UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF RICE STARCH-FISH GELATIN EDIBLE FILMS

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FSTM 2015 40

DEVELOPMENT OF RICE STARCH-FISH GELATIN EDIBLE FILMS

By

ROZHIN AHANGARI

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

March 2015

DEDICATION

Specially dedicated to,

My beloved mother, Shahnaz Ghari Rasi

For her invaluable love, precious support and encouragement from the beginning of my study

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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March 2015

Chairman: Professor Jamilah Bt. Bakar, PhD

Faculty: Food Science and Technology

Edible films of protein or carbohydrate have overall suitable mechanical properties but generally exhibit poor barrier properties. The use of a biopolymer like starch can be an interesting because this polymer is relatively cheap, abundant, biodegradable, and edible. The aim of this study was to incorporate fish gelatin and tannic acid (TA) into rice starch based edible films and investigate barrier, physicochemical and antimicrobial inhibition properties towards *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* by disc diffusion test in 25 and 4 ^{o}C. Film forming solutions of different concentrations of rice starch to fish gelatin (0 to 4 %) were prepared. The best film (fish gelatin: 1 %) containing sorbitol was incorporated with different concentration of TA (0.15, 0.3, 0.45, and 0.6 %) in acidic and alkaline pH. Film containing 0.45 % TA was used to wrap sausage in order to extend shelf life in chilled storage (4 ^{o}C) in compare with industrial package (LDPE) as a control. The addition of fish gelatin has a significant effect ($p<0.05$), resulting in lower tensile strength (TS), higher elongation at the break (EAB), water vapor permeability (WVP), oxygen permeability (PO_2), and film solubility (FS), but made films slightly darker than rice starch edible film (control). The barrier properties of incorporated TA films were found to improve significantly ($p<0.05$) as compared to the non-treated. The addition of TA increased TS (10.83-62.68 MPa), decreased the permeability values, solubility and EAB (4.44 to 1.02 g.mm.m^{-2}.day^{-1}.KPa^{-1}), (0.136 to 0.0025 %), and (27.97 to 16.82 %), respectively. The possible interactions between the two main components were evaluated by X-ray diffractometer (XRD) and Fourier-transform infrared spectroscopy (FTIR). The amide I and II band the most useful peak of the secondary structure of fish gelatin molecule shifted from 1534.54 to 1647.3 cm^{-1} in the edible films. In the TA incorporated films the characteristic peak of starch at 1536 cm^{-1} shifted to 1427 cm^{-1} and the amide peak of fish gelatin at 1642–1646 cm^{-1} indicating interaction between the amide groups and hydroxyl groups (-OH) of starch, fish gelatin, and TA. An amorphous state was observed in the film instead of the crystalline structure of starch granule, indicating molecular miscibility. The microstructure of the film showed smoother surfaces with decreasing fish gelatin, while increasing the TA content made the film more compact due to the networking introduced by tannic acid as observed in SEM. The comparison of inhibition zone was *E. coli*>*L. monocytogenes*>*S. typhimurium*> *B. cereus* with the increase of TA concentration. *E. coli* was more sensitive to the TA than *L. monocytogenes*, *S. typhimurium*, and *B. cereus*. The inhibition zoon in *L. monocytogenes*, *S. typhimurium*, and *B. cereus* in lower concentration of TA and control was not significant. However, in higher concentration of TA, there is no significant difference between inhibition zone of *S. typhimurium* and *B. cereus* either in room or in chilled storage. All sausages became drier than commercial wrapped sausages (MC: 69.97-27.55 %) and in relation to water activity; the average initial values were 0.950 and decreased to 0.850 and 0.920 during 4-week storage in sample and control, respectively. The sausages became more reddish (a value: 17.79-23.66) during chilled storage. As water activity decreased significantly ($p<0.05$), the total count decreased during storage (from 4.82 to 4.15 CFU/g in sample). The thiobarbituric acid reactive substances increased (1.90-4.06 µmol MA equivalent/per µmol PL) as well as control (1.90-5.24 µmol MA equivalent/per µmol PL), however; as compared to control there is lower TBARS after week 2. These results indicate that antimicrobial packaging with the inclusion of tannic acid as a natural antimicrobial could

be considered as an effective technique to extend shelf life to 30 days in compare with control ≤ 2 weeks in 4 ^{o}C.

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

PEMBANGUNAN FILEM BOLEH MAKAN DARIPADA KANJI BERAS DAN GELATIN IKAN

Oleh

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Filem boleh dimakan yang berasaskan protein dan karbohidrat mempunyai ciri-ciri mekanikal yang baik, namun kurang berkesan sebagai penghadang. Penggunaan biopolimer seperti kanji menjadi perhatian kerana polimer ini lebih murah, sumbernya yang banyak, biodegradasi dan selamat dimakan. Kajian ini bertujuan untuk menghasilkan filem berasaskan kanji beras yang mengabungkan gelatin ikan dan asid tannik (TA). Ciri-ciri penghadang, fizikal kimia dan kesan filem tersebut terhadap bakteria *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* diuji melalui ujian resapan cakera pada suhu 25 ^{o}C dan 4 ^{o}C. Campuran larutan kanji beras dan gelatin ikan pada kadar kepekatan yang berbeza (0-4 %) telah disediakan untuk membuat filem. Formulasi filem terbaik (1% gelatin ikan) yang mengandungi sorbitol kemudiannya dicampurkan dengan TA pada kepekatan yang berbeza (0.15, 0.30, 0.45 dan 0.60 %) pada pH yang berasid dan beralkali. Filem yang mengandungi 0.45 % TA telah digunakan untuk membalut sosej dengan tujuan meningkatkan tempoh penyimpanan di suhu sejuk (4 ^{o}C) manakala pembungkus industri LDPE digunakan pada sosej sebagai rujukan kawalan. Penambahan gelatin ikan memberi kesan yang ketara ($p<0.05$) dalam merendahkan kekuatan regangan (TS), meningkatkan pemanjangan takat putus (EAB), kebolehtelapan wap air (WVP), kebolehtelapan oksigen (PO_2) dan kebolehlarutan filem tetapi menghasilkan warna yang lebih gelap daripada filem bolehmakan kanji beras (kawalan). Namun, penambahan gelatin ikan menyebabkan filem yang terhasil berwarna lebih gelap. Filem yang mengandungi TA didapati mempunyai ciri-ciri penghadang yang lebih baik ($p<0.05$) berbanding dengan filem yang tidak mengandungi TA. Penambahan TA meningkatkan TS (daripada 10.83 hingga 62.68 MPa), menurunkan WVP (daripada 4.44 ke 1.02 $g.mm.m^{-2}.hari^{-1}.kPa^{-1}$), kebolehlarutan (daripada 0.136 ke 0.0025 %) dan EAB (daripada 27.97 ke 16.82 %). Sinar-X diffractometer (XRD) and spektrometer inframerah jelmaan Fourier (FT-IR) telah digunakan untuk mengkaji hubungan intraksi antara dua komponen tersebut. Jalur amida I and amida II yang merupakan struktur sekunder penting bagi molekul gelatin ikan telah berganjak daripada 1534.54 ke 1647.30 sm^{-1} di dalam filem eksperimen. Sementara filem yang mempunyai bahan TA menunjukkan ganjakan puncak kanji daripada 1536 sm^{-1} ke 1427 sm^{-1}, dengan puncak amida gelatin ikan pada 1642-1646 sm^{-1}. Keadaan ini menunjukkan wujudnya interaksi antara kumpulan amida dengan kumpulan hidroksil kanji, gelatin ikan dan TA. Bentuk amorfus dapat dilihat di dalam struktur filem berbanding struktur kristal yang menunjukkan kebolehcampuran molekul-molekul. Analisis SEM menunjukkan permukaan mikrostruktur filem semakin licin dengan penurunan kepekatan gelatin ikan. Sementara itu, peningkatan TA menjadikan mikrostruktur filem lebih padat disebabkan oleh rangkaian struktur yang diperkenalkan oleh TA. Kajian antimikrob mendapati peningkatan kepekatan TA menunjukkan zon perencatan yang paling tinggi pada *E. coli*, diikuti dengan *L. monocytogenes*, *S. typhimurium* dan *B. cereus*. Bakteria *E. coli* didapati lebih sensitif pada TA berbanding *L. monocytogenes*, *S. typhimurium* dan *B. cereus*. Zon perencatan bakteria *L. monocytogenes*, *S. typhimurium* dan *B. cereus* didapati kurang ketara pada sosej kawalan dan filem yang mengandungi kepekatan TA yang lebih rendah. Tiada perbezaan ketara didapati pada zon perencatan bakteria *S. typhimurium* dan *B. cereus* yang disimpan pada suhu bilik atau suhu penyimpanan dingin, walaupun kepekatan TA digunakan pada kadar yang tinggi. Selepas 30 hari tempoh penyimpanan, semua sosej didapati menjadi kering dengan kandungan air menurun daripada 69.97 % ke 27.55

%. Kajian penyimpanan mendapati aktiviti air filem telah menurun daripada 0.950 kepada 0.850 (sampel filem) dan 0.920 (sosej kawalan), masing-masing. Sosej menjadi merah sepanjang penyimpanan pada suhu sejuk dangan peningkatan nilai ‘a’ daripada 17.79 sehingga 23.66. Jumlah kiraan bakteria menurun daripada 4.82 CFU/g kepada 4.15 CFU/g (sampel filem) masing-masing semasa penyimpanan, memandangkan aktiviti air menurun dengan ketara ($p<0.05$). Kedua-dua filem (1.90-4.06 µmol MA kesetaraan/per µmolPL) dan sosej kawalan (1.90-5.24 µmol MA equivalent/per µmolPL) menunjukkan peningkatan nilai TBARS. Walaubagaimanapun, filem eksperimen menunjukkan nilai TBARS yang lebih rendah pada minggu kedua berbanding sosej kawalan. Keputusan ini menunjukkan bahawa potensi TA (bahan antibakteria semulajadi) dalam penghasilan pembungkusan antibakteria merupakan kaedah yang berkesan untuk memanjangkan jangka hayat sosej sehingga 30 hari, berbanding sosej kawalan yang hanya mempunya jangka hayat ≤ dua minggu pada 4 °C.

ACKNOWLEDGEMENTS

At the outset, I would like to thank God who is the Most Merciful, for all His blessings, without which I wouldn't be able to achieve this feat.

I would like to take this opportunity to express my profound appreciation to my supervisor, Prof. Dr. Jamilah Bt. Bakar from the Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia. Her remarkable level of knowledge and support has led me to work confidently and her critical comments and suggestions instructed the study in the right direction. I could not have finished this dissertation without her generous guidance, and patience.

Special thank goes to my co-supervisors Prof. Dr. Russly Abdul Rahman and Assoc. Prof. Dr. Roselina Karim for their supportive supervisions and invaluable advice throughout the entire progress of this project.

I would like to thank my family and friends. My beloved mother (Shahnaz Ghari Rasi) who gave me life and love in the first place; her great trust, dedication and generosity have backed me in my entire life. With special regards to my dear brothers, Mohammad Amin Ahangari and Ehsan Ahangari, who support and encourage me to proceed to completion.

There are many more who deserve to be thanked whose names I may have forgotten to mention, but their priceless help, friendship and advice will always been appreciated.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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LIST OF ABBREVIATIONS

A&I	Active and intelligent
ANOVA	Analysis of variance
ASTM	American society for testing and materials
ATCC	American type culture collection
a_w	Water activity
B. cereus	*Bacillus cereus*
CMC	Carboxy methylcellulose
CFU	Colony forming unit
CO_2	Carbon dioxide
C_2H_4	Ethylene
ΔH_m	Melting enthalpy
DSC	Differential scanning calorimetry
EAB	Elongation at the break
EAS	Electronic article surveillance
E. coli	*Escherichia coli*
EFSA	European food safety authority
ERH	Equilibrium relative humidity
FS	Film solubility
FTIR	Fourier-transform infrared spectroscopy
GA	Glutaraldehyde
GLY	Glycerol
GRAS	Generally recognized as safe
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropyl methylcellulose
IMF	Intermediate moisture foods

L. monocytogenes	*Listeria monocytogenes*
LDPE	Low density polyethylene
MA	Malonaldehyde
MAP	Modified atmosphere packaging
MC	Methylcellulose
O_2	Oxygen
OTR	Oxygen transmission rate
PA	Proanthocyanidin
PO_2	Oxygen permeability
PL	Phospholipid
RFID	Radio frequency identification
RH	Relative humidity
S. typhimurium	*Salmonella typhimurium*
SEM	Scanning electron microscopy
SOR	Sorbitol
SD	Standard deviation
SO_2	Sulfur dioxide
TA	Tannic acid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
T_m	Melting temperature
T_g	Transition temperature
TS	Tensile strength
TEP	1, 1, 3, 3-tetraethoxypropane
TSA	Tripton soy agar
TSB	Tripton soy broth
TTI	Time-temperature integrator

UV	Ultra violet
WVP	Water vapor permeability
WVTR	Water vapor transmission rate
XRD	X-ray diffractometer

CHAPTER 1

INTRODUCTION

1.1 Food packaging technology

One of the essential processes to keep quality of food products for storage and transportation is packaging (Kelsey, 1985). It delays quality deterioration and helps distribution and advertising. Also, the basic purposes of packaging are protection, containment, information and convenience (Kelsey, 1985). Good packaging considerably contributes to a business profit in addition to preserve food quality. In the process of delivery, the quality of a food product declines biologically and chemically, beside the more obvious physical and noticeable visual damage. The attribute of the packaged food is directly related to the food and packaging material qualities. Most food products' quality decline because of mass transfer, such as the migration of packaging constituents into the food, absorption of moisture, oxygen permeability, flavor loss, and absorption of unpleasant odor (Debeaufort, Quezada-Gallo, & Voilley, 1998; Kester & Fennema, 1986).

1.2 Developments in food packaging

In recent time, about 150 million tons of plastics are being manufactured annually, all over the world. As a result, it had led to critical environmental pollution because of wasted and non-degradable polymers. The recycling of wasted plastics is limited and plastics cannot be reused forever, that is, wasted plastics are finally set to be burnt or buried in ground (Parra, Lugao, & Ponce, 2004). The continuous growing of consumer demand in convenience and food quality has supported more research in edible films and biopolymers coatings (Rodriguez, Oses, Ziani, & Mate, 2006). Edible films and coatings are useful materials produced these days from edible biopolymers and food-grade additives. Most biopolymers are naturally occurring polymers, either proteins or carbohydrates (Gennadios, Hanna, & Kurth, 1997). Plasticizers and other additives are incorporated with the film-forming biopolymers to improve film physical characteristics or to form extra functionalities (Kester & Fennema, 1986).

The application of edible films and coatings is an easy technique to develop the physical property of the food products (Cuq, Gontard, & Guilbert, 1995). Food products can be protected from oxidation, moisture absorption/desorption, microbial growth, and other chemical reactions (Kester & Fennema, 1986). The most common purposes of edible films and coatings are that they are barriers against oils, gas or vapors, and also they are carriers of active materials such as antioxidants, antimicrobials, colors and flavors (Guilbert & Gontard, 1995; Krochta & De Mulder-Johnston, 1997).

Preservation, convenience and the other basic purposes of packaging are definitely significant, but its disposal should be treated as an important characteristic of packaging improvement. This problem may challenge package development in the future. The food manufacturing uses a large amount of packaging materials. Therefore, any small decrease in the amount of materials used for each package would cause a substantial cost drop, and may decrease solid waste problems. Packaging technology has done an effort to decrease the volume and weight of materials in order to reduce resources and costs (Testin & Vergano, 1990). Another important subject in food packaging is that it should be natural and environmentally friendly. To plan environmentally friendly packaging systems that are more natural, involves partial replacement of synthetic packaging materials with biodegradable or edible materials. Furthermore, a subsequent decline in the application of entire amount of materials and a growth in the amount of biodegradable and recyclable materials will occur.

1.3 New food packaging technologies

Beyond the barrier function and recyclability, there has been more research and progress about the introduction of new functions to food packaging systems. Among these, important new functional packaging systems include active packaging, modified atmosphere packaging (MAP) and edible films/coatings. The increase of new active packaging functionalities has been promising due to technological progresses in food processing and packaging technology, leading to higher standards of rule, improve sanitation, and better health and safety.

Various new determinations have been presented in active packaging technologies, as well as oxygen-scavenging and atmosphere control, antimicrobial activity, intelligent functions, edibility, biodegradability etc. Food packaging has achieved further success than its conventional barrier characteristic. Active packaging systems prolong the shelf life of food products by preserving their quality longer and enhance their safety by securing foods from pathogens. The decision to consider active packaging for a food or beverage is usually based on factors normally elaborated in any package selection. These considerations consist of economic improvement, process engineering limitations, convenience in use, environmentally friendly effects, and subordinate effects resulting from some other modification in the processing or packaging.

Antimicrobial packaging, a promising form of active packaging is directly related to food microbial safety, as well as shelf-life extension by preventing the growth of spoilage and/or pathogenic microorganisms. The growth of spoilage microorganisms reduces the food shelf life, while the growth of pathogenic microorganisms endangers public health. Antimicrobial active packaging systems consist of packaging materials, in-package atmospheres and packaged foods, and are able to kill or inhibit microorganisms that cause food-borne illnesses (Han, 2000; Han, 2003).

Quality may consist of different characteristics such as sensory quality, toxicological quality, and microbial quality, based on deterioration factors and determination procedures. Microbial food quality relates to all three sets of factors, since the growth of bacteria produces undesirable odors and serious toxins, changes the color, taste and texture of food, and also decreases the shelf life of products. Due to the most food packaging systems consist of the food products, the headspace atmosphere and the packaging materials. Any of these three elements of food packaging systems could have an antimicrobial component to enhance antimicrobial efficiency. Antimicrobial active packaging can destroy or prevent target microorganisms and it can be made by applying antimicrobial packaging materials and/or antimicrobial agents inside the package space or inside foods (Han, 2000; Han, 2003).

For the consumers' tendency for foods without chemical preservative, food producers are now applying natural antimicrobials to sterilize and/or prolong shelf life of foods. Herbal extracts have multiple natural compounds, and are recognized to have an extensive antimicrobial range against different microorganisms. Various natural antimicrobial agents such as organic acid, enzymes, and plant extracted essential oils incorporated into packaging materials. Organic acids are usually applied as natural antimicrobial agents because their effectiveness is commonly well understood and cost effective. The precise selection of organic acids is necessary to have effective antimicrobial agents.

Edible films and coatings which carry active elements have been introduced as an active food packaging application (Cuq et al., 1995; Han, 2000, and 2001). Actually, edible films and coatings which are used for packaging of food without active constituents is also regarded as an active food packaging, as these films are edible and biodegradable not be present in conventional packaging constituents (Cuq et al., 1995; Han, 2002).

One of the important factors is the solubility and reactivity of the antimicrobial agents and polymers to the solvents. The reactivity relates to the activity loss of the reactive antimicrobial agents, even though the solubility relates to the homogeneous distribution of the agents in the polymeric materials. Thus, the compatibility of antimicrobial agent and packaging material is a critical factor. The pH of the system is also important. Most antimicrobial chemicals modify their activity with different pH. The pH of the packaging system is generally influenced by the pH of the packed foods, and thus consideration of the food composition with the naturally chemical antimicrobial agent is essential, along with consideration of the packaging substantial reaction with the chemical nature of the agents (Han, 2003). Storage and distribution conditions are further important factors, as well as the storage temperature and time. The microbial growth profile, chemical reaction kinetics, and the distribution profile of antimicrobial agents in the food were affected by the time-temperature combination. In order to inhibit microbial growth, storage at the favorable temperature range for microbial growth should be prevented or decreased for the entire period of storage and distribution.

The incorporation of antimicrobial agents can affect the physical and mechanical properties of packaging materials. A considerable amount of the agent may permeate into the packaging material without any decline of its physical and mechanical properties, if the antimicrobial agent is compatible with the packaging components (Han & Floros, 1997). However, further antimicrobial agent that is not capable of being combined with packaging components will decline physical strength and mechanical properties (Cooksey, Gremmer, & Grower, 2000).

1.4 Scope of study

Plastic polymers are not biodegradable and recycling is energy intensive and leads to critical environmental pollution. The plastic materials can also impose health hazard due to monomer leaching and migration into foods. The food manufacturing uses a large amount of variety of packaging materials (cost) and single edible film has shortcomings such as poor mechanical properties and fewer barriers to moisture. So composite tannic acid incorporated rice starch-fish gelatin edible film is a good modification to overcome above problems. There is no research on this combination for food active packaging application.

1.5 Objectives of study

I. Using rice starch and fish gelatin to produce edible and biodegradable films with glycerol and sorbitol as plasticizers and evaluate the functional properties

II. Evaluate the cross-linking capacity of tannic acid by evaluating both the structural improvement and its influence on physicochemical properties

III. Determine effectiveness of tannic acid incorporated rice starch-fish gelatin films on quality and microbiological characteristics of sausage during refrigerated storage

This thesis consists of three working chapters, rice starch-fish gelatin edible film plasticized with glycerol and sorbitol produced, characterized and its physicochemical properties investigated in first working chapter. In second working chapter, tannic acid were incorporated into the best rice starch-fish gelatin edible film plasticized with sorbitol and then physicochemical properties and inhibition properties against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* of incorporated tannic acid film were investigated. Best rice starch-fish gelatin edible film plasticized with sorbitol incorporated with tannic acid were applied to wrap sausage and stored in chilled temperature. Quality and microbiological characteristics of sausage during refrigerated storage determined and compared with commercial wrapped sausage in the last working chapter.

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PUBLICATIONS

Bakar, J., Ahangari, R., Abdul Rahman, R., & Karim, R., (2014). Edible packaging film. Universiti Putra Malaysia Patent. (PI2014702538)

Ahangari, R., Bakar, J., Abdul Rahman, R., & Karim, R., (2014). Properties of rice starch edible films incorporated with fish gelatin, Food Hydrocolloid (submitted)

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