DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS FROM SAGO STARCH/SURIMI BY-PRODUCTS FISH GELATIN BLENDS

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by

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Ahmed,

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LIST OF SYMBOLS / ABBREVIATION

Symbol/Abbreviation	Caption
a*	Redness
ANOVA	one-way analysis of variance
a_w	water activity
b*	blueness
CFG	commercial fish gelatin
DSC	differential scanning calorimetry
E	Young's modulus
EFG	extracted fish gelatin
FFS	Film forming solution
FTIR	Fourier transform infrared spectroscopy
G	glycerol
G'	storage modulus
G″	loss modulus
HBG	halal bovine gelatin
L*	lightness
IEP	isoelectric electric point
RH	relative humidity
S	sorbitol
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	scanning electron microscopy
T	transparency

TA	texture analyzer
T_g	glass transition
T_{gel}	gelling temperature
TGs	transglutaminase
TS	tensile strength
T_m	melting temperature
%EAB	percentage of elongation at break
TNBS	trinitrobenzenesulfonic acid
β-ME	β-mercaptoethanol
MR	Maillard reaction
WVP	Water vapor permeability
ΔH	Enthalpy

LIST OF PUBLICATIONS & SEMINARS

Seminar & Exhibition

1. A.H. Ahmed, M.H. Norziah and A.B. Khairulnizam. Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglautaminase on gel properties. Paper presented at the International Hydrocolloids Conference 2008, p 67, 15-19 June, 2008, National University of Singapore, Singapore.

2. Norziah, M.H., Khairulnizam, A.B., Ahmed, A., Fazilah, A., and Norita, M. Characterization of Fish Gelatin Extracted from Surimi Processing Wastes. In proceedings of the International Conference on Environmental Research and Technology (ICERT 08), p 52-55, May 28-30, 2008, Penang, Malaysia.

3. M.H. Norziah, A.B. Khairulnizam, A. A. Al-Hassan, M.M Mordi (2009). Thermal characterization of fish gelatin extracted from surimi processing by-products in Gums and Stabilizers for the Food Industry Conference, 22-25 June 2009, Glywndr University, Wales, UK.

Publications

1. Norziah, M. H., Al-Hassan, A., Khairulnizam, A. B., Mordi, M. N., & Norita, M. (2009). Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties. *Food Hydrocolloids, 23*(6), 1610-1616.

2. M.H. Norziah, A.B. Khairulnizam, A. A. Al-Hassan, M.N Mordi (2010). Thermal characterisation of fish gelatin extracted from surimi processing by-products. Chapter in Gums and Stabilisers for the Food Industry 15, P.A. Williams & G.O Philips (eds.), pg 37-50. Publisher: The Royal Society of Chemistry, Cambridge, ISBN 978-1-84755-199-3.

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PEMBANGUNAN DAN PENCIRIAN FILEM BOLEH MAKAN DARIPADA CAMPURAN KANJI SAGU/GELATIN IKAN HASILAN SAMPINGAN SURIMI

ABSTRAK

Sifat-sifat fizikokimia dan termal gelatin ikan terekstrak (EFG) yang diperolehi daripada hasil sampingan surimi telah dikaji dan dibandingkan dengan gelatin ikan (CFG) dan gelatin lembu halal (HBG) komersial. Gelatin ikan yang diekstrak telah dianalisis untuk sifat-sifat fizikokimia seperti warna, kandungan asid amino, kandungan mineral, analisis struktur dengan FTIR dan untuk sifat-sifat termal. Daripada kajian dengan kalorimetri pengimbasan perbezaan (DSC), suhu lebur dan suhu mengel EFG yang diperolehi adalah 16.2 ± 0.2 °C dan 5.27 ± 0.4 °C, masing masing dan daripada pengukuran reologi, adalah 16.7 ± 0.1 °C dan 5.1 ± 0.1 °C. Kedua-dua keputusan untuk EFG adalah lebih rendah daripada CFG dan HBG. Adalah didapati kelikatan lebih rendah secara signifikan bagi EFG dibandingkan dengan gelatin lain. Untuk meningkatkan sifat-sifat fizikal dan termal EFG, transglutaminase (TGs) telah ditambahkan dalam amoun yang berbeza (0.5, 1.0, 3.0 dan 5.0 mg/g gelatin) ke dalam sampel EFG. Sampel gelatin yang dirawat menunjukkan pengurangan kumpulan asid amino daripada ujian silang paut dan kehadiran jalur pempolimeran dikesan dalam analisis SDS-PAGE dalam semua sampel yang dirawat. Disamping itu, imbasan FTIR menunjukkan penurunan keamatan bagi puncak yang melibatkan ε-amina daripada *lysine*. TGs meningkatkan suhu lebur EFG yang dirawat sehingga 19 ± 0.7 °C daripada imbasan DSC. Daripada pengukuran reologi, suhu lebur meningkat sehingga 17.5 ± 0.5 °C manakala suhu mengel meningkat sehingga 5.7 ± 0.1 °C berbanding dengan sampel yang tidak dirawat (5.1 ± 0.1 °C). Kekuatan gel meningkat sehingga 101.1 g dan 90.56 g dengan penambahan TGs sebanyak 1.0 dan 3.0 mg/g, masing-masing. Walau bagaimanapun, dengan penambahan 5.0 mg/g enzim, kekuatan gel menurun (75.06 g) tetapi masih

lebih tinggi berbanding dengan sampel yang tidak dirawat. Dalam penyediaan filem boleh makan daripada campuran kanji sagu/gelatin ikan terekstrak, larutan membentuk filem (FFS) dengan nisbah kanji sagu dan gelatin ikan yang berbeza (1:0, 2:1, 3:1, 4:1, dan 5:1) yang ditambahkan bahan pemplastik gliserol atau sorbitol sebanyak 25, 30 dan 35% w/w. Sifat rheologi FFS serta morfologi dan sifat-sifat fizikokimia, termal dan mekanikal filem yang dihasilkan telah dikaji. Daripada kajian reologi, FFS adalah larutan yang dipengaruhi oleh frekuensi dan bersifat bukan Newtonian. Kebolehtelapan wap air (WVP) telah berkurangan dengan penambahan gelatin ikan dalam filem dengan 25 dan 30% gliserol. Imbasan DSC menunjukkan filem dengan bahan pemplastik dan protein (gelatin) mengurangkan takat peralihan kaca (T_g), suhu lebur (T_m) dan entalpi lebur (ΔH_m). Pemerhatian hanya satu nilai T_g menunjukkan keserasian polimer-polimer kanji sagu dan gelatin ikan untuk membentuk filem pada kepekatan yang digunakan. Secara umum, kekuatan tegangan (TS) menurun apabila kandungan protein adalah lebih tinggi apabila salah satu bahan pemplastik digunakan, dan tiada tren diperhatikan bagi nilai % *elongation-at-break*. Kesan penambahan TGs dan rawatan haba ke atas larutan pembentuk filem kanji/gelatin (3:1) dengan 30% gliserol juga dikaji. Kadar silang paut menunjukkan penurunan kumpulan asid amino yang terdapat dalam larutan pembentuk filem selepas rawatan oleh transglutaminase atau pemanasan. Disamping itu, spectra FTIR juga menunjukkan penurunan dalam serapan jalur bagi kumpulan amida I, II dan III akibat daripada silang paut berbanding dengan sampel yang tidak dirawat. Kedua-dua rawatan menunjukkan perbezaan yang ketara dalam sifat-sifat mekanikal, warna, ketelusan, kelarutan dan isotermal *sorption-desorption*, tetapi tiada kesan positif terhadap WVP filem tersebut.

DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS FROM SAGO STARCH/SURIMI BY-PRODUCTS FISH GELATIN BLENDS

ABSTRACT

Physicochemical and thermal properties of extracted fish gelatin (EFG) obtained from surimi wastes were investigated and compared with those of fish gelatin (CFG) and halal bovine gelatin (HBG) from commercial preparations. Extracted fish gelatin was characterized for its physicochemical properties such as colour, amino acid composition, elemental content, structural analysis by FTIR and for its thermal properties. From differential scanning calorimetry (DSC) experiments, melting and gelling temperatures of EFG obtained were 16.2 ± 0.2 °C and 5.27 ± 0.4 °C, respectively and from rheological measurements, they were 16.7 ± 0.1 °C and 5.1 ± 0.1 °C, respectively. Both results obtained for EFG were much lower than that of CFG and HBG. Significantly lower viscosity of EFG was observed compared to other gelatins. To enhance the physical and thermal properties of EFG, transglutaminase (TGs) was added in different amounts (0.5, 1.0, 3.0 and 5.0 mg/g gelatin) to the EFG samples. Treated gelatin samples showed reduction in the amino acid groups from degree of crosslinking test and polymerization bands were observed in SDS-PAGE analysis in all treated samples. Likewise, FTIR scans showed a decrease in the intensity of the peaks involving the ε-amine of lysine. TGs increased the melting temperature of treated EFG up to 19 ± 0.7 °C from DSC scans. From rheological measurements, melting temperature increased to 17.5 ± 0.5 °C whilst the gelling temperatures increased up to 5.7 ± 0.1 °C compared to untreated samples (5.1 ± 0.1 °C). Gel strengths increased to 101.1 g and 90.56 g with TGs of 1.0 and 3.0 mg/g, respectively. However, with addition of 5.0 mg/g enzyme the gel strength decreased (75.06 g) but was still higher than untreated samples. In the preparation of

edible films from sago starch/extracted fish gelatin blends, film forming solutions (FFS) of different ratios of sago starch to fish gelatin (1:0, 2:1, 3:1, 4:1 and 5:1) plasticized with 25, 30 and 35% (w/w) glycerol or sorbitol were used. The rheology of FFS and morphology, physicochemical, thermal and mechanical properties of the developed films were investigated. From rheological studies, FFS were frequency dependent and behaved as non-Newtonian solutions. Water vapor permeability (WVP) reduced with addition of fish gelatin in films with 25 and 30% glycerol. DSC scans showed that films with plasticizers and protein (gelatin) reduced the glass transition (T_g), melting temperature (T_m) and the melting enthalpy (ΔH_m). The observation of a single T_g was an indication of the compatibility of sago starch and fish gelatin polymers to form films at the concentration levels used. The tensile strength (TS) decreased with higher protein content when either plasticizer were used in general and no trend was observed in % elongation-at-break. The effects of addition of TGs and heat treatment on starch/gelatin (3:1) film forming solution with 30% glycerol were also investigated. Degree of crosslinking showed a reduction in amino acid groups available in the film forming solutions after treatment by either transglutaminase or heating. Likewise, FTIR spectra showed a reduction in the bands absorbance of amides I, II and III as a result of the crosslinking compared to un-treated sample. Both treatments showed significance differences in mechanical properties, colour, transparency solubility and sorption-desorption isotherm, but no positive effect on WVP of the films.

CHAPTER 1
INTRODUCTION

1.1 Introduction

Edible films and coating materials are intended to extend shelf-life and improve food qualities by reducing the moisture transfer, oxygen, carbon dioxide, lipids oxidation, flavor and aroma between food components and the surrounding atmosphere (McHugh, 2000). Edible films and coating materials are defined as thin, continuous layer of edible material formed or placed on or between foods or food components (Torres, 1997). The main film materials that have been used and investigated for improvement of food quality and shelf life are polysaccharides, proteins, lipids and their derivatives or mixtures. Polysaccharides and protein films have good O_2 and CO_2 barriers properties but more hydrophilic presenting high water vapor permeability (WVP) with poor mechanical properties (Arvanitoyannis et al., 1996; Baldwin et al., 1995; García et al., 2000a). Films composed primarily of proteins or polysaccharides are highly sensitive to moisture therefore, films may swell, dissolve, or disintegrate upon contact with the water when applied to high moisture contents food products (Gomez-Guillon et al., 2009). Blending these polymers with hydrophobic materials such lipids reduce the hydrophilicity of the films and therefore reduce the WVP however, lipids oxidation may occur which limits lipids use in edible films (Garcia et al., 2000b). Films with lipids present higher moisture-resistant, but are usually opaque, relatively stiff, and more vulnerable to oxidation (Gomez-Guillon et al., 2009).

Enhancement of the mechanical properties of edible films from protein, polysaccharides and their derivatives or mixtures have been investigated with addition of additives and different types of modifications including physical, chemical and

enzymatic. Many studies have covered the production of films and coatings materials from protein or polysaccharides, their derivatives and mixtures as well. As a result of poor mechanical strength and high moisture sensitivity exhibited by polysaccharides films such starch edible films, several studies have investigated various additives, type of modifications, source of starches, and process parameters in order to improve these weaknesses (Mali et al., 2006; Viga-Santos et al., 2007). Due to the strong economic incentives and since additives must be safe to eat or generally recognized as safe (GRAS), blending polymers is an effective method for modification by conventional processing techniques that may produce edible films with enhanced properties (Su et al., 2010). However, there is still poor understanding of the phenomena that occurred when two biopolymers are blended together such as protein and polysaccharides. Thus, more research need to be conducted , to investigate the compatibility and incompatibility of these polymers in form of films.

Edible films from sago starch and extracted fish gelatin from surimi by-product from fish processing industry and plasticized with polyols namely; glycerol and sorbitol here investigated in this study. Production of fish gelatin (from skins, bones and fins) and their industrial applications is relatively new approach compared to the well-known sources as bovine and porcine. Several studies have investigated the replacement of mammalian gelatins with different sources including plant protein, polysaccharides, fish gelatin, their derivatives, mixtures and modifications but still more research need to be done in this area. Religious or ethical reasons limited some people from eating bovine or porcine gelatins as well as their based products. The out break of Bovine spongiform encephalopathy (BSE) known as mad cow disease has raised great attention and interest in order to find an alternative safe sources of gelatin (Fernández-Díaz et al., 2003; karim & Bhat, 2009). Likewise, much attention given to

the issue of Halal products in the Islamic worlds has increased focus to finding new alternative sources that can replace products containing non-Halal gelatins (such as foods, drug capsules, casing and packaging materials). Fish gelatin is regarded to be an alternative to mammalian sources; however, due to the 'superior' properties in mammalian gelatin, much attention has focused into modifying the properties of fish gelatin either by applying modification treatment on fish gelatin or blending it with other polymers or gums.

Gelatin being a protein is biodegradable and has many applications in the food and pharmaceutical industries. The concerns of environmental management in producing possible biodegradable packaging materials has increased the attention of using such materials from biopolymers including protein, polysaccharides and their derivatives. Recently, some studies have attempted to produce gelatin from fish waste from seafood processing industries such as the surimi processing industry. By definition, surimi is a white odorless past from fish meat "fish pulp" that is made after a process of fish deskin and debone. Management and optimization of waste from seafood processing industries such as surimi wastes (fish skins, bones, meats, heads and fins) are of concern to these industries. Studies have also been carried out especially on the extraction, characterization and modifications of gelatin from many different marine sources.

Surimi by-products from the surimi processing industries are abundant, locally available, and inexpensive. However, these wastes are dumped back to the sea every year (7-8 metric tonnes of surimi waste per day discarded; personal communication with a manager of a local surimi industry plant, Malaysia). Fish gelatin extraction from surimi by-product to our knowledge has never been carried out and therefore such extraction will introduce a new source of fish gelatin to be used in

foods and pharmaceutical industrial applications. Fish gelatin has the properties of lower bloom strength, lower melting and gelling temperatures and retain fish odor when compared to mammalian gelatins and the utilization of fish gelatin is limited. Fish gelatin has been modified to overcome the weakness of its properties. In recent years, physical, chemical and enzymatic modifications of fish gelatin have been the subject of many researches. Transglutaminase enzyme has been used in many different proteins including fish gelatin to enhance their properties such as gel strength and gelation properties. Some studies have proved that the use of transglutaminase enzyme, chemical reagents, mixing with polysaccharides or gums and heat treatments can enhance gelatin properties such as gelling and melting temperatures and gel strength.

Starch has been the subject of many studies such as on its application in edible and coating materials. Starch is abundant, cheap, biodegradable and edible polymer. Several studies have been accomplished to analyze starch in terms of properties of different starches, effect of modifications on starch properties, and the use of starch in edible and biodegradable films (Alves et al., 2007). Sago starch a well-known starch in south East Asia is cheap, available locally and has good film forming ability.

Films that are composed primarily of proteins or polysaccharides are highly sensitive to moisture with poor WVP properties, films may swell, dissolve, or disintegrate upon contact with water. Due to these reason, the current trend in food packaging is to combine different biopolymers (Gomez-Guillon et al., 2009). Mixing two biopolymers, such as fish gelatin and sago starch based-films may enhance its physical and mechanical properties. Edible films and coating materials from fish gelatin cannot replace that of mammalian sources due to lower mechanical properties

and higher water vapor permeability. Likewise, starch-based films have some major limitations due to their poor mechanical properties and high water permeability (Veiga-Santos et al., 2007). Polysaccharides films including starches, cellulose derivatives and plant gums have been reported as edible films and coatings for food packaging and preservations.

Plasticizers play a major role in the properties and qualities of edible and coating material. Several studies investigated the effect of the addition of such plasticizers including water, glycerol, sorbitol and many others. Mixing polysaccharides, protein, their derivatives and their blends with plasticizers namely, glycerol or sorbitol overcomes their brittleness but may retard their physical and mechanical properties.

Nevertheless, there is limited reported data about edible films and coating materials based on starch and fish gelatin mixtures. A thorough understanding of the mechanism of the interactions between these two biopolymers would propose new and effective materials to be used in edible films, coating, food packaging and pharmaceutical products including hard and soft capsules which could lead to alternatives to use of gelatin from mammalian sources.

Part of this study is to extract fish gelatin from wastes obtained from a surimi processing industry and the fish gelatin is then used as one of the biopolymer in this research. Tons of surimi fish wastes end up unprocessed and dumped back to the sea every year (600 000 metric tonnes of surimi produced annually in south East Asia; SEAFDEC (2009). However, the waste produced by fish filleting can account up to 75% of the total weight of catches (Shahidi, 1994). Fish gelatin cannot substitute the role of mammalian gelatin in foods as well as in films and coating materials due to its inherited properties. Therefore, fish gelatin can be modified by chemical, physical

or enzymatic means in order to improve its properties and to compete the mammalian gelatin. In general, starch films and protein films have the properties of being hydrophilic films presenting higher water vapor transmittance and lower mechanical properties compare to synthetic ones. Starch itself normally crystallizes with aging and could cause an adverse effect on film properties. Composite films of sago starch with fish gelatin may result in improve mechanical and physical properties. When the two biopolymers are mixed, they may have different structures depending on each polymer behavior alone and the nature of their interactions. Modification of film forming solution of sago starch/fish gelatin by transglutaminase enzyme or through maillard-induced crosslinking may improve the films physical and mechanical properties.

This thesis covers three aspects namely, characterization of extracted fish gelatin (EFG) from wastes of fish Herring species (*Tenualosa ilisha*) described in chapter 3; followed by chapter 4 which focused on the treatment of EFG with transglutaminase enzyme and evaluation of the thermal and physical properties of treated fish gelatin; development of sago starch/fish gelatin edible films is described in chapter 5 and finally the modification of the sago starch /fish gelatin (3:1) films by transglutaminase enzyme (chapter 6) and heat treatment (chapter 7). The flow chart of this study is presented in Fig. 1.1.

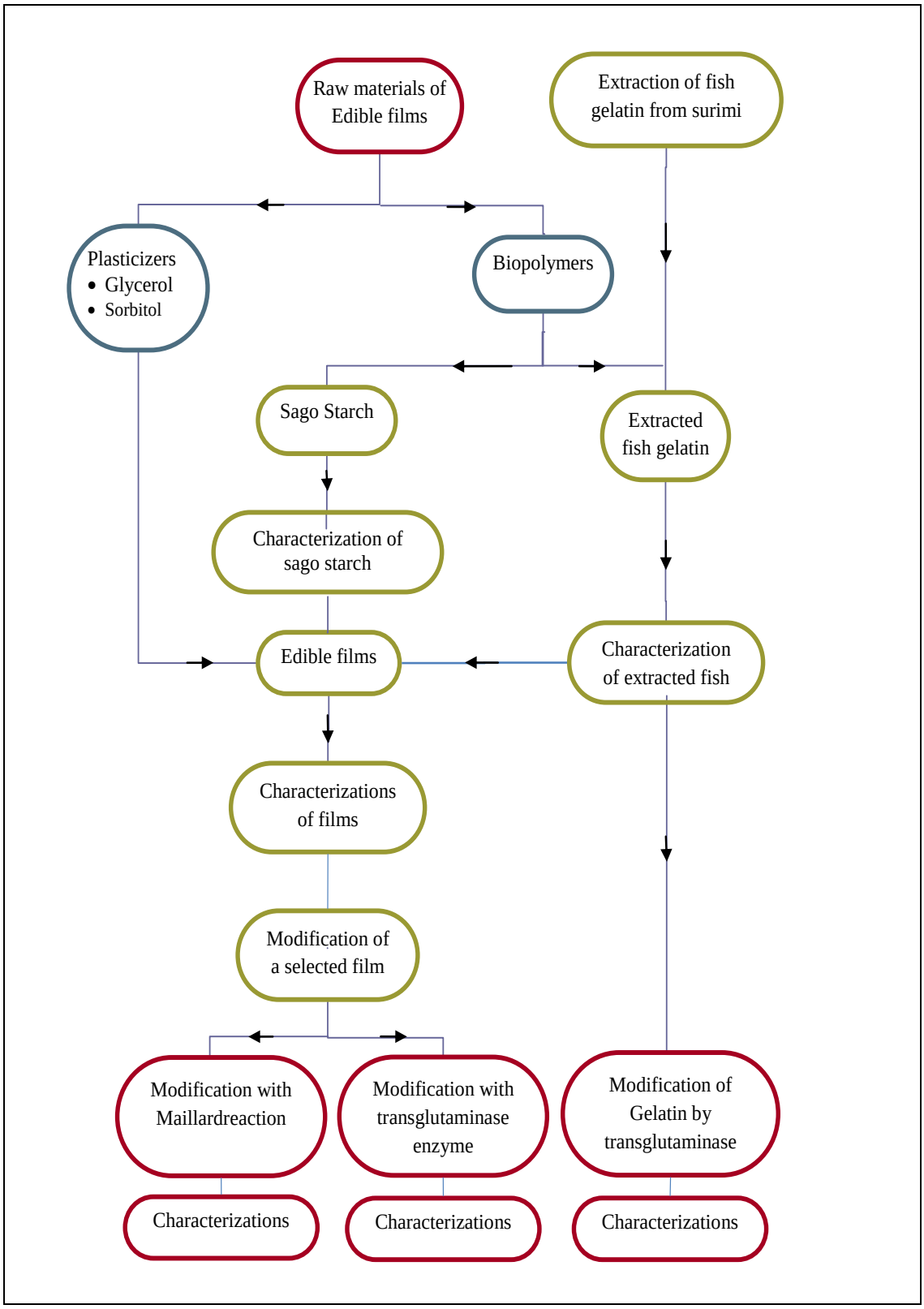


Figure 1.1 Overall methodology.

1.2 Objectives of study

1. To extract and characterize the physicochemical and thermal properties of fish gelatin from surimi by-products.

2. To evaluate the effects of transglutaminase on the properties of extracted fish gelatin.

3. To develop edible films from sago starch/surimi by-products fish gelatin with plasticizers, glycerol and sorbitol.

4. To study the effects of transglutaminase and Maillard-induced crosslinking on the physicochemical and thermal properties of sago starch/surimi by-products fish gelatin film.

CHAPTER 2
LITERATURE REVIEW

In this chapter, the literature review cover sago starch (physical and functional properties), protein (structure, physical and functional properties) and gelatin (extraction, physicochemical and functional properties). In addition, transglutaminase enzyme (reaction mechanism), biopolymer films and plasticizers (starch and protein based films) and two major modification treatments of protein based films (crosslinking by transglutaminase enzyme) and protein-carbohydrate Maillard induced cross-linking (Maillard reaction mechanism and use).

2.1 Starch

Starch exhibits a unique viscosity behavior with changes of temperature, concentrations and shear rate (Nurul et al., 1999). Starch is a carbohydrate structured from granules containing two polysaccharides namely amylose and amylopectin (Fig. 2.1). Amylose is made up of glucosidic chains linked via α1-4 glucosidic bonds with few branches by α1-6 glucosidic bonds (Fig. 2.2-A). Amylopectin is the major component of all starches; a branched molecule of glucosidic chains linked via α1-4 glucosidic bonds and branched by α1-6 glucosidic bonds (Fig. 2.2-B). Kennedy et al. (1983) stated that chain lengths of amylose are commonly in excess of 6000 D-glucopyranose units with molecular weight between 150 000-600 000 Da and amylopectin a highly branched molecule with an average of 17–26 D-glucosyl units linked by (α1-6) branch points. They added, the molecular size of amylopectin is almost too large to be determined accurately, but light scattering studies indicate a value of 106 D-glucosyl residues per molecule, which makes amylopectin one of the largest naturally occurring macromolecules.

Different starch has different ratio of amylose to amylopectin. For example, corn starch has approximately 28% amylose, genetically manipulated high amylose corn starch can contain about 70% amylose while genetically modified waxy corn contains 90%–100% amylopectin (Kennedy et al., 1983). Types of starches used in industrial basis are typically derived from cereals (corn, wheat, rice, sorghum), tubers (potato, sweet potato), roots (cassava), and legumes (mung bean, green pea) Sago starch is perhaps the only example of commercial starch derived from another source as extracted from the stem of palm (sago palm) (Karim et al., 2008). Sago starch (*Metroxylon sagu*) is produced from sago palm (*Metroxylon spp*.) initially presents in South East Asia (Mohamed et al., 2008). It is estimated that about 60 million tones extracted sago starch from sago palms per annum in south-east Asia (Wang et al., 1996). In comparison of sago starch yield per area unit to other starch types could be about 3 to 4 times higher than that of rice, corn, or wheat, and about 17 times higher than that of cassava (Karim et al., 2008).

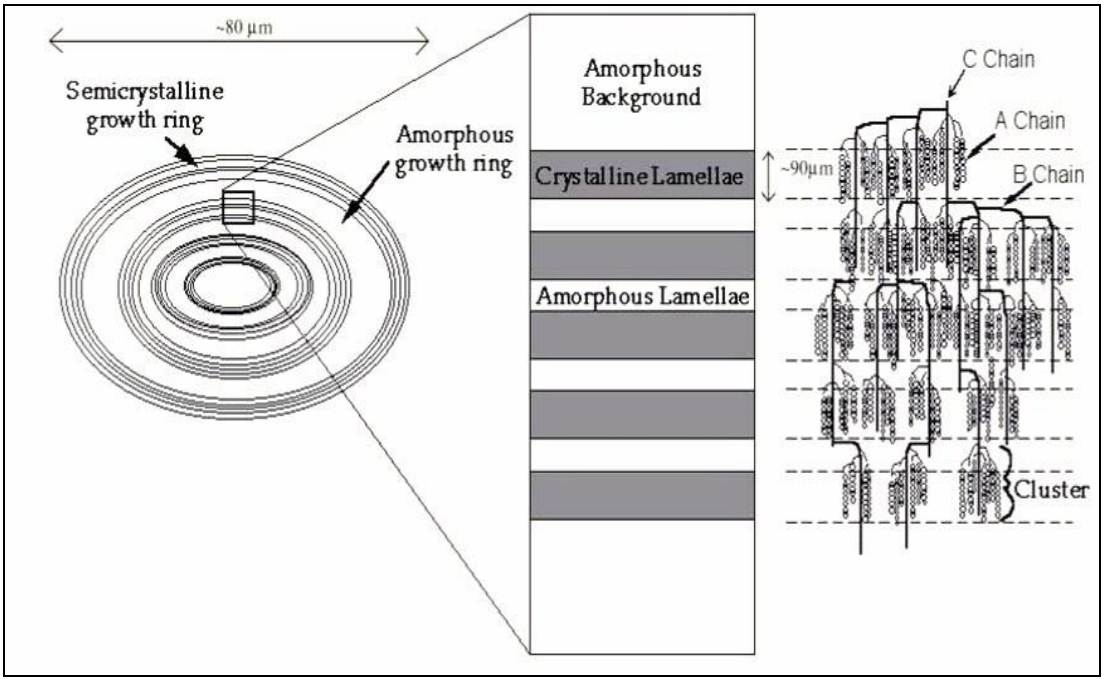


Figure 2.1 Schematic view of the structure of a starch granule, with alternating amorphous and semi crystalline zones constituting the growth rings (adopted from: Oates, 1997).

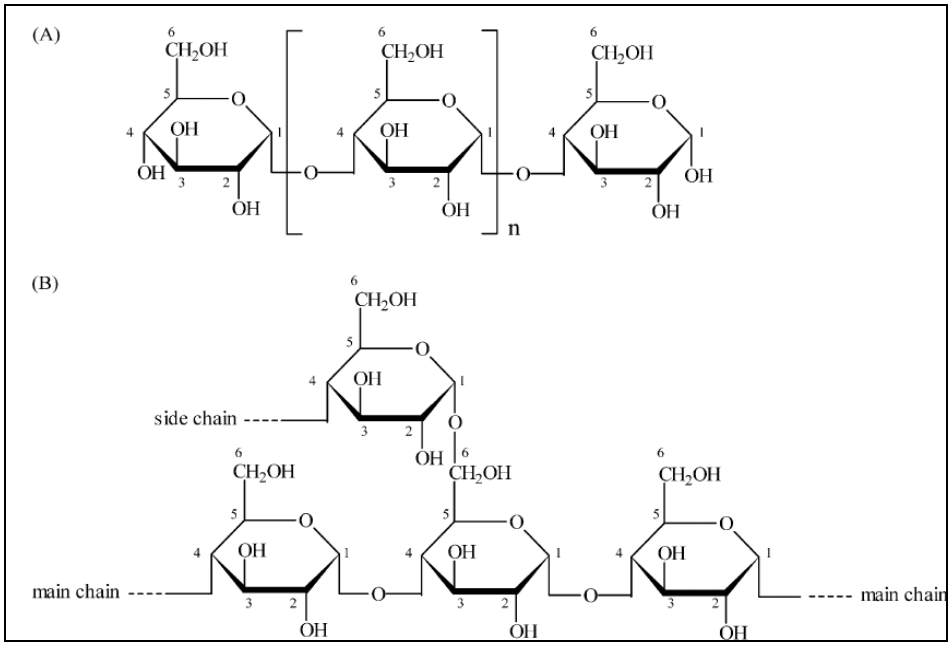


Figure 2.2 Structures of amylose (A) and amylopectin (B); shows the different types of chain linkages, (adopted from: Herrero-Mart´ınez et al., 2004).

2.1.1 Physical properties of sago starch

Sago starch has various applications in food productions including sago meals, biscuits (e.g., “tabaloi”), noodles, vermicelli, bread, crackers and desserts (Karim et al., 2007). Due to Sago starch physicochemical as well as thermo-rheological properties makes it as an excellent starch resource with possible applications in many food processing (Mohamed et al., 2008).

2.1.1.1 Component ratios

The total amylose contents (lipid free starch) in sago starches ranged between 24-31% (Mohamed et al., 2008). However, Amylose content of sago starch from the lower part of the trunk is higher than that from the upper part, which is further increases with growth (Elgadir et al., 2009).

2.1.1.2 Granule size and distribution

Figure 2.3 shows the granule shape and size of sago starch as investigated by Ahmad et al. (1999). They reported that 11 different sago starches from different countries including Malaysia, Indonesia and Thailand had the same shape and average diameter where no significant differences were observed among the various samples. Furthermore, they concluded that sago starch consists of oval granules with diameters in the range of 20–40 μm.

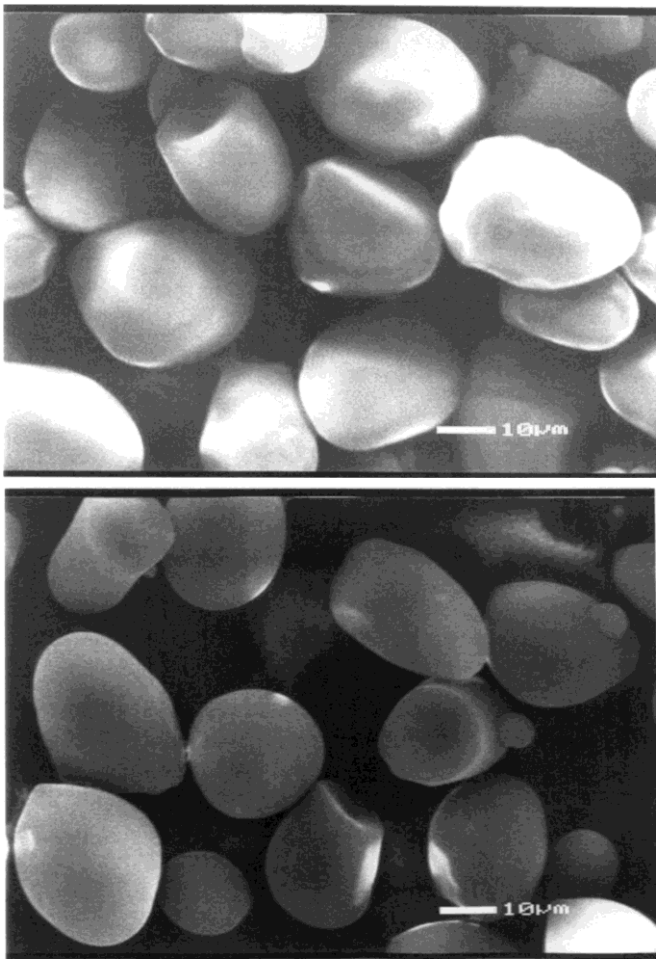


Figure 2.3 Granule shape of sago starch. (adopted from: Ahmad et al., 1999).

2.1.1.3 Fat and minor components

Sago starch has small amount of lipids and protein. Ahmad et al. (1999) concluded that sago starch like other starches has trace amount of lipids and proteins therefore, the proximate analysis of these starches showed that moisture content varied between 10.6% and 20.0%, ash between 0.06% and 0.43%, fiber between 0.26% and 0.32% and crude protein between 0.19% and 0.25%. Furthermore, crude fat of these sago starches ranged from 0.10% to 0.13% compare to potato starch

0.12% and corn starch which was 0.20%. They concluded that crude protein of the starches studied was found to be lower compared to that of pea, potato and corn starches. These different sago starches contained about 0.5%-0.6% free fatty acid, which complex with the amylose (Ahmed et al., 1999; Schoch, 1942) and need hot methanol or ethanol extraction to give fat-free starches rather than normal fat solvent such as hexane. However, trace amounts of fatty acid glycerides usually present in commercial starches with less than 0.1% and can be removed by Soxhlet extraction using ether or hexane. The pH of these investigated sago starches ranged from 3.69-6 depends on the sago starches however, most of these sago starches showed pH around 5–6 with exception of two Malaysian starches which had pH 3.69 and 3.94.

2.1.1.4 Swelling power and solubility

Heating starch in the presence of enough water makes starch granule swells and its volume increases. Tester and Karkalas (1996) have stated that the starch granule swelling occurs due to heating in water breaks the hydrogen bonds that stabilizing the structure of the double helices in crystallites and replace them by hydrogen bonds of water. Lii et al. (1996) suggested that the relationship between starch amylose content and its granular swelling is the rigidity of starch granular structure might be proportional to its amylose content which is conversely proportional to granular swelling degree.

The swelling power of sago starch is effected by presence of sodium chloride which act as swelling inhibitor where some alcohol groups protons in the starch granule exchanged by sodium ions (Maaurf et al., 2001). The swelling power of sago starch was studied by Lutfor et al. (2001) and reported that the maximum swelling was 192 g water g^{-1} in a concentration of 31% sago starch in the absorbent

however, the swelling power decreased with the increase of sago starch concentration. Karim et al. (2007) conducted a study on native and alkali-treated starches samples and concluded that with time of alkaline treatment; there is a progressive increase in swelling power and solubility. In addition, they stated that treating sago starch with 0.5% NaOH had a significantly higher swelling power and solubility than those of starch treated with 0.1% NaOH. Mohamed et al. (2008) stated that swelling power provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. Hoover (2001) reported that amylose/amylopectin ratio and the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching and conformation influence the interaction between starch chains within the amorphous and crystalline domains. Tester and Karkalas (1996) concluded that swelling behavior is a property of the amylopectin content of native starches such as sago starch however; inhibition of swelling might occur especially when amylose-lipid complex forms.

2.1.1.5 Granule surface structure

X-ray diffraction diagram of different sago starches Malaysia, Indonesia and Thailand as indicated that the spectra suggest C-type diffraction pattern of these starches, and the crystalline type is intermediate to that of cereal or potato starches however, all the studied starches showed very similar degree of crystallinity (Ahmad et al., 1999).

2.1.2 Functional properties of sago starch

2.1.2.1 Digestibility

Digestibility of sago starch like other starches depends on the enzyme susceptibility that is effected by several factors including botanical structure, physical texture and granular structure. The most important factor is the granular structure (Cui & Oates, 1997; Granfeldt et al., 1995; Holm et al., 1988). Starch granular structure disrupts by heating and mixing which increase the enzyme availability for digestion (Snow & O Dea, 1981). When gelatinized starch is cooled down and stored, amylose retrogradation occurs as well as recrystallization of amylopectin that reduces the digestibility of the starch (Eerlingen et al., 1994).

Investigation of native, gelatinized and retrograded sago starch for its digestibility by the hydrolysis enzyme (porcine pancreatic α-amylase) showed a significantly different reactivity to the enzyme. Greatest susceptibility to the enzyme was observed in freshly gelatinized sago starch with hydrolysis of 78.3% that was obtained after 8h incubation. Significantly lowered enzymatic susceptibility of gelatinized starch was observed after retrogradation (Cui & Oates, 1997).

2.1.2.2 Thermal behavior

Thermal behavior of sago starch depends on some factors including starch microstructure and amylose content. Ahmad et al. (1999) investigated 11 different sago starches from Malaysia, Indonesia and Thailand and found that the gelatinization temperature for sago starches ranged from 69.48 °C to 70.18 °C with enthalpy range from 15.1-16.7 J/g. They concluded that starch gelatinization temperature and

enthalpy depend on the starch microstructure, crystallinity degree within the granule, granule size and the amylose to amylopectin ratio. In addition, they mentioned that investigated sago starches had higher gelatinization temperatures compared to corn, pea and potato. Likewise, Tomoko et al. (2000) mentioned that sago starch gelatinization T_o from the upper part of the trunk is in the range of 65.3 to 68.2 °C, with a gelatinization conclusion temperature T_c of 75 to 76 °C where sago starch from lower part had lower gelatinization T_o and higher gelatinization conclusion temperatures. Sago palm age has an effect on crystallinity growth in the granules in which the older the sago palms the higher relative crystallinity present. They also mentioned that higher relative crystallinity in a 14.5 years old than a 9 years old palm, also added that the upper part of the trunk has higher crystallinity to the lower part as well.

2.3 Protein

Protein is a key element of life like carbohydrate and fats. It is a polymer that presents in most foods containing 20 different amino acids (Table 2.1) as the backbone of protein linked to each other through peptide bonds (Fig. 2.4) however, asparagine and glutamine could be found in the form or aspartic acid and glutamic acid. Nine out of these 20 amino acids are essential amino acids that cannot be synthesized by human body (phe, val, leu, ile, met, try, his, lys & thr). Not all food rich protein provides the correct balance of amino acids. Plant proteins are deficient in one or more essential amino acids. Functional protein ingredients in food are coagulation, gelation and stabilization. All amino acids are L-isomer (Fig. 2.5) with exception to proline and hydroxyproline (imino acids). The physicochemical properties of amino acids such as net charge, solubility, chemical reactivity and hydrogen bonding potential depend on the R group however; the properties of amino acids determines protein properties (Fennema, 1996).

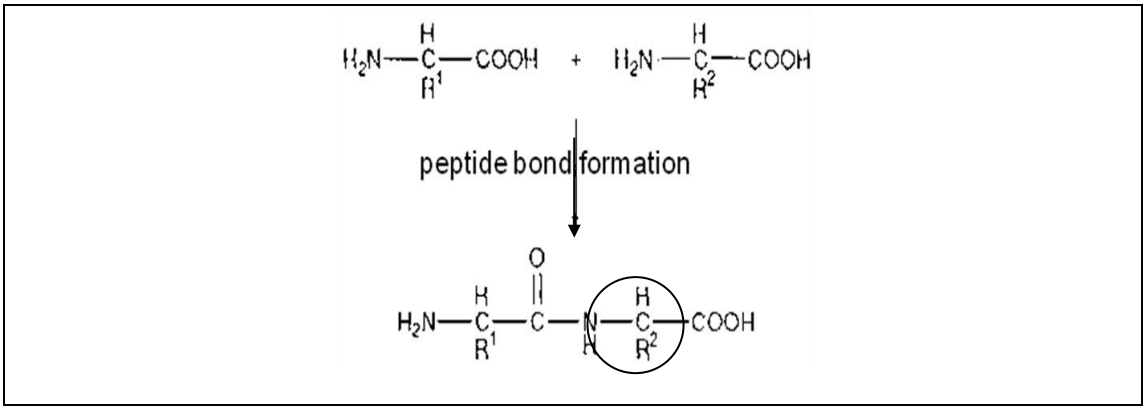


Figure 2.4 Peptide bond formation (adopted from: Belitz et al., 2009).

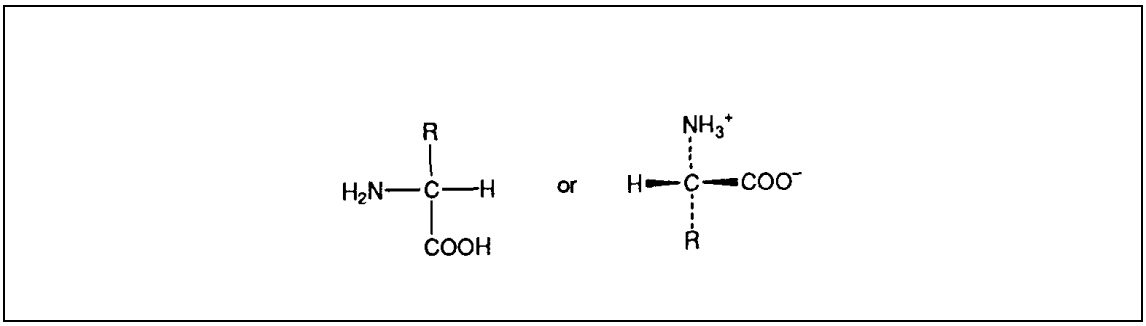
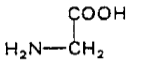
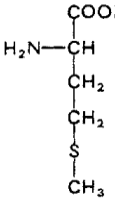
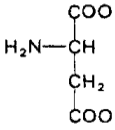
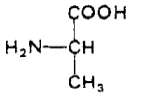
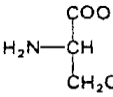
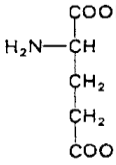
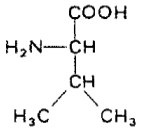
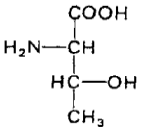
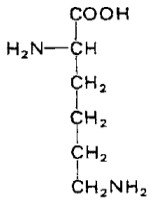
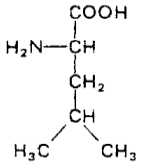
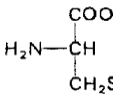
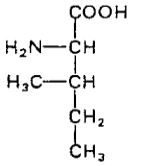
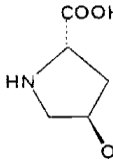
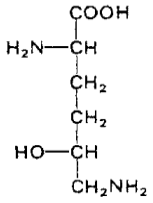
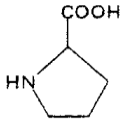
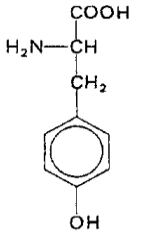
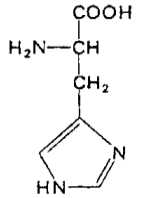
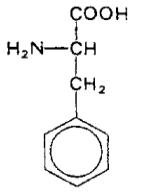
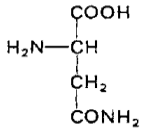
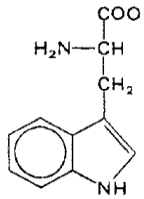
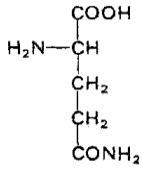
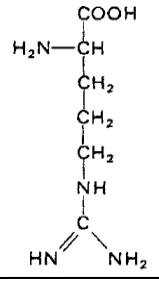


Figure 2.5 L-isomer of an amino acid (adopted from: Fennema, 1996).

Table 2.1 Amino acids side chain (R group) with their corresponding three and one-letter symbols; (adopted from: Belitz et al., 2009).

 Glycine (Gly. G)	 L-Methionine (Met. M)	 L-Aspartic acid (Asp. D)
 L-Alanine (Ala. A)	 L-Serine (Ser. S)	 L-Glutamic acid (Glu. E)
 L-Valine (Val. V)	 L-Threonine (Thr. T)	 L-Lysine (Lys. K)
 L-Leucine (Leu. L)	 L-Cysteine (Cys. C)	
 L-Isoleucine (Ile. I)	 L-4-Hydroxy-proline	 L-5-Hydroxy-lysine
 L-Proline (Pro. P)	 L-Tyrosine (Tyr. Y)	 L-Histidine (His. H)
 L-Phenylalanine (Phe. F)	 L-Asparagine[a] (Asn. N)	
 L-Tryptophan (Trp. W)	 L-Glutamine[a] (Gln. Q)	 L-Arginine (Arg. R)

2.3.1 Structure of protein

There are four different levels of protein structures as a result of polypeptide bonds existed as primary, secondary, tertiary and quaternary. Primary (1°) structure is a sequence of amino acids linked covalently via peptide bonds. Secondary (2°) structure refers to the periodic spatial arrangement of amino acids with twists and turns of polypeptide chain, e.g. α-helix, β-sheet, ϒ-turn, b-barrel, etc. Tertiary (3^o) structure is a secondary structure with further overall folding into a compact 3-dimintional form structure of protein, e.g. globular, fibrous (collagen and gelatine), disordered. Quaternary (4^o) structure is an association of several polypeptides, e.g., protein dimmers and haemoglobin tetramers, etc (Fig. 2.6) (Fennema, 1996).

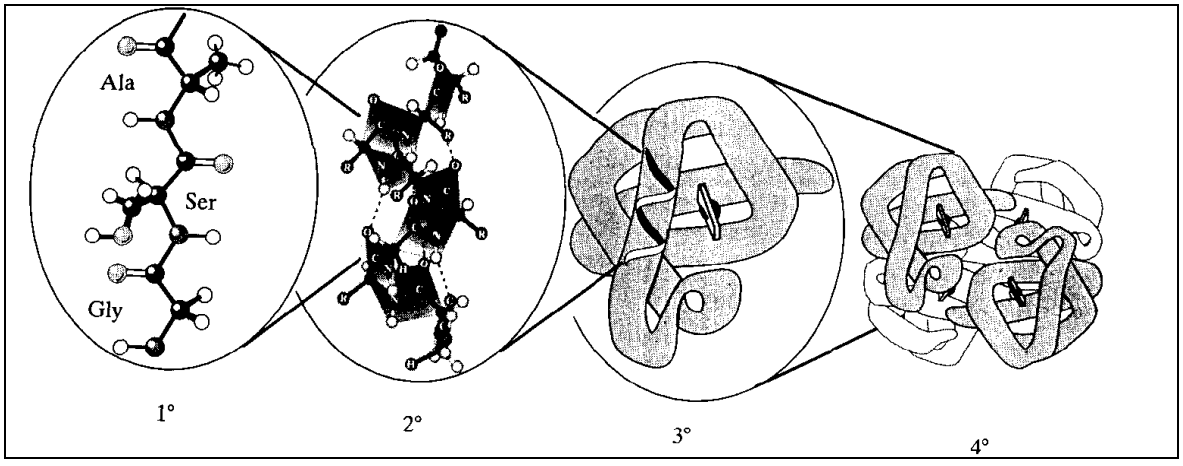


Figure 2.6 Level structure of protein [Primary 1°, Secondary 2°, Tertiary 3^o & Quaternary 4^o]. (adopted from: Fennema, 1996).

Side chains interaction of a protein can form different ways of bonding. The forces that contribute to protein folding are based on the intramolecular interaction originated from forces intrinsic to the protein molecule. Also, forces from intermolecular interaction affected by the surrounding solvent. The forces responsible to stabilize the unique three-dimensional protein structure such as gelatin are including H-bonding (NH, CO, OH), Covalent bonding, electrostatic (NH_3^+, COO^-), hydrophobic (side chains cluster together away from H_2O), salt bridges, Covalent disulphide cross-linking (via cysteine) the only covalent side-chain cross-links found naturally in protein and Van der Waal (Fennema, 1996). In most cases, heating weaken the side chain interaction of a protein with exception of hydrophobic bonds where heating strength them. High temperature can break such bonding including the covalent bond. Likewise, Heating, increasing ionic strength and high ε (polarity of the medium) can weaken such bonds. Besides heating in disulphide bond, this type of bond can be broken by reducing agents such as β-mercaptoethanol, dithiothreitol, ascorbic acid. On the other hand, cooling strengthen most of these boning with exception to hydrophobic bonds (Fennema, 1996).

H-bonding between NH & CO groups of different peptide bonds that helps to stabilize many 2^o structures, e.g. α-helix (Fig. 2.7) and β-sheet structure (Fig. 2.8). H-bond occurs as a results of the interaction of a hydrogen atom that is covalently attached to an electronegative atom with another electronegative atom and involves an interaction between peptide groups, unionized carboxyl groups, phenolic or hydroxyle group and carboxyl groups, phenolic or hydroxyle group and peptide cabonyle groups, and between side chain amide groups. Van der Waal interaction occurs when two atoms approach each other and each one induces a dipole in the other via polarization of the electron cloud that results in an interaction between these induced

dipoles resulting in an attractive and a repulsive component as well. At natural pH, protein has relative number of negative and positive charged residues responsible of the electrostatic interaction in which repulsive interaction between like charges occurs or attractive interaction between positively charged groups and both contribute in stabilizing protein structure. Hydrophobic interaction presents in aqueous solution in which the major force contributes in protein folding comes from interaction between nonpolar groups. However, hydrogen bonding and electrostatic interaction between polar groups in polypeptide chains do not have enough energy to contribute in driving forces for protein folding. Disulphide bonds occur in both intramolecularly and intermolecularly as a result of protein folding through two cysteine residues as in figure 2.9 (Fennema, 1996).

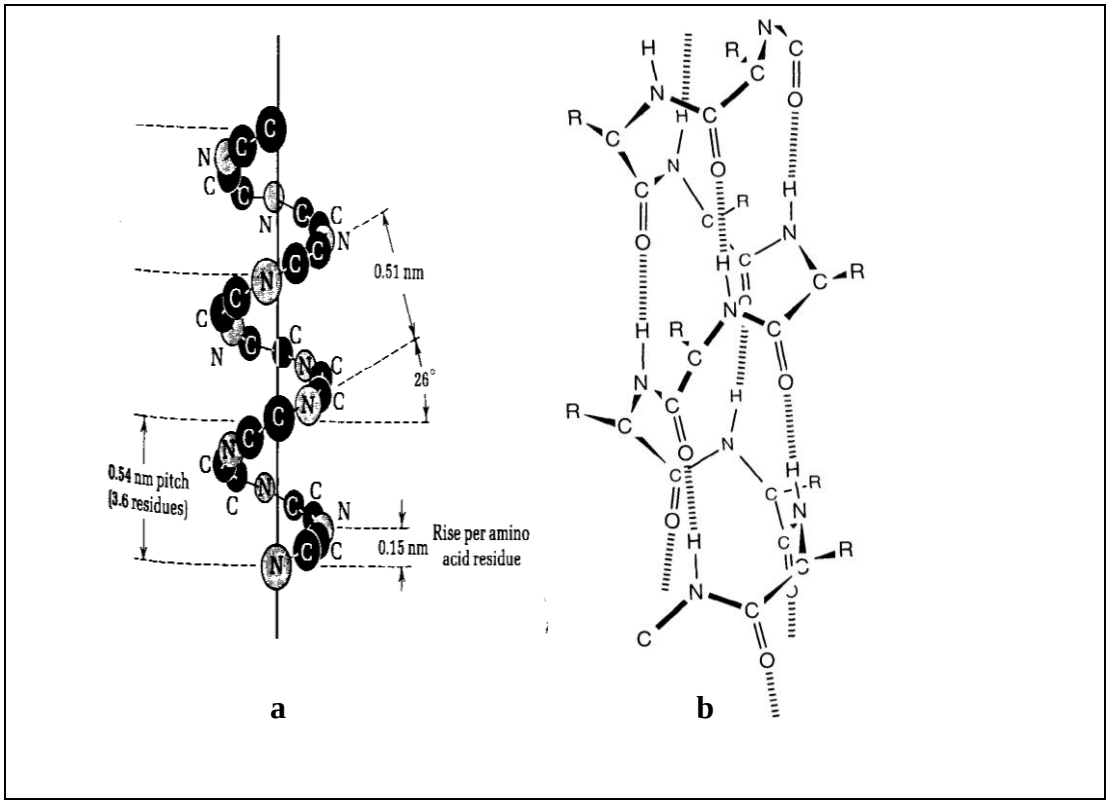


Figure 2.7 α-helix of protein (most common helix), (adopted from: Fennema, 1996).

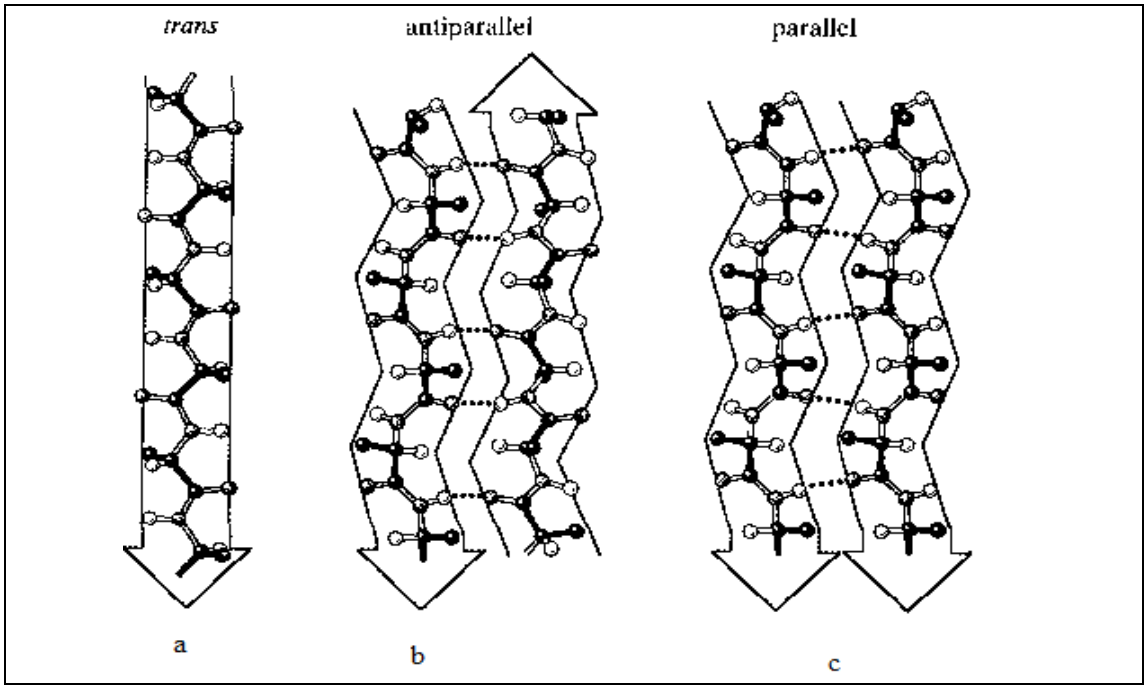


Figure 2.8 Sheet structure, (a) trans conformation (polypeptide), (b) β-sheet (two trans conformation anti-parallel) and (c) β -sheet (two trans conformation parallel), (adopted from: Fennema, 1996).

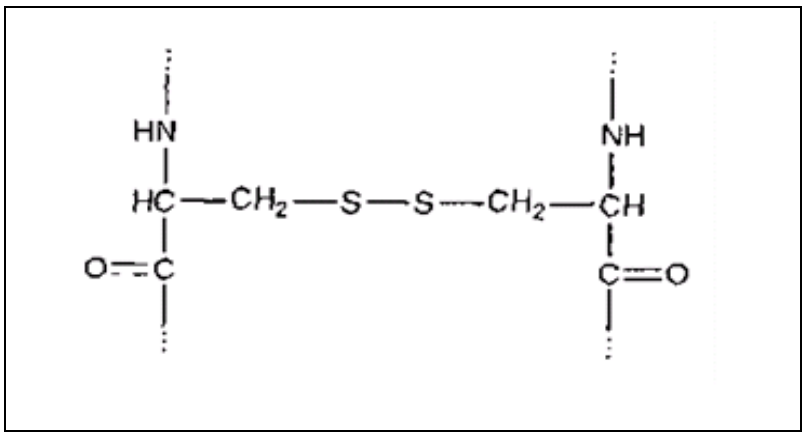


Figure 2.9 Covalent disulphide cross-linking (via cysteine), (adopted from: Fennema, 1996).