UNIVERSITI PUTRA MALAYSIA

PREPARATION AND CHARACTERIZATION OF COMMERCIAL COLD WATER FISH GELATIN-BASED COMPOSITE FILM FOR PACKAGING RED TILAPIA (Oreochromis niloticus Linnaeus)

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By

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Chairman : Assoc. Prof. Seyed Hamed Mirhosseini, PhD
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Food packaging is traditionally limited to protect the food from physical, biological and chemical deterioration before consumption. In the recent years, the production of eco-friendly natural polymers as packaging material has many attractions for food manufacturers. The natural biodegradable polymers are more preferred to non-biodegradable plastic packaging polymers, which cause the biological pollution. Gelatin is one of the most common types of packaging polymer derived from bovine and porcine sources. In recent years, gelatin from marine fish species has been introduced as an alternative to mammalian gelatin. Cold water fish skin gelatin show worse rheological properties, low stability, gelling and melting points in comparison with mammalian and warm water fish gelatin. Gelatin films also have good oxygen barrier properties, but possess poor water barrier.

The main goal of this study was to improve the characteristics of gelatin from the cold water fish skin. The first objective was to investigate the effect of type and concentration of different polysaccharide gums (i.e. pectin (P), sodium alginate (SA), and karaya gum (KG), 1% and 2% w/v) as well as glycerol content (30% and 40%) on the characteristics of fish skin gelatin film. The differences among different films (FSG, FSG-P, FSG-SA and FSG-KG) were assessed by determining the physical, mechanical, barrier, microstructure, and thermal properties. The second objective was to compare the efficiency of the most desirable composite film and two commercial films on the quality and shelf life of the red tilapia fish fillets. The efficiency of the most desirable composite films and two commercial films (i.e. low- and high density polyethylene, LDPE and HDPE) were compared by assessing the moisture content, pH, a_w, colour (L*, a* and b*), texture properties (hardness, cohesiveness, springiness), rancidity degree and microbial quality of red tilapia fish fillets in the fresh form and after 28 days storage at 4 °C.

The addition of polysaccharide gum to the native fish skin gelatin led to improve its melting point, tensile strength (TS), and barrier properties (i.e. water vapour and oxygen). The tensile strength (TS) and melting point were decreased; while the elongation at break (EAB), water vapour permeability (WVP), and oxygen permeability were increased by increasing the percentage of glycerol (as a plasticizer) in the film formulation. Also, FSG based composite films containing 2% karaya gum, sodium alginate, and pectin decreased solubility from 99% to 32%, 39%, and 46%, respectively at 30% glycerol content. The composite film containing 2% polysaccharides gum (especially sodium alginate and karaya gum) along with 30% glycerol had the most desirable characteristics among all composite films. In the present study, karaya gum and pectin induced the most and least desirable effect on the characteristics of FSG film. Among all samples, the composite films (FSG-KG and FSG-SA)

containing 30% glycerol had the most desirable characteristics in terms of physical-, mechanical- and barrier properties. Therefore, they were chosen for wrapping red tilapia fish and storage study.

For shelf life testing and storage study, the tilapia fish fillets were wrapped with the most desirable composite films and two commercial films (LDPE and HDPE) and stored for 28 days at 4 °C. All wrapped samples were analyzed by the interval of 7 days in terms of microbial quality, physical and chemical characteristics, and texture. TBA of fish fillet wrapped with commercial films showed the highest changes (0.03-0.99); while FSG-KG wrapped fish fillet had the lowest TBA changes (0.03-0.36) among all samples. Tilapia fish fillets wrapped with the composite films had lower colour and texture changes than the samples wrapped with commercial films. In this study, the composite films (FSG-KG and FSG-SA) more efficiently retarded the microbial growth of red tilapia fish than the native FSG film and target commercial films (i.e. LDPE and HDPE).

Abstrak tesis yang dikemukeken kepada Senat Universiti Putra Malaysia sebegai memenuhi keperluan untuk Ijazah Master Sains

PENYEDIAAN DAN PENCIRIAN DAGANG IKAN AIR SEJUK GELATIN BERASASKAN FILM KOMPOSIT BAGI PEMBUNGKUSAN TILAPIA MERAH *(Oreochromis niloticus Linnaeus)*

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Pembungkusan makanan tradisional adalah terhad untuk melindungi makanan dari kemerosotan fizikal, biologi dan kimia sebelum penggunaan. Pada tahun-tahun kebelakangan ini, pengeluaran polimer semula jadi mesra alam sebagai bahan pembungkusan mempunyai banyak daya tarikan kepada pengeluar makanan. Polimer terbiodegradasi semula jadi lebih disukai ramai berbanding polimer pembungkusan plastik tidak terbiodegradasi, yang menyebabkan pencemaran biologi. Gelatin adalah salah satu jenis yang paling biasa dalam pembungkusan polimer yang diperolehi daripada lembu dan sumber babi. Dalam tahun-tahun kebelakangan ini, gelatin dari spesies ikan laut telah diperkenalkan sebagai alternatif kepada gelatin mamalia. Gelatin kulit Ikan air sejuk menunjukkan sifat reologi lebih teruk, kestabilan yang rendah, pengegelan dan takat lebur berbanding dengan mamalia dan gelatin ikan air tawar. Filem Gelatin juga mempunyai sifat-sifat penghalang oksigen yang baik, tetapi mempunyai ciri-ciri penghalangair yang kurang baik.

Matlamat utama kajian ini adalah untuk meningkatkan ciri-ciri gelatin dari kulit ikan air sejuk. Objektif pertama adalah untuk mengkaji kesan jenis dan kepekatan gam polisakarida yang berbeza (iaitu pectin (P), natrium alginat (SA), dan gam karaya (KG), 1 % dan 2 % w / v) dan juga kandungan gliserol (30 % dan 40 %) terhadap ciri-ciri filem gelatin kulit ikan. Perbezaan antara filem yang berbeza (FSG, FSG-P, FSG-SA dan FSG-KG) telah dinilai dengan menentukan fizikal, mekanikal, halangan, mikrostruktur dan sifat haba. Objektif kedua adalah untuk membandingkan kecekapan filem komposit yang paling dikehendaki dan dua filem komersial pada kualiti dan jangka hayat filet ikan Talapia merah. Kecekapan filem komposit yang paling dikehendaki dan dua filem komersial (iaitu kepadatan rendah dan tinggi polietilena, LDPE dan HDPE) dibandingkan dengan menilai kandungan kelembapan, pH, a_w, warna (L *, a*, dan b*), tekstur (kekerasan, kesepaduan, kekenyalan), tahap bau hanyir dan kualiti mikrob filet ikan tilapia merah dalam bentuk yang segar dan selepas 28 hari penyimpanan pada 4 °C.

Penambahan gula polisakarida di dalam gelatin kulit ikan asli membawa kepada peningkatan takat lebur, kekuatan tegangan (TS), dan ciri-ciri halangan (iaitu wap air dan oksigen). Kekuatan tegangan (TS) dan takat lebur telah menurun; manakala pemanjangan pada waktu rehat (EAB), wap air kebolehtelapan (WVP), dan kebolehtelapan oksigen telah meningkat dengan meningkatkan peratusan gliserol (sebagai bahan pemplastik) dalam penggubalan filem. Juga, filem komposit berasaskan FSG yang mengandungi 2 % gam karaya, natrium alginat, dan pektin masing-masing berkurang kelarutan dari 99 % kepada 32%, 39%, dan 46%, 30% kandungan gliserol. Filem komposit yang mengandungi 2% polisakarida gam (terutama natrium alginat dan gam karaya) bersama-sama dengan 30% gliserol mempunyai

ciri-ciri yang paling dikehendaki di kalangan semua filem komposit. Dalam kajian ini, gam karaya dan pektin menyebabkan kesan paling dan kurang dikehendaki atas ciri-ciri filem FSG. Antara semua sampel, filem-filem komposit (FSG-KG dan FSG-SA) yang mengandungi 30% gliserol mempunyai ciri-ciri yang paling dikehendaki dari segi sifat-sifat fizikal, mekanikal dan halangan. Oleh itu, mereka telah dipilih untuk membungkus ikan tilapia merah dan kajian penyimpanan.

Untuk ujian jangka hayat dan kajian penyimpanan, filet ikan tilapia telah dibalut dengan filem komposit yang paling wajar dan dua filem komersial (LDPE dan HDPE) dan disimpan selama 28 hari pada 4 °C . Semua sampel dibungkus dan dianalisis dalam tempoh 7 hari dari segi kualiti mikrob ,ciri-ciri fizikal dan kimia, serta tekstur. TBA daripada isi ikan yang dibalut dengan filem komersial menunjukkan perubahan tertinggi (0.03-0.99); manakala FSG-KG dibalut fillet ikan mempunyai perubahan TBA terendah (0.03-0.36) di kalangan semua sampel. Filet ikan tilapia yang dibalut dengan filem komposit mempunyai perubahan warna dan tekstur lebih rendah daripada sampel yang dibalut dengan filem komersial. Dalam kajian ini, filem-filem komposit (FSG-KG dan FSG-SA) lebih cekap membantutkan pertumbuhan mikrob ikan tilapia merah daripada filem FSG yang asli dan filem-filem komersial sasaran (iaitu LDPE dan HDPE).

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This Thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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^{-}OH	:	Hydroxyl group
/	:	Per
%	:	Percentage
°C	:	Centigrade
~	:	Around
≤	:	Equal or less than
≥	:	Equal or larger than
Ala	:	Alanine
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
Arg	:	Arginine
Asx	:	Aspartic acid or Asparagine
a_w	:	Water activity
BSE	:	Bovine spongiform encephalopathy
cm	:	Centimetre
CO_2	:	Carbon dioxide
COO^-	:	Carboxyl group
CRD	:	Completely randomized design
Da	:	Dalton
DE	:	Degree of esterification
DSC	:	Differential Scanning Calorimetry
e.g	:	Exempli gratia
EAB	:	Elongation at break
et al	:	Et alibi
etc	:	Et cetera
FAO	:	Food and agriculture organisation
FCMs	:	Food contact materials
FPI	:	Fish protein isolate
FSG	:	Fish skin gelatin

FTIR	:	Fourier transform infrared spectroscopy
G	:	Glycerol
g	:	Gram
Glx	:	Glutamic or Glutamine
h	:	Hour
H^+	:	Hydrogen ion
HDPE	:	High-density polyethylene
His	:	Histidine
HMP	:	High methoxyl pectin
Hyl	:	Hydroxylysine
Hyp	:	Hydroxyproline
i.e	:	id est
Ile	:	Isoleucine
KG	:	Karaya gum
kN	:	kilonewton
LDPE	:	Low-density polyethylene
Leu	:	Leucine
LMP	:	Low methoxyl pectin
log CFU/g	:	Log of the colony-forming units per gram of sample
Lys	:	Lysine
m^2	:	Square Meter
MDA	:	Malondialdehyde
Met	:	Methionine
mg	:	Milligram
min	:	Minute
mL	:	Millilitre
mm	:	Millimetre
mm/ min	:	Millimetre per Minute
MPa	:	Mega Pascal
mPa/s	:	Milli Pascal-Second

N	:	Newton
N_2	:	Nitrogen
NaCl	:	Sodium chloride
ND	:	Not detected
nm	:	Nanometer
O_2	:	Oxygen
OH^-	:	Hydroxide ion
OP	:	Oxygen permeability
OTR	:	Oxygen transmission rate
P	:	Pectin
P.a	:	Pascal
PA	:	Polyamide
PE	:	Polyethylene
PET	:	Polyethylene terephthalate
pH	:	Hydrogen ion exponent
Phe	:	Phenylalanine
PP	:	Polypropylene
PS	:	Polystyrene
PTC	:	Psychotropic bacteria
PVC	:	Polyvinyl chloride
QPM	:	Quaternary polymethacrylate
RH	:	Relative humidity
rpm	:	Revolutions per minute
s	:	Seconds
Ser	:	Serine
S^{-1}	:	Reciprocal seconds
SA	:	Sodium alginate
SEM	:	Scanning electron microscope
SPI	:	Soy protein isolate
Thr	:	Threonine

TBA	:	Trichloroacetic acid
TBARS	:	Thiobarbituric Acid Reactive Substances
T_m	:	Melting temperature
TPA	:	Texture Profile Analysis
TS	:	Tensile strength
TVC	:	Total viable counts
Tyr	:	Tyrosine
uL	:	Microliter
Val	:	Valine
v/v	:	Volume/volume
w/v	:	Weight/volume
w/w	:	Weight/weight
WHC	:	Water holding capacity
WVP	:	Water vapour permeability
WVTR	:	Water vapour transmission rate
κ	:	Kappa
µm	:	Micrometer

CHAPTER 1

INTRODUCTION

Packaging plays a significant role in the conservation, distribution and marketing of food. In the modern food chain system, it is hardly conceivable to distribute foodstuffs without packaging. Today, packaging is regarded as a significant part of food technology for protecting foods from microbial, chemical, and biological deterioration by processing and environmental factors such as temperature, light, moisture, etc. Food packaging can extend the shelf life of food products. Marine food is an important source of the human diet in different societies, especially in eastern Asia where many foods include different amounts of fish because of its health and nutritional benefits (Burger et al., 2003). However, fish is more perishable than most other fresh foods because of its chemical composition, and biological contamination, leading to safety issues as well as economic and health issues (Fernández-Saiz et al., 2013). Thus, fish must be stored properly or consumed immediately (Kilincceker et al., 2009). There are several packaging materials mainly used as packaging material for fish and marine products.

Non-biodegradable films are usually used as packaging materials in food systems. They are produced by using synthetic polymers. Petrochemical plastic is one of the most common synthetic packaging materials widely used in food systems because of its low cost and high preservative quality. However, this kind of plastic is basically non-biodegradable polymer that results in environmental contamination (Tharanathan, 2003). Thus, researchers and scientists have attempted to discover eco-friendly, biodegradable and recyclable materials as an alternative for synthetic polymer. In this regard, biodegradable and natural products such as gums and animal protein, as non-pollutant materials can be considered as reliable substitutes for synthetic packaging. However, due to their current high cost, they are mostly used for packaging of high-priced food products (Debeaufort et al., 1998). Thus, the biopolymer packaging has not become popular in the market. Indeed, further research is required for its commercialization and development.

Biodegradable films are thin layered films containing natural elements. They are formed as a solid sheet through different approaches such as casting, molding and extrusion. The main mechanisms in film-forming of biopolymers are interaction forces including covalent and electrostatic bonds, hydrophobic and ionic interactions (Han & Gennadios, 2005). Biodegradable film is a protective layer typically used on the surface of food products that are vulnerable to microbiological, physical and chemical activities. In addition, it can protect the food staff against water and gas flow. The characteristics of biodegradable films are mainly influenced by their composition (i.e. type and content of plasticizers and other additives) and film-forming conditions (i.e. pH and temperature) (Rojas-Graü et al., 2009a).

Biodegradable films are typically categorized based on their polymer structure. The major polymers for film formation are either proteins (such as gelatin, whey protein, casein, and wheat gluten) or polysaccharide gums (such as pectin, chitosan, sodium alginate and starch), or lipids (such as waxes and fatty acid and their blends) (Falguera et al., 2011). Moreover, the composite films are generated by mixing two or more polymers such as protein, lipid and polysaccharide gum. This combination can lead to the improvement of the weak properties of each polymer (Falguera et al., 2011). These films can emerge as a bi-layer or a cluster (Falguera et al., 2011). In the bi-layer type, one layer is protein; while another layer includes the polysaccharide gum or lipid. In cluster composite film, all combinations of components occur throughout the film forming solution. The most attractive films are protein-based edible films because of their nutritional values. They possess more desirable mechanical and barrier properties than lipid- and polysaccharide gums-based edible films (Ou et al., 2004).

Gelatin is a high molecular weight water-soluble protein. It is usually one of the basic components in many biodegradable films. It is typically extracted from bones, skin and connective tissue of cattle through thermal denaturation of collagen. However, replacing mammalian gelatin with a more economical alternative is highly desired. Gelatin has various applications in food and non-food industries. Physical properties of gelatin depend on its molecular weight aggregates, amino acid composition and sources. Fish skin is the waste of the fish industry. It is an important source of gelatin (Badii & Howell, 2006). Recently, gelatin, which is extracted from cold and warm water fish skin, has been found to be a suitable alternative to mammalian gelatin because of health, religious and social issues.

Problem Statement

Gelatin is a major component in protein-based biodegradable films. It has a three-dimensional network with areas of intermolecular microcrystalline junctions, leading to brittle films because of the dehydration of its structure (Vanin et al., 2005). The addition of plasticizer is suggested to improve the film fragility and develop its flexibility (Barreto et al., 2003). Due to the hydrophilic nature of gelatin, its film often represents a suitable gas barrier. However, its poor water barrier properties limit its application. Furthermore, the cold-water fish skin gelatin show lower melting points and weaker gelling properties than warm-water fish skin and mammalian gelatin. This weakness is basically due to its lower hydroxyproline and proline contents (Haug et al., 2004). This weakness also limits its industrial applicability. Fish is an important source of protein in Malaysia. Red tilapia is an important farmed freshwater fish in Malaysia. Freshwater aquaculture production statistics showed that tilapia topped the list for several years. On the choice of colour, the Malaysian aquaculture industry has always preferred the red hybrids. Red tilapia is usually sold freshly as a raw product which is easy spoiled. This study provided the background on various aspects of the preservation of perishable fish in respect of proper packaging and possible types of packaging materials that could ensure the quality and safety of the fish before consumption. It was hypothesized that the coating properties of gelatin film could be improved by combining with various hydrocolloids and polysaccharide gums.

Polysaccharide gums are complex carbohydrates, composed of several monosaccharides linked via glycosidic bonds. They are biodegradable polymers with sustainable and bio-safety features (Rana et al., 2011). Polysaccharide gums are utilized in food, cosmetics and pharmaceutical industries for different purposes such as texture modifiers, dietary fibre, gelling agents, thickeners, emulsifiers, stabilizers, coating agents, and packaging films (Anderson & Andon, 1998; Williams & Phillips, 2000; Mirhosseini & Tan, 2010a). Due to hydrophilic properties of the polysaccharide gum, their films have a high amount of oxygen. In addition, the polysaccharide gum-based films have very weak moisture barrier properties. This issue may be solved by combination with a protein (like gelatin).

Research Objectives

The main objective of this study was to investigate the effect of different polysaccharide gum and plasticizer on functional properties of cold fish skin gelatin. Subsequently, the application of the most desirable films for packaging of tilapia fish was investigated. The specific objectives were as follows:

- To investigate the effect of type and content of different polysaccharide gums and plasticizer on the characteristics of cold water fish skin gelatin film.
- To compare the efficiency of the most desirable composite films and various commercial films for maintaining the quality of red tilapia fish fillet during 28 days storage at refrigerator (4 ± 1 °C).

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