้านเชื้อแบคทีเรีย (Chitosan-Coated Food Preserving Film Surface Modified by Using DBD Plasma Technique for Antimicrobial Property Improvement) อ.ที่ปรึกษา : รศ. คร.รัตนา รุจิรวนิง, และ ศ.คร.เซอิจิ โทคุระ 80 หน้า

ในการเพิ่มจุณสมบัติการด้านเชื้อแบคทีเรียบนพื้นผิวแผ่นฟิล์มถนอมอาหาร พื้นผิวของ แผ่นฟิล์มสี่ชนิด ได้แก่ พอลิไวนิลคลอไรด์ พอลิเอทิลีน พอลิพรอลิลีน และพอลิแลกติกแอซิด จะ ผ่านการดัดแปลงพื้นผิวด้วยพลาสมาแบบไดอิเลีกทริกแบริเออร์ดิสชาร์จเพื่อเพิ่มการมีปฏิสัมพันธ์ ระหว่างแผ่นฟิล์มกับไคโตซาน โดยแผ่นฟิล์มที่ผ่านการดัดแปลงพื้นผิวแล้วได้ทำการจุ่มลงใน สารละลายไคโตซานอะซิเตทที่มีความเข้มข้นต่างกัน ทั้งนี้ก่าระดับการกำจัดหมู่อะซิทิลและมวล โมเลกุลของไคโตซานที่สกัดได้กือ 97 เปอร์เซนต์และ 807 กิโลดาลตัลตามลำดับ เวลาที่เหมาะสม ในการดัดแปลงพื้นผิวแผ่นฟิล์มกือ 10 วินาทีซึ่งทดสอบโดยก่ามุมสัมผัสหยดน้ำ จากผลของเวลา ในการดัดแปลงพื้นผิวด้วยพลาสมาต่อความขรุงระได้ทำการตรวจสอบโดยกล้องจุลทรรศน์แรง อะตอม (AFM) พบว่าหลังจากการดัดแปลงพื้นผิวแผ่นฟิล์มทั้งสี่จะมีความขรุงระเพิ่มขึ้น จากการ วิเคราะห์ทางเคมีด้วยเทคนิก X-ray photoelectron spectroscopy (XPS) พบว่ามีหมู่ฟังก์ชัน O=C–O⁻ และ C–O⁻ บนพื้นผิวแผ่นฟิล์มเกิดขึ้น ด้วยเหตุนี้สามารถกล่าวได้ว่าพลาสมาแบบไดอิ เล็กทริกแบริเออร์ดิสชาร์จสามารถเพิ่มจุณสมบัติการดูดซับน้ำ (Hydrophilicity) บนพื้นผิว แผ่นฟิล์ม และในการทดสอบจุณสมบัติการยับยั้งแบคทีเรียพบว่าแผ่นฟิล์มที่ค่านการดัดแปลง พื้นผิวด้วยพลาสมาและเคลือบด้วยไคโตซานแล้ว จะมีประสิทธิภาพในการด้านเชื้อแบกทีเรียชนิด *Escherichia coli* และ Staphylococcus aureus ได้อย่างยอดเยี่ยม

ACKNOWLEDGEMENTS

Beyond the list of individuals and program, I wish to extend my thanks to whom are not in the limit of this format. I would like to thank the following persons and programs for the guidance, encouragement, and support.

First of all, the author is deeply indebted to Assoc. Prof. Ratana Rujiravanit, my thesis advisor, for providing useful recommendation, creative comment, and encouragement throughout the course of her work. The author also would like to express my thanks to Prof. Seiichi Tokura, for his invaluable suggestion and criticism.

The author would like to thank Assoc. Prof. Sumaeth Chavadej and Dr. Panya Wongpanit, for their advice and for being on the thesis committee.

I am grateful for the scholarship and partial funding of the thesis work provide by Center for Petroleum and Petrochemicals College; and the National Center of Excellence for Petroleum, Petrochemicals, and Advanced Materials, Thailand.

This work was also support by Master Research Grants Program from Thailand Research Fund (TRF-MRG). Special thank to surapon food public company for their support of shimp shells, the raw material used throughout this work.

I am indebted to my many student colleagues for providing a stimulating and fun environment to learn and grow. Particularly thanks are given to Anyarat, Tuspon, Ponya, and Pannee for available themselves to me. Also, I simply say thanks to friends who made the two years such a memorable experience.

I would like to express my special thanks to Mr. Shinki Maki for his kindness in giving me useful XPS information and also to Faculty of Chemistry, Materials and Bioengineering and High Technology Research Centre, Kansai University.

Finally, I cannot finish without saying how grateful I am with my family. I would like to thank my family for their love, spirit, and understanding during my study and research work.

TABLE OF CONTENTS

				PAGE
	Title I	Dage		i
	Abstr	act (in English)		iii
	Abstr	act (in Thai)		iv
	Ackn	owledgements		v
	Table	of Contents		vi
	List o	f Tables	;	ix
	List o	f Figures		x
CHA	CHAPTER			
	I	INTRODUCTION		1
	II	LITERATURE REVIEW		3
	III	EXPERIMENTAL		23
		3.1 Chemical and Materials		23
		3.1.1 Materials		23
		3.1.2 Chemicals		23
		3.1.3 Air Gas for Reaction		23
		3.2 Equipment		24
		3.2.1 Capillary Viscometer		24
		3.2.2 Contact Angle Measurement		24
		3.2.3 Lloyd Tensile Tester		24
		3.2.4 Thermogravimetric Analyzer	(TGA)	24
		3.2.5 Atomic Force Microscopy (Al	FM)	24
		3.2.6 X-ray Photoelectron Spectroso	copy (XPS)	25
		3.2.7 Fourier Transformed Infrared	Spectroscopy (FTIR)	25

	3.3 Methodology	25
	3.3.1 Preparation of Chitin and Chitosan	25
	3.3.2 Characterization of Chitin and Chitosan	26
	3.3.3 Plasma Treatment and Sample Preparation	28
	3.3.4 Characterization of Chitosan-Coated on Polymeric	
	Films	29
	3.3.5 Antimicrobial Evaluation	30
		11
IV	RESULTS AND DISCUSSION	32
	4.1 Preparation and Characterization of Chitosan	32
	4.1.1 Chitosan Production	32
	4.1.2 Determination of the Degree of Deacetylation (%DD)	
	of Chitosan	33
	4.1.3 Determination of the Molecular Weight of Chitosan	35
	4.2 Characterization of Modified Surfaces	36
	4.2.1 Effect of Plasma Treatment Time on Contact Angle	36
	4.2.2 Effect of Plasma Treatment Time on the Mechanical	
	Properties	37
	4.2.3 Chemical Composition of DBD Plasma Treated	
	Polymeric Surface	39
	4.2.4 Effect of DBD Plasma Treatment on Surface	
	Morphology	43
	4.2.5 Effect of The DBD Plasma Treatment on the	
	Functional Group of the Plasma Treated Surfaces	46
	4.3 Characterization of Chitosan-Coated Polymeric Films	48
	4.3.1 Thermogravimetric Analysis	48
	4.3.2 FTIR Spectra of Chitosan-Coated Polymeric Films	
	in Different Concentration	49
	4.3.3 Effect of The DBD Plasma Treatment on The	
	Functional Group of the Plasma Treated Surfaces	50

	4.3.4 Effect of Number of Washing Cycle on Amount	
	of Deposited Chitosan	52
	4.3.5 Chitosan Content Determination	53
	4.3.6 Staining of Chitosan Deposited on Plasma-Treated	
	Polymeric Surfaces	55
	4.4 Antimicrobial Activity of Chitosan-Coated Polymer Films	57
/	CONCLUSIONS	60
	REFERENCES	62
	APPENDIX	68
	Appendix A Determination of the Molecular Weight	
	of Chitosan	68
	Appendix B Contact Angle Measurement	76
	Appendix C Effect of Plasma Treatment Time on	
	the Mechanical Properties	77
	Appendix D Kjeldahl Method	78
	CURRICULUM VITAE	80

viii

LIST OF TABLES

TABLE

4.1	Yield of chitin and chitosan production from shrimp shell	32
4.2	Characteristics of chitosan treated samples	35
4.3	Percent peak area of XPS C1s spectra of before and after	
	DBD plasma in air of the PVC, PE, PE and PLA polymeric	
	films for 10 s	42
4.4	Relative chemical composition and atomic ratios of fourth	
	polymeric films determine by XPS	43
4.5	The root mean square roughness (RMS) of before and after	
	plasma-treated films	46
4.6	Bacterial reduction (BRR%) of chitosan-coated polymeric	
	films	59

PAGE

LIST OF FIGURES

FIGUF	Æ	
2.1	Schematic view of (a) plasma and (b) discharge.	3
2.2	General structure of a glow discharge.	8
2.3	Schematic diagram of a capacitively-coupled plasma (CCP)	
	plasma source with an equivalent electrical circuit.	9
2.4	Schematic drawing of Microwave discharge.	10
2.5	Phases of gliding arc evolution: (A) initial gas break-down;	
	(B) equilibrium heating phase; (C) non-equilibrium reaction	
	phase.	10
2.6	The corona discharge-inhomogeneous discharge at atmospheric pressure.	12
2.7	Experimental set up of dielectric barrier discharge plasma	
	(DBD)	13
2.8	Schematic diagram of parallel-plate DBD plasma source	
	configurations.	13
2.9	Schematic diagram of cylindrical DBD plasma source	
	configurations.	14
2.10	3D views of AFM images of PET textile surface. (a)	
	Untreated surface; (b) 60 s treated surface; (c) 120 s treated	
	surface.	16
2.11	SEM images of the polyester fibers, (a) untreated; (b) air/Ar	
	plasma treated. The sample were treated at a dielectrics	
	space of 3 mm for 150 s.	18
2.12	Chemical structures of chitin and chitosan.	20
3.1	Simplified representation of preparation of chitin and	
	chitosan.	26
3.2	Schematic show of the experimental setup of DBD plasma	28
3.3	Flow chart of the entire experimental procedure.	31

FIGURE

4.1	The influence of plasma treatment time on the tensile	
	strength of four polymeric films.	34
4.2	FTIR spectra of chitin and chitosan: (a) chitin, (b) 1 st	
	deacetylation, (c) 2 nd deacetylation, (d) 3 rd deacetylation,	
	and (e) 4 th deacetylation.	34
4.3	Variation curve of water contact angle versus DBD plasma	
	treatment time of four polymeric films; (a) PVC films, (b)	
	PE films, (c) PP films, and (d) PLA films.	37
4.4	The influence of plasma treatment time on the tensile	
	strength of four polymeric films.	38
4.5	The influence of plasma treatment time on percent	
	elongation at break of four polymeric films.	39
4.6	Deconvoluted XPS C1s core level spectra of PVC films; (a)	
	before and (b) after plasma treated for 10 s.	40
4.7	Deconvoluted XPS C1s core level spectra of PE films; (a)	
	before, and (b) after plasma treated for 10 s.	41
4.8	Deconvoluted XPS C1s core level spectra of PP films; (a)	
	before, and (b) after plasma treated for 10 s.	41
4.9	Deconvoluted XPS C1s core level spectra of PLA films; (a)	
	before, and (b) after plasma treated for 10 s.	42
4.10	Three-dimensional AFM images of the PVC surface: (a)	
	before; and (b) after plasma treatment for 10 s.	44
4.11	Three-dimensional AFM images of the PE surface: (a)	
	before; and (b) after plasma treatment for 10 s.	44
4.12	Three-dimensional AFM images of the PP surface: (a)	
	before; and (b) after plasma treatment for 10 s.	45

FIGURE

413	Three-dimensional AFM images of the PLA surface: (a)	
	before plasma treatment; and (b) after plasma treatment for	
	10 s.10 s.	45
4.14	ATR-FTIR spectra of four polymeric films before and after	
	plasma treatment for 10 s; (a) PVC films, (b) PE films, (c)	
	PP films, and (d) PLA films.	48
4.15	TGA thermogram of four polymeric films before and after	
۰.	coating with 2% chitosan concentration; (a) PVC films, (b)	
1	PE films, (c) PP films, (d) PLA films.	49
4.16	The ATR-FTIR spectra of treated PVC films, chitosan and	
	chitosan-coated PVC films.	50
4.17	The ATR-FTIR spectra of treated PE films, chitosan and	
•.	chitosan-coated PVC films.	51
4.18	The ATR-FTIR spectra of treated PP films, chitosan and	
•	chitosan-coated PVC films.	51
4.19	The ATR-FTIR spectra of treated PLA films, chitosan and	
	chitosan-coated PVC films.	52
4.20	Effect of number of washing cycle on amount of chitosan	
	deposited on four polymeric films.	53
4.21	Relation between the concentrations of chitosan and the	
	amount of chitosan deposited on untreated films and plasma-	
	treated films of four polymeric films; (a) PVC films, (b) PE	
	films, (c) PP films, and PLA films.	54
4.22	Photographic of the neat polymeric films, the polymeric	
	coated with 0.5 % chitosan concentration and the polymeric	
	coated with 2% chitosan concentration, after they had been	
	immersed in the aqueous solution of the dye for 12 h.	56

PAGE

.

FIGURE

4.23 Number of colonies of neat polymeric films and chitosan-coated all polymeric films; (a) PVC films, (b) PE films, (c) PP films and (d) PLA films, containing 0.25%, 0.75% and 2.0% of concentration of chitosan and the corresponding bacterial reduction rate (BRR) values against Gram-positive *Staphylococcus aureus* and Gramnegative *Escherichia coli*.

PAGE