

**A STUDY ON THE EXTRACTION & APPLICATIONS
OF CHITOSAN IN THE FOOD INDUSTRY**

N.D. Wanniarachchi

(09/8095)



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

Degree of Master of Science

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

June 2013

A STUDY ON THE EXTRACTION & APPLICATIONS OF CHITOSAN IN THE FOOD INDUSTRY

N.D. Wanniarachchi

(09/8095)



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

Thesis/Dissertation submitted in partial fulfillment of the requirements for the degree
Master of Science

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

June 2013

DECLARATION

I declare that this is my own work and this thesis does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any University or other institute of higher learning and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where the acknowledgement is made in the text.

I hereby grant the University of Moratuwa the right to archive and to make available my thesis or dissertation in whole or part in the University Libraries in all forms of media, subject to the provisions of the current copyright act of Sri Lanka. I retain all proprietary rights, such as patent rights. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Date:



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

We have supervised and accepted this thesis for the award of the degree.

Signature of the supervisor

Date

.....

Dr. F. M. Ismail

.....

Dr. S. H. P. Gunawardena

.....

Mr. A. M. P. B. Samarasekera

ABSTRACT

Chitin and chitosan are renewable natural biopolymers having extensive applications in food and many other industries due to their low toxicity, biocompatibility, biodegradability, etc. The main industrial raw material for chitin production is waste from seafood industries that consist of exoskeleton remains of crustaceans.

Isolation of chitin from prawn shells involved demineralization, deproteinization and purification processes. Chitin was converted to chitosan through deacetylation using 45% NaOH by different physical treatments; steeping at ambient temperature, thermal heating and microwave radiation. Chitosan produced from chitin was characterized using FTIR analysis. Antimicrobial activity of chitosan against *E-coli* and *Aspergillus niger* was investigated by agar dilution assay where as an indirect method of cellulose yield was tested for *Acetobacter xylinum*. Properties of purified chitosan dehydrated by different methods (freeze, vacuum and normal oven drying) were determined by FTIR, viscometry and antibacterial activity.

Prawn shells contain mainly chitin, protein and minerals; the chitin content was approximately 22 %. Among the different physical treatments for deacetylation, conventional thermal heating and microwave radiation methods gave DDA values in the range of 69 – 99 % where as steeping did not give significant DDA value even after 10 days. Microwave radiation was the most energy, time and cost effective method for deacetylation as it reduced reaction time significantly.

Chitosan had effective antimicrobial properties against three microorganism species studied. Antimicrobial activity of chitosan depends on its DDA, concentration and microorganism species.

Purification of chitosan by dissolving in acetic acid reduced DDA by approximately 10 %. The dehydration method used to dry purified chitosan had an effect on antimicrobial properties since freeze drying gave higher antimicrobial activity because of better retainment of active amine sites. However the method of drying has not influenced the molecular weight and DDA.

This study ultimately addressed a major concern in Sri Lankan seafood industry by adding value to a waste product.

Keywords: Chitin, Chitosan, DDA, Antimicrobial Activity

DEDICATION

I lovingly dedicate this thesis to my parents and husband,



who supported me each step of the way.
University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

ACKNOWLEDGEMENT

I am very much grateful to Dr. (Mrs.) F. M. Ismail, Senior Lecturer, Department of Chemical and Process Engineering, University of Moratuwa for being the main supervisor of this research project. I am also grateful to Dr. (Mrs.) S. H. P. Gunawardena, Senior Lecturer, Department of Chemical and Process Engineering, University of Moratuwa and Mr. A. M. P. B. Samarasekera, Senior Lecturer, Department of Material Science and Engineering, University of Moratuwa for being co-supervisors of this project.

I am thankful to Dr. F. M. Ismail for giving me the moral support and guidance throughout this research work and Dr. S. H. P. Gunawardena for her helpful advices and giving me words of encouragements. I wish to thank to Mr. A. M. P. B. Samarasekera for sharing his valuable experience and facilitating me in obtaining FTIR spectroscopy measurements. Further I would like to thank Prof. (Mrs.) B. M. W. P. K. Amarasinghe, Senior Lecturer, Department of Chemical and Process Engineering, University of Moratuwa for her valuable advices being the chairperson of the progress reviews.

My sincere thanks also go to Prof. K. K. D. S. Ranaweera, Director, Bandaranaike Memorial Ayurveda Research Institute and Prof., A. A. P. De Alwis, Senior Lecturer, Department of Chemical and Process Engineering, University of Moratuwa for serving on my final defence committee and for their encouragement and insightful comments.

I would like to extend my sincere appreciation to the Atomic Energy Authority of Sri Lanka for facilitating me in determining the molecular weight of chitosan by viscosity measurements. My special thank to their scientific staff, especially Scientific Officer Mr. K. R. C. De Silva, Senior Scientific Officer Ms. D. C. K. K. Dissanayake and Ms. S. S. Kulatunga, Head, Radiation Processing Section,

for helping me in carrying out the experiments and for sharing their valuable experience.

I wish to express my gratitude to Technical Officer Mrs. I.K Athukorala, Senior Staff Technical Officers; Ms. A.S Wahalathanthri, Mr. R. T. Masakorela and Mr. Jayaweera Wijesinghe, Lab Assistant Mr. W. L. Fernando and all the other technical officers and lab assistants of Department of Chemical and Process Engineering, University of Moratuwa for their assistance in carrying out experiments.

This M.Sc. thesis was supported by University of Moratuwa Senate Research Grant Number SRC/LT/2009/39.

Finally, I would like to express my heartiest thanks to my parents, husband, brothers, all my family members and friends who always gave me courage and helped me in numerous ways in different stages of the project, which was of extreme importance in bringing out this effort a success.



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

2.9.2.2	Antimicrobial Properties	28
2.10	Reactions of Chitin and Chitosan	29
2.11	Applications	30
2.11.1	Applications of chitosan in the food industry	32
Chapter 3:	Materials & Methods	34
3.1	Extraction of Chitin	34
3.1.1	Raw Material Preparation	34
3.1.2	Demineralization	36
3.1.3	Deproteinization	36
3.1.4	Purification	37
3.2	Determination of the Composition of Prawn Shells	37
3.3	Preparation of Chitosan (Deacetylation)	37
3.3.1	Steeping in 45% NaOH	38
3.3.2	Thermal Heating with 45% NaOH	38
3.3.3	Thermal heating followed by steeping with 45% NaOH	38
3.3.4	Microwave Radiation with 45% NaOH	38
3.4	Testing of Degree of Deacetylation	39
3.5	Investigation of the Antimicrobial Efficacy of Chitosan	40
3.5.1	Antibacterial Efficacy	40
3.5.2	Antifungal Efficacy	42
3.5.3	Antibacterial Efficacy Against <i>Acetobacter Xylinum</i> Bacteria	42
3.6	Comparison of Methods of Dehydration of Purified Chitosan	43
3.6.1	Purification of Chitosan	43
3.6.2	Dehydration of Purified Chitosan	43
3.6.2.1	Freeze Drying	44
3.6.2.2	Vacuum Drying	44
3.6.2.3	Normal Oven Drying	44
3.6.3	Testing of the Characteristics of Purified Chitosan	44



3.6.3.1	Testing of DDA	44
3.6.3.2	Molecular Weight Measurement	45
3.6.3.3	Antibacterial Properties	45
Chapter 4:	Results and Discussion	46
4.1	Isolation of Chitin	46
4.1.1	Effect of Prawn Shell Size Reduction	46
4.1.2	Effect of Demineralization	46
4.1.3	Effect of Deproteinization	47
4.1.4	Chitin Purification	48
4.2	Composition of Prawn Shells	49
4.3	Synthesis of Chitosan from Chitin	50
4.4	Economic Aspects	51
4.5	Analysis of FTIR Spectra of Chitin and Chitosan	52
4.6	Deacetylation of Chitin in 45 % NaOH by Different Physical Treatments	56
 4.6.1	Effect of Steeping in 45% NaOH on Deacetylation	57
4.6.2	Effect of Thermal Heating on Deacetylation	58
4.6.3	Effect of Microwave Radiation with 45% NaOH on Deacetylation	61
4.7	Investigation of the Antimicrobial Efficacy of Chitosan	65
4.7.1	Effect on <i>E-coli</i> and <i>Aspergillus Niger</i>	65
4.7.2	Effect on <i>Acetobacter Xylinum</i>	67
4.8	Potential Applications in the Food Industry	69
4.9	Comparison of Methods of Dehydration of Purified Chitosan	70
4.9.1	Determination of DDA of Purified Chitosan	71
4.9.2	Determination of Molecular Weight of Chitosan	72
4.9.3	Antimicrobial Activity	73
Chapter 5:	Conclusions & Recommendations	75
References		77

LIST OF FIGURES

	Page
Figure 2.1 Structures of glucosamine (monomer of chitosan) and glucose (monomer of cellulose)	6
Figure 2.2 Structures cellulose, chitin and chitosan	7
Figure 2.3 Chemical structural representation of chitin and chitosan depicting the co-polymer character of the biopolymers	8
Figure 2.4 The antiparallel chain arrangement in α -chitin	9
Figure 2.5 The parallel chain arrangement in β -chitin	9
Figure 2.6 Shrimp Production in Sri Lanka	12
Figure 2.7 Isolation of chitin and preparation of chitosan by chemical process	14
Figure 2.8 The method of determining the absorption	21
Figure 2.9 Illustration of the possible reaction sites in chitin and chitosan	29
Figure 3.1 (a) Indian tiger prawn (<i>Penaeus Indicus</i>) and (b) Flowery (Green tiger) prawn (<i>Penaeus Semisulcatus</i>)	34
Figure 3.2 Prawn processing at a sea food processing factory (a) cleaned body parts of prawns and (b) waste heads of prawns	35
Figure 3.3 Raw material preparation (a) removal of meat residues, (b) removed meat residues, (c) separated prawn shells and (d) dried prawn shells	35
Figure 3.4 Isolation of chitin	36
Figure 3.5 Isolation of chitin (a) demineralization and (b) deproteinization	37

Figure 3.6	FTIR spectrometer (Model: Bruker)	39
Figure 3.7	An Inoculated agar plate in which chitosan discs have been inserted	40
Figure 3.8	(a) A Petri lid in which glass rods have been fixed (b) An Agar Plate with wells	41
Figure 4.1	Demineralised prawn shells	47
Figure 4.2	Deproteinised prawn shell power (before purification)	48
Figure 4.3	Chitin isolated from prawn shells	48
Figure 4.4	Composition of prawn shells	49
Figure 4.5	Chitosan	51
Figure 4.6	FTIR spectra of transmittance of synthetic chitin and chitin isolated from prawn shells	53
Figure 4.7	FTIR spectrums of chitosan prepared from prawn shell chitin	54
Figure 4.8	FTIR spectra of chitin and chitosan	55
Figure 4.9	FTIR spectrum of chitosan H-7 S-16	56
Figure 4.10	FTIR spectra of chitin that was steeped in 45 % NaOH solution for different time intervals	57
Figure 4.11	FTIR spectra of chitosans prepared by thermal heating method	59
Figure 4.12	DDA % Vs. reaction time of chitosans prepared by thermal heating method	60
Figure 4.13	FTIR spectra of chitin that was exposed to microwave radiation at power level 60% for different time intervals	62



Figure 4.14	FTIR spectra of chitin that was exposed to microwave radiation at power level 80% for different time intervals	63
Figure 4.15	Degree of deacetylation of chitosan prepared by microwave radiation method at 80 % power level	64
Figure 4.16	Bacterial cellulose pellicles produced	68
Figure 4.17	Bacterial cellulose yields against chitosans with different DDA values	68
Figure 4.18	Dehydrated purified chitosans (a) freeze dried (b) vacuum oven dried (c) normal oven dried	70
Figure 4.19	FTIR spectra of purified chitosans dehydrated by different methods and respective non purified chitosan	71
Figure 4.20	DDA values of purified chitosans dehydrated by different methods and respective non purified chitosan	72
Figure 4.21	Bacterial cellulose yields against purified chitosans dehydrated by different methods and respective non purified chitosan	74



University of Moratuwa, Sri Lanka.
 Electronic Theses & Dissertations
 www.lib.mru.ac.lk

LIST OF TABLES

	Page
Table 2.1 Sources of chitin and chitosan	10
Table 2.2 Applications of chitin, chitosan and their derivatives	30
Table 4.1 Antimicrobial index of chitosan against <i>E-coli</i>	66
Table 4.2 Antimicrobial index of chitosan against <i>Aspergillus Niger</i>	66
Table 4.3 Viscosity average molecular weight of purified chitosans dehydrated by different methods and respective non purified chitosan	73



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk

LIST OF ABBREVIATIONS

Abbreviation	Description
DDA	Degree of Deacetylation
FTIR	Fourier Transformation of Infrared
HMWC	High Molecular Weight Chitosan
HPLC	High Performance Liquid Chromatography
IR	Infrared
LMWC	Low Molecular Weight Chitosan
NMR	Nuclear Magnetic Resonance
RNA	Ribonucleic Acid



University of Moratuwa, Sri Lanka.
Electronic Theses & Dissertations
www.lib.mrt.ac.lk