CHARACTERIZATION AND OPTIMIZATION OF BIODEGRADABLE CHITOSAN-SAGO BASED FILMS FOR FOOD PACKAGING

FADZILLAH BINTI MUHIDIN

UNIVERSITI TEKNOLOGI MALAYSIA

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FADZILLAH BINTI MUHIDIN

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical and Energy Engineering
Universiti Teknologi Malaysia

SEPTEMBER 2016

To my beloved parents, siblings and friends and most importantly Allah S.W.T

ACKNOWLEDGEMENT

Firstly, I would like to express my gratitude to Allah S.W.T for His love, care and blessings, and for directing my life into becoming a postgraduate student. This once in a lifetime opportunity had given me the chance to improve myself in terms of knowledge and maturity, and this experience had humble me more towards becoming a better servant. Insha'Allah.

Secondly, I am vastly indebted to all the people who had helped and inspired me into completing my research especially, deepest appreciation to my supervisor, Prof Mohd Ghazali Bin Mohd Nawawi for his guidance and continuous supports. I have nothing but to thank him for his time, his patience, his knowledge and encouragement for me, and know that I am thankful to get to work with such an easygoing and humble yet dedicated person. Meanwhile, my special gratitude goes to Dr. Nik Azmi Nik Mahmood, for his guidance and knowledge, and the opportunity to work with him for my antimicrobial analysis lab work.

Many thanks for the CLEAR staff, En. Zulkifli Bin Mansor and Bioprocess Engineering Laboratory staff, En. Ya'akop Bin Sabudin, for all their helps and guidance. I am extremely thankful for the continuous support and love that were given by my good friends, especially my lab mate, Nurhazerin Bt Md Wahi for being with me through thick and thin. Special love for my best friend, Norhidayah Bt Harun and Nur Aina Bt Zaidan for their moral support and care throughout this challenging times.

Finally, I wish to express my greatest appreciation for my family, especially my mother, Fatimah Bt Mat Lasim and my father, Muhidin Bin Mohd Shari, for being very patience and understanding, for giving me the strength and support to continue this research. To them, I owe everything.

ABSTRACT

Petroleum based plastics have been used in many applications especially in food packaging industries for decades. These plastics are favorably used because they are cheaper and easy to develop yet the materials are high in toxic and non-biodegradable. The accumulations of these plastics wastes have increased every year, reaching billions of tons undegraded and untreated plastics thus contributing to great environmental pollution. An alternative method of producing biodegradable plastics made from natural and renewable materials was developed in order to counter the environmental pollution issue. This research focused on the development and characterization of chitosan and sago biodegradable films incorporated with glycerol, sorbitol and lemongrass oil. The relationships between all components in the film formulations were studied and the optimization process using response surface methodology (RSM) and analysis of variance technique (ANOVA) were carried out. The chitosan-sago based films were characterized through various analysis, for instance, scanning electron microscopy was used for morphological study of the chitosan-sago films. Meanwhile, the resulting Fourier transform infrared spectra validated the functional groups interactions between components in the films. The antimicrobial susceptibility assay had efficiently inhibited the growth of *Escherichia coli* microbes, through the incorporations of lemongrass essential oil using agar and broth dilution method. Furthermore, the chitosan-sago blend formulations were analyzed using central composite design (CCD), RSM and ANOVA techniques, in order to investigate the interactions between process variables and the resulting film properties such as the water vapor permeability (WVP), water solubility (WS), tensile strength (TS), elastic modulus (EM) and elongation at break (EAB). The data fitting from RSM and ANOVA indicated that the quadratic model used in CCD and the variables-response interactions was significant ($p<0.05$) with the values for the coefficient of determinations, R^2 of 0.9037, 0.9435, 0.8717, 0.8733, and 0.8711 for WVP, WS, TS, EM and EAB, respectively. The independent variables of chitosan and sago blend significantly increased the WVP, WS, TS and EM values of chitosan-sago films while glycerol, sorbitol and lemongrass oil decreased the WVP, TS and EM values. The addition of glycerol and sorbitol increased the values of EAB of chitosan-sago based films and the addition of lemongrass oil exhibited an increased in WS values. An optimum set of film formulations was generated in this research; 100 wt.% chitosan/sago, 25 wt.% glycerol/sorbitol and 0.5 wt.% lemongrass oil has yielded an optimum response of $7.637\text{x}10^{-11}$ g/Pa.s.m^2 for WVP, 208.407 MPa for TS, $4.329\text{x}10^5$ MPa for EM, and 17.682 % for EA, with an overall desirability of 0.793. The resulted optimum values were compared between current thin plastic films and from other researchers, thus indicating that the chitosan-sago based films developed in this study were adequate and potentially met the requirements for food packaging films properties.

ABSTRAK

Plastik berasaskan petroleum telah digunakan dalam pelbagai aplikasi terutamanya dalam industri pembungkusan makanan sejak berdekad lamanya. Plastik jenis ini lebih digemari untuk digunakan di seluruh dunia kerana ianya lebih murah dan mudah untuk dihasilkan namun bahan-bahan plastik ini tinggi toksik dan tidak boleh terurai. Pengumpulan sisa plastik ini telah meningkat mencapai berbilion tan setiap tahun lalu menyumbang kepada pencemaran alam sekitar. Satu kaedah alternatif iaitu menghasilkan plastik daripada bahan semulajadi dan diperbaharui yang boleh terurai telah dibangunkan bagi mengatasi isu pencemaran ini. Kajian ini menumpukan kepada penghasilan dan pencirian filem daripada kitosan dan sagu yang digabungkan dengan gliserol, sorbitol dan minyak serai. Hubungan di antara semua komponen yang ada di dalam formulasi filem telah dikaji dan proses pengoptimuman menggunakan kaedah gerak balas permukaan (RSM) dan teknik analisis varian (ANOVA) telah dijalankan. Filem berasaskan kitosan-sagu dicirikan melalui pelbagai analisis seperti mikroskop elektron imbasan, yang digunakan untuk mengkaji morfologi filem kitosan-sagu. Sementara itu, spektra yang terhasil dari analisis inframerah transformasi Fourier mengesahkan interaksi kumpulan berfungsi yang ada di antara komponen-komponen di dalam filem. Teknik rencatan antimikrobial telah terbukti berkesan bagi menghalang pertumbuhan mikrob *Escherichia coli*, melalui gabungan minyak serai menggunakan kaedah pencairan agar dan kaldu. Tambahan pula, formulasi campuran kitosan-sagu dianalisa menggunakan teknik reka bentuk komposit pusat (CCD), RSM dan ANOVA untuk menyiasat interaksi di antara pembolehubah proses dengan ciri-ciri filem yang terhasil seperti kebolehtelapan wap air (WVP), kelarutan air (WS), kekuatan tegangan (TS), modulus elastik (EM) dan pemanjangan pada tahap patah (EAB). Penyesuaian data dari RSM dan ANOVA menunjukkan bahawa model kuadratik yang digunakan dalam CCD dengan interaksi antara pembolehubah-respon adalah ketara ($p<0.05$) dengan nilai regrasi R^2 masing-masing 0.9037, 0.9435, 0.8717, 0.8733, dan 0.8711 untuk WVP, WS, TS, EM and EAB. Pembolehubah bebas kitosan dan sagu telah sewajarnya meningkatkan nilai-nilai WVP, WS, TS dan EM filem kitosan-sagu manakala gliserol, sorbitol dan minyak serai telah menurunkan nilai-nilai WVP, TS dan EM. Penambahan gliserol dan sorbitol telah meningkatkan nilai EAB filem kitosan-sagu dan penambahan minyak serai mempamerkan peningkatan bagi nilai WS. Satu set formulasi filem yang optimum telah dijana dari kajian ini; 100 wt.% kitosan/sagu, 25 wt.% gliserol/sorbitol dan 0.5 wt.% minyak serai telah menghasilkan respon optimum, 7.637×10^{-11} g/Pa.s.m^2 untuk WVP, 208.407 MPa untuk TS, 4.329×10^5 MPa untuk EM, dan 17.682 % untuk EA, dengan kebaikan keseluruhan sebanyak 0.793. Nilai optimum yang terhasil telah dibandingkan dengan filem plastik nipis sedia ada dan daripada kajian lain sekaligus menunjukkan bahawa filem berasaskan kitosan-sagu yang dihasilkan dari kajian ini adalah mencukupi dan berpotensi untuk memenuhi ciri-ciri filem pembungkusan makanan pada masa kini.

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LIST OF ABBREVIATIONS

AM	-	Antimicrobial
ANOVA	-	Analysis of Variance
AP	-	Adequate Precision
ASTM	-	American Society for Testing and Materials
ATP	-	Adenosine triphosphate
CCD	-	Central Composite Design
CV	-	Coefficient of variance
D	-	Desirability
DD	-	Degree of deacetylation
DHA	-	Docosahexaenoic acid
DNA	-	Deoxyribonucleic acid
DOE	-	Design of Experiment
DP	-	Partial water vapor pressure differences
E	-	Percentage elongation
EAB	-	Elongation-at-break
EBSD	-	Electron backscatter electron
EDX/EDS	-	Energy Dispersive X-ray
EM	-	Elastic Modulus
EMR	-	Electromagnetic radiation
EO	-	Essential oil
EPA	-	*Eicosapentaenoic acid*
EVOH	-	Ethylene vinyl alcohol
FDA	-	Food and Drug Administration
FI	-	Factor interaction
FTIR	-	Fourier Transform Infrared
HDPE	-	High density polyethylene
IR	-	Infrared

LDPE	-	Low density polyethylene
LHS	-	Latin hypercube sampling
MAP	-	Modified atmosphere packaging
MC	-	Monte Carlo
MHA	-	Mueller Hinton agar
MHB	-	Mueller Hinton broth
MI	-	Michelson interferometer
NSP	-	Non-starch polysaccharide
OA	-	Orthogonal array
PEN	-	Polyethylene naphthalate
PET/ PETE	-	Polyethylene terephthalate
PLA	-	Polylactide
PP	-	Polypropylene
PS	-	Polystyrene
PUFA	-	Polyunsaturated fatty acids
PVC	-	Polyvinyl chloride
RH	-	Relative humidity
RSM	-	Response Surface Methodology
SEM	-	Scanning Electron Microscopy
SSR	-	Sum of squares due to regression
SST	-	Total sum of squares
TPS	-	Thermoplastic starch
TS	-	Tensile strength
TTI	-	Time-temperature indicator
UK	-	United Kingdom
UTM	-	University Technology Malaysia
UV	-	Ultraviolet
WS	-	Water solubility
WVT	-	Water vapor transmission
WVP	-	Water vapor permeability

LIST OF SYMBOLS

α	-	Alpha
A	-	Area
β	-	Beta
E	-	Mean relative deviation modulus
$FeCl_3$	-	Iron (III) chloride
G	-	Weight change
HCl	-	Hydrochloric acid
k	-	Number of independent variables
µ	-	Micro
N	-	Number of data points
NaOH	-	Sodium hydroxide
n_0	-	Number of center points
O	-	Oxygen
O_5O_4	-	Osmium tetroxide
R^2	-	Coefficient of determination
S	-	Saturation vapor pressure
t	-	Time interval
X	-	Independent variable
Y	-	Dependent variables
X_1	-	Concentration of chitosan-sago
X_2	-	Concentration of glycerol-sorbitol
X_3	-	Concentration of lemongrass oil
wt.	-	Weight
w/v	-	Weight over volume
θ	-	Angle
λ	-	Wavelength
%	-	Percentage

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

The plastic packaging materials are generally derived from non-renewable and non-biodegradable petroleum-based fuels. Due to the increasing demands in the plastic usage especially in food packaging industry, the petroleum based plastics are vastly produced in line with the extensive growth of human populations (Mollah *et al*., 2016). The non-biodegradable property of petroleum based plastic materials render the plastics to be improperly disposed, hence leaving an enormous amount of unrecyclable and untreated plastic wastes to pollute the entire world. For this reason, biodegradable films derived from natural polymers such as polysaccharides were very favorable, granted that the polymer based films can be developed from renewable resources (Dai *et al*., 2008).

In fact, it is mandatory to consider the biological recycling of polymers, as the world is dying to be cleaned from the existing plastic wastes. Therefore, new kind of polymers must be synthesized in order to enhance the recyclable property, so that the polymers can returned to the natural biological cycle state after being utilized. Certainly, natural biopolymers from agricultural resources that can easily degrade, fulfilled this criteria and helped solved these current problems, were given more interest from the researches over the last decade. Moreover, increased attentions towards the biodegradable natural polymers from renewable resources had scientist all around the world to devote the time, energy and funding into developing polymer-based food-packaging materials in order to help replace the current non-biodegradable petroleum-based films. Furthermore, overwhelming demand regarding biodegradable polymer based films with added properties for instance higher quality, longer shelf life, increased safety, and cost-efficient were indispensable in the food packaging industry.

Chitosan polymer, derived from chitin compound was extensively used in many industries, including food packaging, pharmaceutical and in agriculture fields due to its promising properties of biodegradability, biocompatibility, and bioactivity as well as the antimicrobial and film-forming ability (Garcia *et al*. 2009). On the other hand, starch is another one of promising renewable raw materials sources from variety of different crops, can be used to generate biodegradable films to substitute the current petroleum-based plastics. It is abundantly available, low in cost, renewable, and most importantly possessed biodegradable and degradation characteristics. However, starch was highly hydrophilic and direct expose to water will tend to reduce its mechanical integrity (Mathew and Dufresne, 2002). Therefore, in order to surpass this problem, starch can be mixed with other polymeric materials such as chitosan to develop a favorable food packaging films.

Plenty of studies regarding starch-based films have been produced especially about starches from corns, potatoes, wheats, rice, tapiocas, and cassavas but not so much on sago starch. For instance, Mathew and Abraham (2008) and Tonny *et al*. (2014) had developed biodegradable packaging films using chitosan and potato starches in order to studied their physical and mechanical properties while Singh and Kamlesh (2014) had prepared a biodegradable crosslinked films mainly from chitosan and corn starches in order to evaluate the films' properties and utilizations for controlled drug released. On the other hand, Maran *et al*. (2013) had presented studies regarding tapioca starch-based edible films in order to investigate the films' optical and barriers properties meanwhile Assefa and Admassu (2013) had developed antimicrobial packaging films using taro starch as the films base in order to evaluate the physicochemical and its mechanical properties. Therefore, in this present research, chitosan based edible and biodegradable films incorporated with sago starch with optimum combination of desirable mechanical properties and biodegradation performances were developed. In this study, the chitosan-sago based films were prepared through casting/solvent evaporation method. The film-forming solutions that contain chitosan and sago starch with different content was dispersed in distilled water. Plasticizers and antimicrobial was added in the solutions in order to upgrade the films' mechanical properties and serve as a barrier for surface-contaminating microorganisms. The main objective of this research was to assess the effects of plasticizers and antimicrobial concentrations towards chitosan-sago based biodegradable films and to analyze the interactions among the present constituents. The interactions will be evaluated using different means; physical and mechanical properties as well as antimicrobial activities. This research was hoped to offer an alternative packaging option, obtained from renewable resources, which does not contribute to environmental pollution and safely used to replace petroleum based plastics in food packaging industry.

1.2 Problem Statement

Current existing synthetic packaging materials such as petroleum-based plastics were typically used in industries worldwide for instance in food packaging, fish processing, agriculture industry and dairy industry. Petroleum-based plastics are extensively and favorably use to pack food products because it can be obtain in large amount at low cost, lightweight and can easily be design into many shapes. Nevertheless, despite all that, the plastic wastes cannot be degraded and difficult to dispose thus the accumulation of wastes contribute to a large amount of environmental pollution each year.

In this research, one type of biodegradable packaging film was developed, mainly from abundant renewable sources such as chitosan and sago in order to fulfill the demands for disposable packaging material. However, the problem with this chitosan and sago blend film, it is always brittle and cannot tolerate high temperature condition. Thus, slight modification was made to this film by adding plasticizers such as glycerol and sorbitol during the films blending in order to enhance the mechanical properties as well prolonged the shelf life of food products. In the meantime, the antimicrobial agent was also added to the chitosan and sago blend film to provide the film with protections from food borne bacteria. Most importantly, the film produced in this research will hope to offer a reduction to the environmental pollution caused by petroleum based plastic due to its biodegradable ability and can be used in many applications in the food industries.

1.3 Objectives of Research

The development of chitosan-sago based films in this study was carried out in order to fulfill these listed aims:

i) To develop and characterize the biodegradable chitosan-sago film formulations in terms of physical and mechanical properties as well as the antimicrobial activities.

ii) To investigate the relationship between the concentrations of chitosan-sago, glycerol-sorbitol and lemongrass oil towards the physical, mechanical and the antimicrobial properties of biodegradable chitosan-sago based film formulations and to generate an optimum set of formulation for input variables and output responses of chitosan-sago based films using RSM and ANOVA technique.

1.4 Scopes of Research

In order to achieve the objectives of this research, this following action will be executed:

i) Development and characterization of biodegradable chitosan-sago based films

Biodegradable films from chitosan and sago with varied composition were developed through solvent-casting evaporation method. Modification of the film was performed chemically through the addition of plasticizer and antimicrobial agents in order to improve the mechanical properties as well as enhancing its biological capacity. The biodegradable films was analyzed to evaluate its physicochemical properties. Surface morphologies of chitosan-sago films were conducted through Scanning Electron Microscopy (SEM) analysis. Meanwhile, functional groups and chemical bonding existed in chitosan-sago based films was identified using Fourier Transform Infrared (FTIR) analysis. Then, the physical properties of the film were elucidated through water vapor permeability, water solubility and mechanical testing; and finally antimicrobial inhibition activity of the film was tested against the growth of *E.coli* microbes.

ii) Relationship between different concentrations of independent variables towards the dependent variables as well as the optimization of chitosan-sago blend formulations

In this present study, the independent variables; the concentrations of chitosan-sago, glycerol-sorbitol and lemongrass oil was manipulated in order to evaluate their effects towards the dependent variables such as the values of water vapor permeability, water solubility, tensile strength, elastic modulus and elongation at break as well as the antimicrobial activity. The chitosan-sago concentration was ranged from (50-100) wt. % while glycerol-sorbitol concentration was ranged between (25-50) wt. % and finally the concentration of lemongrass essential oil was differed between (0.5-1.0) wt. %.

The optimization process of biodegradable chitosan-sago based film formulations was performed to determine the significance of second-degree polynomial model based on the formulations design developed from Central Composite Design (CCD) techniques. The response surface methodology (RSM) and the analysis of variance (ANOVA) were employed in the statistical analysis using Design Expert Software with pre-determined value of significance probability, to be less than 0.05. Every interaction between the effects of process variables against process responses were visualized using response surface contour plots for chitosan-sago based films in this study. After that, further optimizations were applied to the chitosan-sago based formulations using the software in order to produce the optimum set of values for process variables and responses.

1.5 Significance of Research

There have been increased demands for disposable packaging materials as well as prolonging the food shelf life and improving the food quality in the market nowadays. Since there are not so much studies about sago starch has been done, thus, a research regarding edible and biodegradable chitosan based films incorporated with sago starch with optimum mechanical properties and biodegradation performance was developed. Slight modifications for the chitosan-sago based films was performed by adding the plasticizers and antimicrobial in order to improve the mechanical properties of the film (flexibility and durability) and also serve as barrier for surface-contaminating microorganisms to provide longer shelf life for the food products. The effects of chitosan and sago concentrations, plasticizers and antimicrobial towards the film were assessed through statistical analysis and the interactions between those constituents that present in the films were analyzed through physical and mechanical means as well as the antimicrobial activities. Thus, this research will hopefully offer an alternative packaging material that is safe and will avoid any environmental pollution.

1.6 Thesis Outline

This thesis is based on five chapters that explicate the research in sequential order. First off, Chapter 1 introduced the problem statements, listed the research objectives and scopes, and explained the significance of current research. Meanwhile, Chapter 2 provides the thorough insight regarding the past and presents researches regarding the chitosan and starch based films from other researches from all around the world. Furthermore, Chapter 3 listed the materials and methods utilized in this research guided from the objectives and scopes declared in Chapter 1.

After that, the results from experimental works were presented and elaborated in depth in Chapter 4. The discussions includes the details from the characterizations of chitosan-sago based films using SEM, FTIR and the antimicrobial assay of films, the statistical analysis using RSM and ANOVA including the effects of process variables against process responses, and finally the optimizations of chitosan-sago film formulations using Design Expert Software. Lastly, Chapter 5 summarized the findings from current research and proposed some recommendations for future works.

1.7 Summary

This research focused on the developments and modifications of chitosan based films, incorporated with sago starch, glycerol, and sorbitol as well as lemongrass oil in order to increase the films physical and mechanical properties. Thus, in this chapter, the research background, objectives and scopes, including the thesis outline were comprehensively stated and elaborated.

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