



**PHYSICOCHEMICAL AND RHEOLOGICAL
PROPERTIES OF FISH GELATIN MODIFIED BY
TANNIC ACID**

by

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A handwritten signature in black ink, consisting of several fluid, overlapping loops and curves, positioned above the author's name.

Sazeren Bt Amir

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
C	Degree Celsius
G'	Elastic modulus
FTIR	Fourier Transform Infrared
γ	Gamma
kDa	Kilodaltons
MPa	Megapascals
N	Normality
KBr	Potassium Bromide
SEM	Scanning Electron microscopy
NaOH	Sodium Hydroxide
TA	Tannic acid
G''	Viscous modulus
w/v	Weight per volume

SIFAT-SIFAT FIZIKOKIMIA DAN REOLOGI GELATIN IKAN YANG DIUBAH OLEH ASID TANIK

ABSTRAK

Gelatin ikan mendapat perhatian sebagai alternatif kepada gelatin mamalia yang mempunyai masalah budaya sosial dan juga masalah kesihatan. Walau bagaimanapun, gelatin ikan mempunyai sifat fizikokimia dan reologi yang lemah. Oleh itu, tujuan kajian ini adalah untuk mengkaji kesan asid tanik dengan kepekatan berbeza iaitu 0%, 2%, 4% dan 6% terhadap sifat-sifat fizikokimia dan reologi gelatin ikan. Sifat-sifat fizikokimia dan reologi gelatin ikan seperti warna telah kelikatan, kekuatan gel, sifat aliran dan juga sifat viskoelastik diperhatikan. Analisa warna telah menunjukkan yang penurunan dalam keterangan dan peningkatan dalam kemerahan dan kekuningan apabila kepekatan asid tanik semakin tinggi. Analisa FTIR mendedahkan terdapat peningkatan dalam kawasan gelombang amida bagi gelatin ikan yang telah dirawat dengan asid tanik. Hal ini menunjukkan bahawa asid tanik yang teroksida telah berinteraksi melalui ikatan kovalen dengan kumpulan amino di dalam gelatin. Sebarang perbezaan dari segi struktur mikro gelatin ikan tidak dapat diperhatikan melalui SEM. Kepekatan asid tanik sebanyak 4% sudah memadai untuk meningkatkan kekuatan gel gelatin ikan dengan ketara ($p < 0.05$) dari 7.90 g kepada 18.42 g. Kelikatan gelatin ikan juga meningkat secara ketara ($p < 0.05$) selepas dirawat dengan asid tanik yang berkepekatan 4% dan 6%. Kelikatan gelatin ikan yang dirawat dengan asid tanik adalah seiring dengan peningkatan kepekatan asid tanik. Bagi sifat-sifat reologi dinamik, gelatin ikan yang dirawat asid tanik menunjukkan sifat aliran Newtonian sama seperti gelatin ikan yang tak dirawat. Bagi sifat viskoelastik, kepekatan asid tanik yang rendah tidak mampu untuk meningkatkan keupayaan gelatin gel untuk membentuk gel dibuktikan dengan ketiadaan penyilangan antara modulus elastik (G') dan modulus likat (G''). Bagaimanapun, analisa suhu berayun menunjukkan bahawa 6% asid tanik menunjukkan peningkatan ketara bagi G' . Kajian menunjukkan

bahawa kepekatan asid tanic 6% tidak dapat meningkatkan kemampuan gelatin ikan untuk membentuk gel.

PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF FISH GELATIN MODIFIED BY TANNIC ACID

ABSTRACT

Fish gelatin was gaining attention as an alternative gelatin because mammalian gelatin faced social cultural issue and also health concern. However, fish gelatin has poor physicochemical and rheological properties. Therefore, the purpose of this study was to investigate the physicochemical and rheological properties of fish gelatin treated with tannic acid at different concentration which is 0%, 2%, 4% and 6%. The physicochemical and rheological properties such as colour, viscosity, gel strength, flow and viscoelastic properties of gelatin was observed. Colour measurement shows that there was decrease in lightness and increase in redness and yellowness of fish gelatin as the concentration of tannic acid increase that was treated with tannic acid because fish gelatin turned from pale yellow to dark brown due to tannic acid oxidation. FTIR analysis revealed increment in amide band region in treated fish gelatin indicating that oxidized tannic acid interacts with gelatin by covalent bonding with the amino group in gelatin. However, scanning electron microscopy do not shows any observable differences in microstructure of untreated and treated fish gelatin. 4% concentration of tannic acid was enough to improve gel strength of fish gelatin significantly ($p < 0.005$) from 7.90 g to 18.42 g. Viscosity of fish gelatin increase significantly ($p < 0.005$) by treating the tannic acid with 4% and 6% tannic acid. Viscosity of treated fish gelatin increase as concentration of tannic acid increases. For dynamic rheological properties, fish gelatin treated with 0%, 2%, 4% and 6% tannic acid shows Newtonian behaviour similar with untreated fish gelatin. As for viscoelastic properties, low concentration of tannic acid was unable to improve the gelling ability of fish gelatin as indicated by no cross over of elastic modulus (G') and viscous (G'') modulus from the temperature sweep analysis. However, the temperature sweep analysis

revealed that 6% concentration of tannic acid shows pronounced effect in enhancing the G' modulus. The study revealed that 6% concentration of tannic was unable to improve gelling ability of fish gelatin.

CHAPTER 1 INTRODUCTION

1.1 Research Background

Gelatin is a biopolymer derived from partial thermal hydrolysis of collagen (Shyni et al., 2014). Due to its unique functional properties, the application of gelatin is widely used in the pharmaceutical, cosmetic industries, and also in food industry. Gelatin, it is commonly used in food industry as 63 % of gelatin usage in food industry followed by pharmaceutical industry with 31% and other with 6 % (GME, 2020). Gelatin was commonly incorporated in food processing to improve the quality of food product such as to provide chewiness, texture and foam stabilization to confections product, to provide creaminess, fat reduction and mouthfeel sensation in low-fat spreads products, to provide water-binding in meat product and to provide stabilization and texturization for dairy products (Karim & Bhat, 2009). Desserts, aspics, gummy bears jellies, dairy products (ice cream and yogurt) and also marshmallows were the common product that used gelatin application (Venien & Levieux, 2005).

Majority of gelatin that was used in industry was mammalian gelatin which mainly extracted from pig skin (porcine) and cow hide (bovine). Despite the broad application of the mammalian gelatin, it faced religious sentiment and social-cultural issues as Islam and Judaism prohibited consumption of any pork related product and besides, Hindu also prohibited any cow related product (Alfaro et al., 2015). Moreover, the outbreaks of the bovine spongiform encephalopathy (BSE) which also known as Mad cow disease rose the concern about mammalian gelation among the consumers (Zilhada et al., 2018). Therefore, such factors consequently restrict the application of porcine and bovine gelatin in the food industry and leading to the increasing demand for non-mammalian alternative sources especially fish and poultry (Abedinia et al., 2020).

Interest for the alternative's sources from marine and freshwater fish was growing in the recent year due to its unique functional properties and applications (Bonne & Verbeke, 2008; Das et al., 2017). However, the application of fish gelatin in the industry was limited due to the inferior rheological properties such as lower gelation and melting temperature and also lower gel strength compared to mammalian gelatin (Alfaro et al., 2015; Araghi et al., 2015; Karim & Bhat, 2009). Gel strength, viscosity, gelling, and melting points are the most important properties food application as it will determine the quality and the range of application of gelatin. So the poor rheological properties of fish gelatin consequently limits the fish gelatin application in food industry (Choi & Regenstein, 2000; Karim & Bhat, 2009). The inferior rheological properties of fish gelatin is mainly attributed to the fact that fish gelatin contain low amount of imino acid (hydroxyproline and proline) (Alfaro et al., 2015; Araghi et al., 2015).

This drawback of fish gelatin has spark interest among researcher to find method that will improve the functional properties of fish gelatin. Fish gelatin can be modified enzymatically by Microbial-transglutaminase (MTGase), physically by radiation or chemically by phenolic acid. Compared to enzymatically modification, chemical modification of gelatin received less interest. The common and widely used as chemical cross-linker used in application is glutaraldehyde because it reacts rapidly with gelatin however safety concern arises due to the toxicity (Karim & Bhat, 2009). Plant phenolic compound can also act as cross-linker for gelatin gels and gelatin-based coarcervates for future use as food ingredients (Strauss & Gibson, 2004). Commonly phenolic acid can be found in various plant such as coffee and grape. Therefore, the use of phenolic acid as chemical cross-linker in gelatin was considered practical as coffee and grape or other plant that is sources of phenolic acid was easily available and contain sufficient concentration of phenolic acid (Karim & Bhat, 2009).

Tannin is a polyphenolic compound that found in plant as secondary metabolites. Tannic is also a natural excellent cross-linker due to the sufficient hydroxyls and carboxyls group that can form strong complexes with protein and has antioxidation, low cytotoxicity and antibacterial properties (Kroll et al., 2003; Yu et al., 2020). Combination of tannins with protein solutions under different conditions has been widely investigated in order to improvement of mechanical and gel properties of the proteins (Aewsiri et al., 2010; Anvari & Chung, 2016; Kaewdang & Benjakul, 2015). However, less information is available about using of the tannins to modified the physicochemical and rheological properties of fish gelatin.

1.2 Research objective

The main objective of this study is to improve the rheological and physicochemical properties of fish gelatin using different concentration of tannic acid.

The specific objectives are:

1. To investigate the effect of tannic acid on rheological properties of the fish gelatin
2. To investigate the effect of tannic acid on physicochemical properties of the fish gelatin