OPTIMIZATION OF BIODEGRADABLE SAGO-BASED FILM FORMULATION FOR FOOD PACKAGING

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To my beloved father, mother and my husband.

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ABSTRACT

Millions of tons of food plastic packaging have been produced and deposited for decades. These types of plastics are made of petroleum-based materials and they are called synthetic plastics which are expensive, toxic, totally non-biodegradable and may contribute to environmental pollution. Great concerns regarding environmental issue has produced an alternative idea to replace the synthetic plastic with biodegradable plastics based on sago starch. This research focuses on the synthesis and characterization of biodegradable sago film formulation. In order to obtain the optimal conditions in developing the films, optimization process has been carried out. The sago film formulations were characterized using scanning electron microscopy for the morphology study of film formulation. Fourier transform infrared spectroscopy had successfully shown the chemical structure and bonding while cinnamaldehyde had successfully inhibited *E. coli* bacteria growth by using agar diffusion method. A central composite design technique from response surface methodology was used to investigate the effects of independent variables on biodegradable sago film formulation properties. Biodegradable sago film formulation was analyzed for tensile strength (TS), elongation at break (EB), elastic modulus (EM) and water vapor permeability (WVP). The statistical analysis results show that the data appropriately fitted a second-order polynomial model where the coefficients of determination, R^2 are 0.8192, 0.8451, 0.8036 and 0.8941 for TS, EB, EM and WVP respectively. The independent variables of linear sago/chitosan blend and quadratic cinnamaldehyde had significant effect on TS. The linear cinnamaldehyde and quadratic cinnamaldehyde and glycerol/sorbitol-plasticized had significant effect on EB. Meanwhile, EM was significantly affected by both linear sago/chitosan and cinnamaldehyde and quadratically by glycerol/sorbitol-plasticized. The WVP was significantly affected by both linear sago/chitosan and glycerol/sorbitol-plasticized. The results show that the best film formulations for sago film formulation within the experimental ranges are 16.36 wt.% sago/chitosan, 41.11 wt.% glycerol/sorbitol and 0.7350 wt.% cinnamaldehyde for 135.49 MPa of TS; 57.01 wt.% sago/chitosan, 59.14 wt.% glycerol/sorbitol and 0.6400 wt.% cinnamaldehyde for 0.6012% EB and 41.03 wt.% sago/chitosan, 30.32 wt.% glycerol/sorbitol and 0.8364 wt.% cinnamaldehyde for 8.45×10^6 MPa of EM. Furthermore, for the optimum composition of 1×10^{-7} g/Pa.s.m^2 of WVP, the experimental values are 79.66 wt.% sago/chitosan, 83.64 wt.% glycerol/sorbitol and 0.5430 wt.% cinnamaldehyde. Finally, the present findings indicate that the biodegradable sago-based film can be a potential plastic packing with appropriate optimized formulation values.

ABSTRAK

Berjuta tan pembungkus plastik makanan telah dihasilkan dan disimpan selama beberapa dekad. Plastik jenis ini adalah diperbuat daripada bahan yang berasaskan petroleum dan dipanggil sebagai plastik sintetik di mana ianya mahal, bertoksik dan tidak mesra alam serta menyumbang kepada pencemaran alam sekitar. Kebimbangan besar mengenai isu alam sekitar membawa kepada penyelidikan dalam bidang ini dan memberi idea alternatif untuk menggantikan plastik sintetik dengan plastik mesra alam berasaskan kanji sagu. Kajian ini memberi tumpuan kepada sintesis dan pencirian formulasi filem sagu terurai. Untuk mendapatkan keadaan optimum dalam pembentukan filem, proses pengoptimuman telah dijalankan. Formulasi filem sagu telah dicirikan menggunakan mikroskop imbasan elektron untuk mengkaji morfologi pembentukan filem. Spektroskopi inframerah transformasi Fourier telah berjaya membuktikan kewujudan struktur kimia dan ikatan manakala sinamaldehid berjaya menghalang pertumbuhan bakteria *E. coli* dengan menggunakan kaedah penyerapan agar. Teknik reka bentuk komposit pusat daripada kaedah gerak balas permukaan telah digunakan untuk mengkaji kesan pembolehubah kajian terhadap pembentukan filem sagu. Pembentukan filem sagu telah dianalisis untuk kekuatan tegangan (TS), pemanjangan pada waktu rehat (EB), modulus elastik (EM) dan kebolehtelapan wap air (WVP). Keputusan analisis statistik menunjukkan bahawa data yang diperoleh dengan sewajarnya berpadanan dengan model polinomial tertib kedua dengan nilai pekali, R^2, 0.8192, 0.8451, 0.8036 dan 0.8941 untuk TS, EB, EM dan WVP. Komposisi pembolehubah sagu/kitosan linear dan sinamaldehid kuadratik mempunyai kesan yang besar ke atas TS. Sinamaldehid linear dan sinamaldehid kuadratik serta keplastikan-gliserol/sorbitol telah memberi kesan yang besar ke atas EB. Manakala, EM telah terjejas dengan ketara oleh kedua-dua komposisi sagu/kitosan dan sinamaldehid linear dan keplastikan-gliserol/sorbitol kuadratik. WVP telah terjejas dengan ketara oleh kedua-dua sagu/kitosan dan keplastikan-gliserol/sorbitol linear. Keputusan menunjukkan bahawa komposisi pembentukan filem sagu terbaik adalah dalam julat eksperimen 16.36 wt.% sagu/kitosan, 41.11 wt.% gliserol/sorbitol dan 0.7350 wt.% sinamaldehid untuk menghasilkan 135.49 MPa TS; 57.01 wt.% sagu/kitosan, 59.14 wt.% gliserol/sorbitol dan 0.6400 wt.% sinamaldehid untuk menghasilkan 0.6012% EB dan 41.03 wt.% sagu/kitosan, 30.32 wt.% gliserol/sorbitol dan 0.8364 wt.% sinamaldehid untuk menghasilkan 8.45×10^6 MPa EM. Tambahan pula, komposisi optimum untuk 1×10^{-7} g / Pa.s.m^2 WVP memerlukan nilai eksperimen 79.66 wt.% komposisi sagu/kitosan, 83.64 wt.% gliserol/sorbitol dan 0.5430 wt.% cinnamaldehida. Akhir sekali, hasil kajian ini menunjukkan bahawa filem mesra alam yang berasaskan sagu boleh menjadi plastik pembungkusan yang berpotensi dengan nilai pembentukan yang optimum.

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LIST OF ABBREVIATIONS

ANOVA	-	analysis of variance
CCD	-	central composite design
E. coli	-	*Escherichia coli*
EOs	-	essential oils
EB	-	elongation at break
EM	-	elastic modulus
FTIR	-	Fourier transform infrared
RSM	-	response surface methodology
S/C	-	sago/chitosan blend
SEM	-	scanning electron microscopy
TS	-	tensile strength
WVP	-	water vapor permeability

LIST OF SYMBOLS

%	-	percentage
wt.%	-	weight percent
^{o}C	-	degree celsius
cm	-	centimeter
g	-	gram
min	-	minute
s	-	second
h	-	hour
kPa	-	kilo Pascal
l	-	liter
ml	-	milliliter
mm	-	millimeter
v/v	-	volume to volume ratio
w/w	-	weight to weight ratio
i.d.	-	internal diameter
o.d	-	outer diameter
k	-	number of independent variables
n_o	-	number of replication
N	-	number of tests
X	-	process variable (independent variable)
Y	-	response
Z	-	coded value of the independent variable
α	-	significance level
β	-	regression coefficient
H_o	-	null hypothesis
R^2	-	coefficient of determination
i, j, 1, 2	-	component identification

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Examples of plastic packaging materials are polyethylene (PE), high density polyethylene (HDPE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS) and polycarbonate (PC). The uses of these typical types of material depend on the applications, which may be containers, bottles, films or coatings. All the typical materials are petrochemical-based and non-biodegradable polymers which are harmful to the ecological environment by creating more wastes that cannot be disposed naturally.

The increasing uses of plastics based on petrochemical, for example polyolefin, polyesters and polyamides are due to their availability in economical quantities and favorable usage quality (Tharanathan, 2003). In addition, these plastics have good tear and tensile strength, favorable barrier characteristics to oxygen as well as aroma compounds, and good heat seal ability. Synthetic plastics such as polyethylene and polypropylene have a very low water vapor transmission rate and they are totally non-biodegradable, and therefore lead to environmental pollution, which give serious ecological problems. Biodegradable polymers are an alternative replacement for traditional petroleum-based non-biodegradable polymer. Development of biodegradable films can offer great potential to enhance food quality, safety and stability. There are numerous natural biodegradable polymers, for example, protein and polysaccharide based films, might be beneficial materials for ecologically friendly packaging.

The increase of customer need for higher quality and longer shelf-life in foods, while reducing disposable packaging materials, has encouraged further research into edible films and edible coatings from natural polymers such as polysaccharides. These needs have caused increased interest in biodegradable films or materials that could be used to enhance the shelf-life and improve the quality of packed food products by providing a mass transfer barrier to moisture, oxygen, carbon dioxide, lipid, flavor and aroma between food components and the surrounding atmosphere (Tharanathan, 2003).

Starch is one of the most important biopolymers and widely used in numerous industrial applications, such as in the paper, textile, food, pharmaceutical and cosmetics industries (Shah *et al.*, 1995). Starch possesses the essentials of having adequate thermal stability with minimal interference of melt properties and insignificant disturbance of product quality and has been deliberated as a material successor in certain thermoplastic usage cause by its known biodegradability, availability and is inexpensive/low-priced (Siracusa *et al.*, 2008).

Basically, starch-based films have high elasticity; nonetheless, starch-based films are too brittle for packaging utilizations (Mani, 1998). Addition of plasticizers in the starch-based film is the common approach to develop and improve the mechanical properties of biopolymer films such as a low molecular weight non-volatile substance, plasticizer in the film may reduce biopolymer chain-to-chain interactions, though resulting in enhanced film flexibility and stretch ability. To increase the film pliability and flexibility, plasticizers are necessary in the biodegradable film formulations. Plasticizers that are hydrophilic compounds such as polyols (glycerol/sorbitol) and polyethylene glycol are commonly used as plasticizers in hydrophilic film formulations.

Food additives may be incorporated in film formulations such as antioxidants, antimicrobial agents and nutrients; these additives were used to achieve based film specific functionalities. The incorporation of antimicrobials into edible films could serve as a barrier for surface-contaminating microorganisms (Fishman *et al.*, 1996). Essential oil is the product specially extracted from plant parts; contain active elements that may perform as antimicrobial compounds against bacteria, yeast, and moulds. The bigger groups of principal components that make essential oils effective antimicrobials include saponins, flavonoids, carvacrol, thymol, citral, eugenol, linalool, terpenes, and their precursors. For example, cinnamon essential oil, which is rich in cinnamic aldehyde, exhibits antimicrobial activity against a broad spectrum of bacteria, such as *Campylobacter jejuni, Listeria monocytogenes,* and *Salmonella enteritidis* (Joerger, 2007). The presence of antimicrobial (essential oils) in the based films makes the films as "active films". Hence, the concept of "active films" is a very promising function and application as it creates new pathways for designing packaging materials especially for food stuffs.

Starch based films that are made of many starches such as high amylase corn, potato and cassava, have been reported by many studies but a very limited work has been reported on sago-starch based film. In this research, sago-starch based biodegradable films with an optimum combination of desirable characteristics and biodegradation performance was developed. The films in this study were prepared by a casting/solvent evaporation method. The film-forming solutions which contained a combination of sago-starch and chitosan with different ratio were manipulated with glycerol/sorbitol mixture and cinnamaldehyde. Plasticizers and antimicrobial are combined in the solution to improve mechanical properties of films and serve as a barrier for surface-contaminating microorganisms.

Response surface methodology (RSM) is the one most commonly used techniques for effect study. In most of the effect studies, the process parameters have been merely investigated by conducting one-factor-at-a-time experiments. The result of one-factor-at-a-time experiments does not reflect the actual changes in the environment as they ignore interactions between factors that are present simultaneously. An alternative is to use empirical modeling approach such as response surface methodology (RSM). RSM is a collection of mathematical and statistical techniques for empirical model building that is an efficient tool for effect study and does not demand a lot of experimental data (Cornell, 1990). RSM were successfully used to link one or more responses to a set of variables when firm interaction is known. This method is an effective and successful technique used to obtain the best value and most influencing variable to a few set of variables that affect the value of any response.

1.2 Problem Statement

Petroleum-based plastic materials has been produced widely because it contributed in economical, processing and technological aspects but these plastic materials caused environmental pollution. Most of these plastic materials had been deposited in the garbage and depots for more than 30 years. A serious ecological problems has occurred because of these petroleum-based plastic materials take a longer time to degrade and are totally non-biodegradable. Plastics that are petroleum-based materials contain toxic chemicals which may cause pollution to the environment. At high temperature or during combustion process, petroleum-based plastics will release dioxin which is a toxic chemical substance. Apart from that, due to these non-biodegradable plastics, the landfill area will have abundance of plastic waste and leads to another issue including lack of landfill area, unsightly view and soil contamination. Hence, it is crucial to find another alternative to replace the petroleum-based plastic materials with biodegradable polymers that are more environmentally friendly.

Concerns regarding environmental issue and the non-biodegradable of petroleum-based plastics have attracted many researchers to investigate about biodegradable plastics. This research focuses on developing and synthesizing biodegradable sago-based plastics based on sago starch. The development of sago-based film alone has few disadvantages, such as the film made was too brittle, low mechanical resistance and also high chances to be attacked by yeast, mould and microbes. Hence, modifications were needed to enhance the properties of sago-based films by modifying with chitosan, addition of glycerol/sorbitol-plasticizers and antimicrobial agent. The optimal biodegradable sago-based film formulation was obtained by conducting the optimization process using a response surface methodology. The optimization process was conducted to show the interaction between the independent variables for production of biodegradable sago-based film formulation. The best or optimal value of variables from optimization process results a better film formulation.

Thus, this research was expected to offer an alternative packaging option, obtained from renewable resources to replaced petroleum-based plastic materials, which do not contribute to the environmental pollution and consequently satisfies a variety of needs and meets specific product challenges for a large number of food applications.

1.3 Objective of Study

The development of biodegradable sago-based film formulation was conducted in order to fulfill these following three objectives:

i. To synthesize and characterize the biodegradable sago film formulations and study the mechanical, physical, chemical properties of sago starch based film as well as their microstructure and antimicrobial activity.

ii. To investigate the effect of varying composition of sago/chitosan blend, glycerol/sorbitol-plasticizers and cinnamaldehyde content on the mechanical properties, water vapor permeability of biodegradable sago based film formulation.

iii. To obtain the optimum conditions of the independent variables for the development of biodegradable sago starch based film formulation by using a response surface methodology.

1.4 Scope of Study

In order to achieve the objectives of this research, the following research activities were executed:

i. Synthesis and characterization of the biodegradable sago film formulations.

Biodegradable film from sago starch was developed by solvent evaporation method. The synthesis of based films was obtained by manipulating both sago starch and chitosan composition. Modification of the film was performed chemically through the addition of glycerol/sorbitol-plasticizer mixture to improve the mechanical properties of film, and antimicrobial agent loadings will enhance its biological capacity.

The biodegradable film was analyzed to evaluate its physicochemical properties. A scanning electron microscopy (SEM) was performed for the morphology study of the film, fourier transform infrared (FTIR) spectroscopy was used to investigate the existence of functional groups and chemical bonding in the film formulation due to chemical modification. Physical properties of the films were also elucidated through water vapor permeability according to ASTM E96 standard method, and mechanical properties was investigated through tensile test for their tensile strength, elongation at break, and elastic modulus according to ASTM D 882-02 standard method. Antimicrobial activity of the film was tested to determine antimicrobial effects of film formulation against *Escherichia coli*. The zones of complete inhibition illustrate the antimicrobial effects of sago starch based biodegradable film incorporated with cinnamaldehyde as antimicrobial agent.

ii. The effect of varying composition for independent variables.

The independent variables in this research are sago/chitosan blend composition, glycerol/sorbitol-plasticizers mixture and cinnamaldehyde content. In this research study, the concentration of sago starch and chitosan used was ranged in

30–70 wt.%. For the glycerol/sorbitol-plasticizers concentration ranged 30–70 wt.% based on wt.% sago starch basis and concentration for cinnamaldehyde used was 4.00 wt.% based on sago starch basis (wt.%). These independent variables were manipulated to investigate the impact on tensile strength, elongation at break, elastic modulus, water vapor permeability, as well as antimicrobial activity.

iii. Optimization approach for biodegradable sago film formulations.

The optimization process of the biodegradable sago film formulations was determined using a response surface methodology (RSM). RSM is a method that basically involves three major steps. First, the design of experiment using statistical approach, which a 2^3 full-factorial central composite experimental design (CCD) was used resulting in 16 experiments in the study. In statistics, a CCD is useful in RSM for building a second order (quadratic) model for the response variable of film formulation without needing to use a complete three-level factorial experiment. Since the independent variables in this study was three variables, so that it is suitable to use CCD in this study because the design require minimum number of treatment combinations which is three. Second, the coefficient based on mathematical model (second degree polynomial equation), showed a relationship between independent variables and responses. Third, the response prediction and finally model adequacy check. The most significant independent variable was shown by pareto chart. The adequacy of the fitted model was checked by analysis of variance (ANOVA).

1.5 Significance of Study

Recently, million tons of plastics and disposable items produced in the world and most of them are deposited in the garbage and the environmental effects of these materials lasting for centuries in landfills or other disposal places. Most plastics are used as a packaging system for food stuffs. This situation has resulted in serious environmental problem. Petroleum-based plastic materials called synthetic plastics are completely non-biodegradable, which pose serious ecological problems. Thus, this research was done to develop biodegradable plastics based on sago-starch and chitosan for food packaging. The best source for starch present in Malaysia is sago starch. It is chosen due to its known biodegradability, high availability and low cost. Chitosan was used because of the properties of biocompability characteristics for food preservation. Thus, the development of edible and easily degradable based films of plastics for food packaging will provide safety assurance, shelf-life extension and quality maintenance on food packed, thus leads to being environmentally friendly.

REFERENCES

Abdorreza, M., Robal, M., Cheng, L., Tajul, A. Y. and Karim, A. A. (2012). Physicochemical, Thermal and Rheological Properties of Acid-hydrolyzed Sago (*Metroxylon Sagu*) Starch. *Food Science and Technology*. 46(1): 135–141.

Abdorreza, M. N., Nafchi, M., Cheng, L. H. and Karim, A. A. (2011). Effects of Plasticizers on Thermal Properties and Heat Sealability of Sago Starch Films. *Food Hydrocolloids*. 25(1): 56–60.

Abdullah, A. A. (2013). Antimicrobial Effect of Cinnamon Bark Hot and Cold Watery Extract against Extra Intestinal Pathogenic *Escherichia Coli* (ExPEC) and *Staphylococcus Aureus*. 3208: 30–35.

Ahmad, F. B., Williams, P. A., Doublier, J. L., Durand, S. and Buleon, A. (1999). Physicochemical Characterisation of Sago Starch. *Carbohydrate Polymers*. 38(4): 361–370.

Aranaz, I., Mengibar, M., Harris, R., Panos, I., Miralles, B., Acosta, N., Galed, G. and Heras, A. (2009). Functional Characterization of Chitin and Chitosan. 203–230.

Araujo-Farro, P. C., Padadera, G., Sobral, P. J. A. and Menegalli, F. C. (2010). Development of Films Based on Quinoa (*Chenopodium quinoa*, Willdenow) Starch. *Carbohydrate Polymers*. 81: 839-848.

Appendini, P. and Hotchkiss, J. H. (2002). Review of Antimicrobial Food Packaging. *Innovative Food Science and Emerging Technologies*. 3(2): 113–126.

ASTM International (1995). *ASTM E96/E96M-12*. Standard Test Method for Water Vapor Transmission of Materials. West Conshohocken, PA.

ASTM International (2002). *ASTM D 882-02*. Standard Test Method for Tensile Properties of Thin Plastic Sheeting. West Conshohocken, PA.

Avella, M., Vlieger, J. J. D., Errico, M. E., Fischer, S., Vacca, P. and Volpe, M. G.

(2005). Biodegradable Starch/Clay Nanocomposite Films for Food Packaging Applications. *Food Chemistry*. 93: 467–474.

Avila-Sosa, R., Palou, E., Jimenez, M. M. T., Nevarez-Moorillon, G. V., Navarro, C. A. R. and Lopez-Malo, A. (2012). Antifungal Activity by Vapor Contact of Essential Oils Added to Amaranth, Chitosan, or Starch Edible Films. *International Journal of Food Microbiology*. 153(1-2): 66–72.

Ball, S., Guan, H., James, M., Myers, A., Keeling, P., Mouille, G., Buleon, A., Colonna, P. and Preiss, J. (1996). From Glycogen to Amylopectin: Minireview: A Model for the Biogenesis of the Plant Starch Granule. *Cell Press*. 86: 349–352.

Bertuzzi, M. A., Armada, M. and Gottifredi, J. C. (2007). Physicochemical Characterization of Starch Based Films. *Journal of Food Engineering*. 82(1): 17–25.

Bhargava, R., Wang, S. and Koenig, J. L. (2003). FTIR Microspectroscopy of Polymeric Systems. *Advance Polymer Science*. 163: 137–191.

Bourtoom, T. (2008). Plasticizer Effect on the Properties of Biodegradable Blend Film From Rice Starch-Chitosan. *Songklanakarin Journal Science Technology*. 30: 149–165.

Bourtoom, T. (2008). Review Article Edible Films and Coatings: Characteristics and Properties. *International Food Research Journal*. 3(15): 237–248.

Brown, S. R. and Melamed, L. E. (1990). *Experimental Design and Analysis*. Sage Publications. Newbury Park, California.

Cao, N., Yang, X. and Fu, Y. (2009). Effects of Various Plasticizers on Mechanical and Water Vapor Barrier Properties of Gelatin Films. *Food Hydrocolloids*. 23(3): 729–735.

Carvalho, A. J. F., Zambon, M. D., Curvelo, A. A. S. and Gandini, A. (2003). Size Exclusion Chromatography Characterization of Thermoplastic Starch Composites: 1. Influence of Plasticizer and Fibre Content. *Polymer Degradation and Stability*. 79: 133–138.

Chana-Thaworn, J., Chanthachum, S. and Wittaya, T. (2011). Properties and Antimicrobial Activity of Edible Films Incorporated with Kiam Wood Cotyleobium lanceotatum Extract. *LWT - Food Science and Technology*. 44(1): 284–292.

CLSI. (2012). *CLSI document M02-A11*. Performance Standards for Antimicrobial

Disk Susceptibility Tests; Approved Standard—Eleventh Edition. Wayne, PA: Clinical and Laboratory Standards Institute.

Colla, E., Sobral, P. J. A. and Menegalli, F. C. (2006). *Amaranthus Cruentus* Flour Edible Films: Influence of Stearic Acid Addition, Plasticizer Concentration and Emulsion Stirring Speed on Water Vapor Permeability and Mechanical Properties. *Journal of Agricultural and Food Chemistry*. 54: 6645-6653.

Coma, V., Deschamps, A. and Martial-Gros, A. (2003). Bioactive Packaging Materials from Edible Chitosan Polymer-Antimicrobial Activity Assessment on Dairy Related Contaminants. *Journal of Food Science*. 68(9): 2788–2792.

Cornell, J.A. (1990). *How to Apply Response Surface Methodology*. (vol.8). American Society for Quality Control (ASQC) Statistics Division. Milwaukee, WI.

Cybulska, G. (2000) *Waste Management in the Food Industry*: *An Overview*. Campden and Chorleywood Food Research Association Group, UK.

Davis, G. and Song, J. H. (2006). Biodegradable Packaging Based on Raw Materials from Crops and Their Impact on Waste Management. *Industrial Crops and Products*. 23: 147–161.

Dutta, P. K., Dutta, J. and Tripathi, V. S. (2004). Chitin and Chitosan: Chemistry, Properties and Applications. *Journal of Scientific and Industrial Research*. 63: 20–31.

Egerton, V. (2005). Physical Principles of Electron Microscopy: An Introduction to TEM, SEM, and AEM. *Springe*r.

Electronic Media and URLs. (n.d.) Retrieved February 14, 2014, from http://www.european-bioplastics.org

Electronic Media and URLs. (n.d.) Retrieved September 16, 2004, from http://www.pubchem.ncbi.nlm.nih.gov

Epure, V., Griffon, M., Pollet, E. and Averous, L. (2011). Structure and Properties of Glycerol-Plasticized Chitosan Obtained by Mechanical Kneading. *Carbohydrate Polymers*. 83(2): 947–952.

Ferruh, E. (2009). *Optimization in Food Engineering*. Boca Raton: CRC Press, Taylor and Francis Group.

Fishman, M. L., Coffin, D. R., Unruh, J. J. and Ly, T. (1996). Pectin/Starch/Glycerol Films: Blends or Composites? *Journal of Macromolecular Science*. 33(5): 639–654.

Flach, M. and Schuiling, D. L. (1998). Revival of an Ancient Starch Crop: A Review of the Agronomy of the Sago Palm. *Kluwer Academic PublisheKl, Dordrecht*. 7: 259–281.

Galdeano, M. C., Mali, S., Grossmann, M. V. E., Yamashita, F. and Garcia, M. A. (2009). Effect of Plasticizers on the Properties of Oat Starch Films. *Materials Science and Engineering*. 29(2): 532–538.

Garcia, M. A., Martino, M. N. and Zaritzky, N. E. (2000). Microstructural Characterization of Plasticized Starch-Based Films. *Starch/Staerke*. 1390(4): 118–124.

Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavarz, B., Cran, M. J. and Khaksar, R. (2013). Physical, Mechanical and Barrier Properties of Corn Starch Films Incorporated with Plant Essential Oils. *Carbohydrate polymers*. 98(1): 1117–26.

Guzman, A., Gnutek, N. and Janik, H. (2011). Biodegradable Polymers For Food Packaging – Factors Influencing Their Degradation and Certification Types – A Comprehensive Review. *Chemistry & Chemical Technology*. 1(5): 115-122.

Han, J. H. (2003). Antimicrobial Food Packaging. Novel Food Packaging Techniques.

Himratul-Aznita, W. H., Mohd-Al-Faisal, N. and Fathilah, A. R. (2011). Determination of the Percentage Inhibition of Diameter Growth (PIDG) of *Piper Betle* Crude Aqueous Extract against Oral Candida Species. *Journal of Medicinal Plants Research*. 5(6): 878-884.

Hotchkiss, J. H. (1997). Food-Packaging Interactions Influencing Quality and Safety. *Food Additives and Contaminants*. 14(6-7): 601–607.

Imre, B. and Pukanszky, B. (2013). Compatibilization in Bio-Based and Biodegradable Polymer Blends. *European Polymer Journal*. 49(6): 1215–1233.

Jenkins, P. J. and Donald, A. M. (1995). The Influence of Amylose on Starch Granule Structure. *International Journal Macromolecules*. 17(6): 315–321.

Joerger, R. D. (2007). Antimicrobial Films for Food Applications: A Quantitative Analysis of Their Effectiveness and Science. *Packaging Technology Science*. 20: 231–273.

Karim, A. A., Tie, A. P., Manan, D. M. A. and Zaidul, I. S. M. (2008). Starch from the Sago (*Metroxylon sagu)* Palm Tree- Properties, Prospects, and Challenges as A New Industrial Source for Food and Other Uses. *Food Science and Food Safety*. 7: 215–228.

Karim, A. and Bhat, R. (2011). Physical and Mechanical Properties of Sago Starch Alginate Films Incorporated with Calcium Chloride. 18(3): 1027–1033.

Kechichian, V., Ditchfield, C., Veiga-Santos, P. and Tadini, C. C. (2010). Natural Antimicrobial Ingredients Incorporated in Biodegradable Films Based on Cassava Starch. *LWT - Food Science and Technology*. 43(7): 1088–1094.

Lavorgna, M., Piscitelli, F., Mangiacapra, P. and Buonocore, G. G. (2010). Study of the Combined Effect of Both Clay and Glycerol Plasticizer on the Properties of Chitosan Films. *Carbohydrate Polymers*. 82(2): 291–298.

Leceta, I., Guerrero, P. and Caba, K. D. (2013). Functional Properties of Chitosan Based Films. *Carbohydrate Polymers*. 93(1): 339–346.

Leceta, I., Guerrero, P., Ibarburu, I., Duenas, M. T. and De La Caba, K. (2013). Characterization and Antimicrobial Analysis of Chitosan-Based Films. *Journal of Food Engineering*. 116(4): 889–899.

Lee, L. H. (1977). *Characterization of Metal and Polymer Surfaces*. Polymer Surfaces Academic Press, New York, USA.

Maaruf, A. G., Che Man, Y. B., Asbi, B. A., Junainah, A. H. and Kennedy, J. F. (2001). Effect ofWater Content on the Gelatinisation Temperature of Sago Starch. *Carbohydrate Polymers*. 46(4): 331–337.

Maizura, M., Fazilah, A., Norziah, M. H. and Karim, A. A. (2008). Antibacterial Activity of Modified Sago Starch-Alginate Based Edible Film Incorporated with Lemongrass (Cymbopogon citratus) Oils. *International Food Research Journal*. 15(2): 223–236.

Mali, S., Sakanaka, L. S., Yamashita, F. and Grossmann, M. V. E. (2005). Water Sorption and Mechanical Properties of Cassava Starch Films and Their Relation to Plasticizing Effect. *Carbohydrate Polymers*. 60(3): 283-289.

Mani, R. and Bhattacharya, M. (1998). Properties of Injection Moulded Starch/Synthetic Polymer Blends - III. Effect of Amylopectin to Amylose Ratio in Starch. *European Polymer Journal*. 34(10): 1467–1475.

Marsh, K. and Bugusu, B. (2007). Food Packaging - Roles, Materials, and Environmental Issues. *Journal of Food Science*. 3(72): 39-55.

Martinez-camacho, A. P., Cortez-rocha, M. O., Ezquerra-brauer, J. M. and Graciano verdugo, A. Z. (2010). Chitosan Composite Films: Thermal, Structural, Mechanical and Antifungal Properties. *Carbohydrate Polymers*. 82(2): 305–315.

Mayachiew, P., Devahastin, S., Mackey, B. M. and Niranjan, K. (2010). Effects of Drying Methods and Conditions on Antimicrobial Activity of Edible Chitosan Films Enriched with Galangal Extract. *Food Research International*. 43(1): 125–132.

Mei, J., Yuan, Y., Guo, Q., Wu, Y., Li, Y. and Yu, H. (2013). Characterization and Antimicrobial Properties of Water Chestnut Starch-Chitosan Edible Films. *International Journal of Biological Macromolecules*. 61: 169–74.

Ming, X.,Weber, G. H., Ayres, J.W. and Sandine, W. E. (1997). Bacteriocins Applied to Food Packaging Materials to Inhibit *Listeria Monocytogenes* on Meats. *Journal of Food Science*. 62(2): 413–415.

Montgomery, D. C. (1997). *Design and Analysis of Experiments*. 4th edition. New York: John Wiley & Sons. New York. 445-474.

Naik, M. I., Fomda, B. A., Jaykumar, E. and Bhat, J. A. (2010). Antibacterial Activity of Lemongrass Cymbopogon Citratus Oil Against Some Selected Pathogenic Bacterias. *Asian Pacific Journal of Tropical Medicine*. 3(7): 535–538.

NorthwoodNort and Oakley-Hill, D. (1999). *Wastebook*. Luton Friends of the Earth, Environment Agency and the Building Research Establishment.

Preechawong, D., Peesan, M., Supaphol, P. and Rujiravanit, R. (2004). Characterization of Starch/Poly(E-Caprolactone) Hybrid Foams. *Polymer Testing, Elsevier*. 23: 651–657.

Preechawong, D., Peesan, M., Supaphol, P. and Rujiravanit, R. (2005). Preparation and Characterization of Starch/Poly(L-lactic acid) Hybrid Foams. *Carbohydrate Polymers, Elsevier*. 59: 329–337.

Pukkahuta, C. and Varavinit, S. (2007). Structural Transformation of Sago Starch by Heat Moisture and Osmotic-Pressure Treatment. *Starch/Staerke*. 59: 624–631.

Rinaudo, M. (2006). Chitin and Chitosan: Properties and Applications. *Progress in Polymer Science*. 31: 603–632.

Rodriguez, A., Batlle, R. and Nertin, C. (2007). The Use of Natural Essential Oils

Antimicrobial Solutions in Paper Packaging. Part II. *Progress in Organic Coatings*. 60: 33–38.

Rojas-Grau, M. A., Avena-Bustillos, R. J., Olsen, C., Friedman, M., Henika, P. R., Martin-Belloso, O., Pan, Z. and McHugh, T. H. (2007). Effects of Plant Essential Oils and Oil Compounds on Mechanical, Barrier and Antimicrobial Properties of Alginate Apple Puree Edible Films. *Journal of Food Engineering*. 81(3): 634–641.

Scott, G. and Wiles, D. M. (2001). Programmed-Life Plastics from Polyolefins: A New Look at Sustainability. *Biomacromolecules*. 2(3): 615–622.

Seydim, A. C. and Sarikus, G. (2006). Antimicrobial Activity of Whey Protein Based Edible Films Incorporated with Oregano, Rosemary and Garlic Essential Oils. *Food Research International*. 39: 639–644.

Shah, P. B., Bandopadhyayb, S. and Bellare, J. R. (1995). Environmentally Degradable Starch Filled Low Density Polyethylene. *Polymer Degradation and Stability*. 47: 165–173.

Shujun, W., Jiugao, Y. and Jinglin, Y. (2005). Preparation and Characterization of Compatible Thermoplastics Starch/Polyethylene Blends. *Polymer Degradation and Stability*. 87(3): 395–401.

Silva, M. A. D., Bierhalz, A. C. K. and Kieckbusch, T. G. (2009). Alginate and Pectin Composite Films Crosslinked with Ca^{2+} Ions: Effect of the Plasticizer Concentration. *Carbohydrate Polymers*. 77: 736–742.

Singhal, R. S., Kennedy, J. F., Gopalakrishnan, S. M., Kaczmarek, A., Knill, C. J. and Akmar, P. F. (2008). Industrial Production, Processing, and Utilization of Sago Palm-Derived Products. *Carbohydrate Polymers*. 72(1): 1–20.

Siracusa, V., Rocculi, P., Romani, S. and Rosa, M. D. (2008). Biodegradable Polymers for Food Packaging: A Review. *Trends in Food Science and Technology*. 19(12): 634–643.

Sivarooban, T., Hettiarachchy, N. S. and Johnson, M. G. (2008). Physical and Antimicrobial Properties of Grape Seed Extract, Nisin and EDTA Incorporated Soy Protein Edible Films. *Food Research International*. 41: 781–785.

Skoog, D. A., Holler, F. M. and Crouch, S. R. (2007). *Principle of Instrumental Analysis.Belmont*, CA: Thomson Brooks/Cole.

Skoog, D. A., West, D. M., Holler, F. M. and Crouh, S. R. (2204). *Fundamental of*

Analytical Chemistry. 8th ed. Belmont, CA: Thomson Brooks/Cole.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D. and Rogues, M. A. (2001). Mechanical Water Vapor Barrier and Thermal Properties of Gelatin Based Edible Films. *Food Hydro. Colloid*. 15(6): 423–432.

Srinivasa, P., Ramesh, M. and Tharanathan, R. (2007). Effect of Plasticizers and Fatty Acids on Mechanical and Permeability Characteristics of Chitosan Films. *Food Hydrocolloids*. 21(7): 1113–1122.

Steinka, I., Morawska, M., Rutkowska, M. and Kukulowicz, A. (2006). The Influence of Biological Factors on Properties of Some Traditional and New Polymers used for Fermented Food Packaging. *Journal of Food Engineering*. 77(4): 771–775.

Suppakul, P., Sonneveld, K., Miltz, J. and Bigger, S. W. (2003). Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and Its Applications. *Journal of Food Science*. 68(2): 408–420.

Tapia-Blácido, D., Mauri, A. N., Menegalli, F. C., Sobral, P. J. A. and Añón, M. C. (2007). Contribution of the Starch, Protein, and Lipid Fractions to the Physical, Thermal, and Structural Properties of Amaranth (*Amaranthus caudatus*) Flour Films. *Journal of Food Science*. 72(5): 293–300.

Tapia-Blácido, D., Sobral, P. J. A. and Menegalli, F. C. (2005). Development and Characterization of Edible Films Based on Amaranth Flour (*Amaranthus caudatus*). Journal of Food Engineering. 67: 215-223.

Tester, R. F., Karkalas, J. and Qi, X. (2004). Starch-Composition, Fine Structure and Architecture. *Journal of Cereal Science*. 39(2): 151–165.

Tester, R. F. and Debon, S. J. J. (2000). Annealing of Starch: A Review. *International Journal of Macromolecules*. 27: 1–12.

Thakhiew, W., Devahastin, S. and Soponronnarit, S. (2010). Effects of Drying Methods and Plasticizer Concentration on Some Physical and Mechanical Properties of Edible Chitosan Films. *Journal of Food Engineering*. 99(2): 216–224.

Tharanathan, R. (2003). Biodegradable Films and Composite Coatings: Past, Present and Future. *Trends in Food Science and Technology*. 14(3): 71–78.

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J. and Gerschenson, L. N.

(2009). Antimicrobial Activity and Physical Properties of Chitosan – Tapioca Starch Based Edible Films and Coatings. *Food Research International*. 42(7): 762–769.

Vieira, M. G. A., Da Silva, M. A., Dos Santos, L. O. and Beppu, M. M. (2011). Natural Based Plasticizers and Biopolymer Films: A Review. *European Polymer Journal*. 47(3): 254–263.

Wang, T. L., Bogracheva, T. Y., Hedley, C. L., Centre, J. I. and Nr, N. (1998). Starch: As Simple as A, B, C? *Journal of Experimental Botany*. 49(320): 481– 502.

Yaacob, B., Amin, M. C. I. M., Hashim, K. and Bakar, B. A. (2011). Optimization of Reaction Conditions for Carboxymethylated Sago Starch. *Iranian Polymer Journal*. 20(3): 195–204.

Yang, J., Yu, J. and Ma, X. (2006). Study on the Properties of Ethylenebisformamid and Sorbitol Plasticized Corn Starch (ESPTPS). *Carbohydrate Polymers*. 66: 110–116.

Ying, G., Xiong, W., Wang, H., Sun, Y. and Liu, H. (2011). Preparation, Water Solubility and Antioxidant Activity of Branched-Chain Chitosan Derivatives. *Carbohydrate Polymers*. 83(4): 1787–1796.

Zhang, S., Tang, R. and Kan, J. (2007). Effect of Magnetic Field and Rare-Earth Ions on Properties of Polyaniline Nanoparticles. *Journal of Applied Polymer Science*. 103: 2286–2294.