CHAPTER 2 THEORY AND LITERATURE REVIRWS

2.1 Fish protein

Fish protein contains three main groups of proteins: sarcoplasmic or water-soluble proteins, myofibrillar proteins and stoma proteins which readily separated by fractional extraction techniques employing water and strong salt solution.

2.1.1 Sarcoplasmic proteins [17]

Sarcoplasmic proteins comprise about 18-25% of the total muscle proteins. Among sarcoplasmic proteins, Myoglobin is presumably the most important protein. Some researchers have indicated that sarcoplasmic proteins, including myoglobin, have an adverse effect on the formation of heat-induced gel by interfering with myosin cross-linking during gel formation. It has been suggested that these proteins may interfere with myosin cross-linking during gel matrix formation because they do not form gels and have poor water-holding capacity. As sarcoplasmic proteins are low molecular weight (40-60 kDa), readily extractable with water, they are ideally suited for separation by electrophoresis.

2.1.2 Myofibrillar proteins [18]

Myosin is the major protein in fish muscle, comprising approximately 55-60% of the myofibrillar protein and is the component responsible for the contractile as well as gelling properties of muscle. In addition, the A bands of myofibrils are composed of thick filaments and the I bands of thin filaments (Figure 2.1). Each thick filament is formed from an ordered arrangement of myosin molecules. Myosin is composed of two 220 kDa heavy amino acid chains and 4 light chain subunits, ranging from 17 to 22 kDa. These amino acid chains are non-covalently attached to the myosin head. In addition, main component of myofibrillar proteins are shown in Table 2.1.

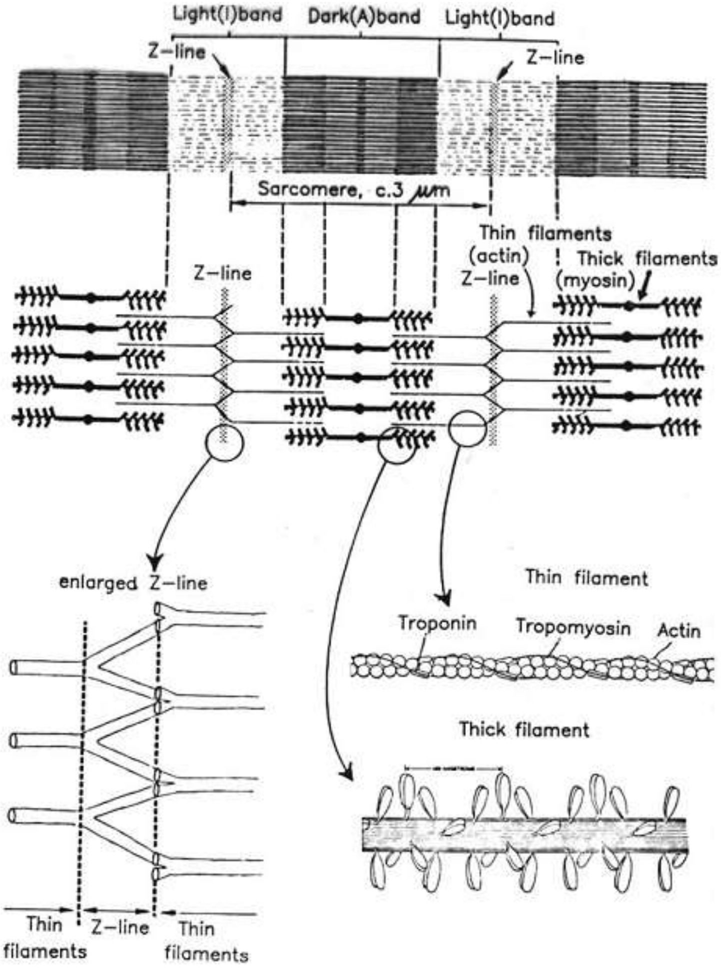


Figure 2.1 Muscle cell and components of protein [19].

Actin is a second major component and constitutes 15-30% of myofibrillar proteins. The monomer form of actin has a globular structure and is commonly referred to as G-actin, with molecular weight of about 43 kDa. In biological systems, G-actin is polymerized together into actin filaments called fibrous actin (F-actin). Other minor proteins are tropomyosin, troponin complex, actinins, M-proteins, and C-proteins associated with either myosin or actin.

Myofibrillar proteins are able to extract in strong salt solution while the former are only extractes in SDS solution often in the presence of high concentrations of urea. Like the sarcoplasmic proteins, myofibrillar proteins are readily denatured by heat.

Table 2.1 Main component of myofibrillar protein [20]

Protein or sub fragment	Amount of total protein (%)	Molecular weight (kDa)	No. of subunits
Myosin	43	500	2
Myosin heavy chain		400	2
Myosin light chain			
Light chain-1		25	1
Light chain-2		20	1
Light chain-3		16	1
Rocl		220	2
LMM		140	2
HMM		340	2
S-1		115	
S-2		62	2
Actin	22	42	
Tropomyosin	5	65-70	2
A-chain		34	
B-chain		36	
Troponins	5		3
Troponin-C		17-18	1
Troponin-I		20	1
Troponin-T		3	1

2.1.3 Stoma protein

Stoma protein comprise about 3-5% of total protein. The stoma proteins of fish skeletal muscle consist mostly of collagen which exists in several polymorphic forms. Collagen is insoluble in low and high ionic strength salt solutions but on heating it breaks down to its denatured form. These proteins in their denatured form, together with myofibrillar proteins, are likely to contribute to the differences in the electrophoretic profiles of total muscle extracts [17].

2.1.4 Amino acid composition of fish protein

Proteins are complex copolymers that are made up of four levels of structure, designated as the primary, secondary, tertiary, and quaternary structures. Proteins are also composed of repeating units, but may contain up to 20 different amino acid monomers forming a polypeptide chain. The amino acid repeat unit contains two carbon atoms as well as nitrogen, differing only in their functional side groups (Figure 2.2) and the amino acid compositions of protein show in Table 2.2. In its natural environment, a protein will be folded into secondary, tertiary and quaternary structures stabilized through hydrophobic interactions, hydrogen bonding and electrostatic interactions between amino acid functional groups. Once folded, the structure maybe stabilized further with strong covalent cross-links.

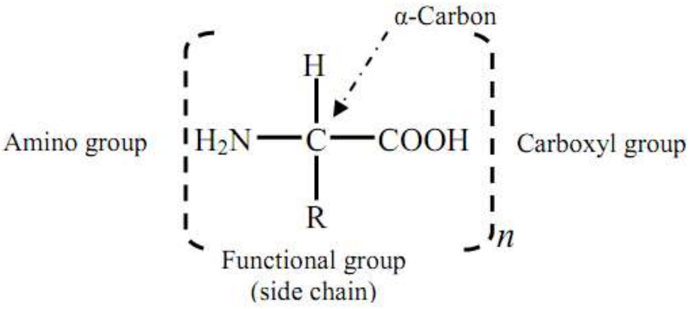


Figure 2.2 General structure of an amino acid [4].

Table 2.2 Amino acid composition of fish muscle [1, 2]

Amino acid	Molecular weight	Content (%)
Aspartic acid	133	6.8
Threonine	119	3.4
Serine	105	3.6
Glutamic acid	147	8.8
Proline	115	3.4
Glycine	75	5.8
Alanine	89	5.9
Cystine	240	2.5
Valine	117	2.5
Methionine	149	2
Isoleucine	131	2.7
Leucine	131	5.1

Table 2.2 Amino acid composition of fish muscle [1, 2] (Continues)

Amino acid	Molecular weight	Content (%)
Tyrosine	181	1.7
Phenylalanine	165	2.1
Tryptophan	204	1.1
Lysine	146	11.7
Histidine	155	3.5
Arginine	174	13.2

Proteins are polymers with individual subunits which containing both polar and non-polar amino acids and side chain, which are able to form numerous intermolecular linkages [21]. Based on structure and solubility, proteins can be placed into three categories: fibrous proteins, globular proteins and membrane proteins.

The final structure is determined by intermolecular interactions, such as hydrophobic interactions, ionic interactions, hydrogen bonding and in some cases even stable covalent disulfide linkages between cysteine residues [4]:

1. Hydrophobic interactions occur between non-polar amino acids, such as leucine, phenylalanine, tryptophan, and valine. The purpose of these interactions is to minimize interactions of the non-polar amino acids with water.
2. Hydrogen bonding between NH and C=O moieties of functional groups contribute significantly to the stability of the secondary and tertiary structures. Hydrogen bonds can occur between polar amino acids, such as serine and tyrosine, and other amino acids.
3. Ionic interactions can occur between positively charged side chains and negatively charged side chains.
4. Covalent disulfide linkages between cysteine residues are often essential to maintain tertiary structures, by restraining the overall conformation of the polypeptide. These bonds are only broken at high temperatures, acidic pH or in the presence of reductants.

In present, the use of proteins in material applications increased significantly. With the discovery of cheaper and more versatile petrochemical-based polymers, the use of such protein polymers was overshadowed. However, increased environmental awareness has renewed interest in sustainable and biodegradable polymers, such as proteins.

Proteins from vegetables and animals have been used to manufacture bioplastics. Some of these proteins are readily available at low cost and are often produced as industrial waste or by-products. Proteins are natural polymers, but, are structurally much more complex, compared to synthetic polymers. The folded conformation is a delicate balance of interactions such as, covalent bonding, hydrophobic bonding, hydrogen bonding, and ionic bonding. In addition, mostly proteins contain the amino acid cysteine. Cysteine (Cys, C) is a sulfur-containing amino acid and can form disulfide (S-S) cystine bond with other intra-molecular or inter-molecular cysteine molecules. Intermolecular cystine bonds are referred to as cross-links. The cross-links plus other protein structural eatures, like crystallinity and hydrogen bonding, usually give the protein high strength and stiffness [22].

For a protein to behave like a synthetic polymer, the protein chain is required in an extended conformation enabling the formation of a three-dimensional network. In order to do this, multiple non-covalent and covalent interactions need to be reduced, allowing chains to unfold and form new interactions and entanglements. The unfolded protein offers unordered and ordered structures similar to that of semi-crystalline commodity plastics.

2.2 Production of biomaterial

Production of biomaterial, biomaterial would not be as useful as they are without plasticizers. A plasticizer is a compound that makes another compound, usually a plastic, more pliable. Plasticizers also increase a plastic’s ease of handling and resilience. Without plasticizers biomaterial would lose some of the properties that make them so useful.

2.2.1 Polymer blending

Blending of two or more polymers provides the ability to improve material properties to achieve specific goals with higher value. While a particular homopolymer will have properties that can be improved by controlling molecular weight and the degree of branching and crosslinking, blending of polymers makes a vastly greater range of potential materials properties available. As well as making simple additive properties accessible, in many instances polymer blending results in high-performance composite materials as a result of synergistic interactions between the components. However, many polymer combinations are immiscible and so exist in two different phases in the polymer matrix. This separation into phases can result in poor stress transfer between the phases, thereby lowering the mechanical properties of the blend to that at least of one of the individual components.

2.2.2 Plasticizer [2, 23, 24]

Plasticizers are organic substance of low volatility that is added to plastics compounds to improve their flexibility, extensibility, and processability. They increase flow and thermoplasticity of plastic materials by decreasing the viscosity of polymer melts, the glass transition temperature (T_g) and the elasticity modulus of finished products due to the mechanical and heat energy.

Plasticizers are particularly used for polymers that are in a glassy state at room temperature. These rigid polymers become flexible by strong interactions between plasticizer molecules and chain units, which lower their brittle-tough transition or brittleness temperature and their T_g value, and extend the temperature range for their rubbery or viscoelastic state behavior.

Glycerol ($C_3H_8O_3$) is the most commonly used plasticizer for reducing intermolecular forces and glass transition temperature, which can significant improve chain mobility of protein [25]. It is a low molecular weight, hydrophilic plasticizer that has been widely used in the thermoplastic processing of proteins [26]. Its high plasticizing effect has been attributed to the ease with which glycerol can insert and position itself within the 3-dimensional biopolymer network.

Figure 2.3 illustrates how plasticization and heating above a material's glass transition and softening point will allow it to become shaped into a marketable product; plasticization reduces both T_g and softening point and when the softening point is below the decomposition temperature, the material should be easily processable.

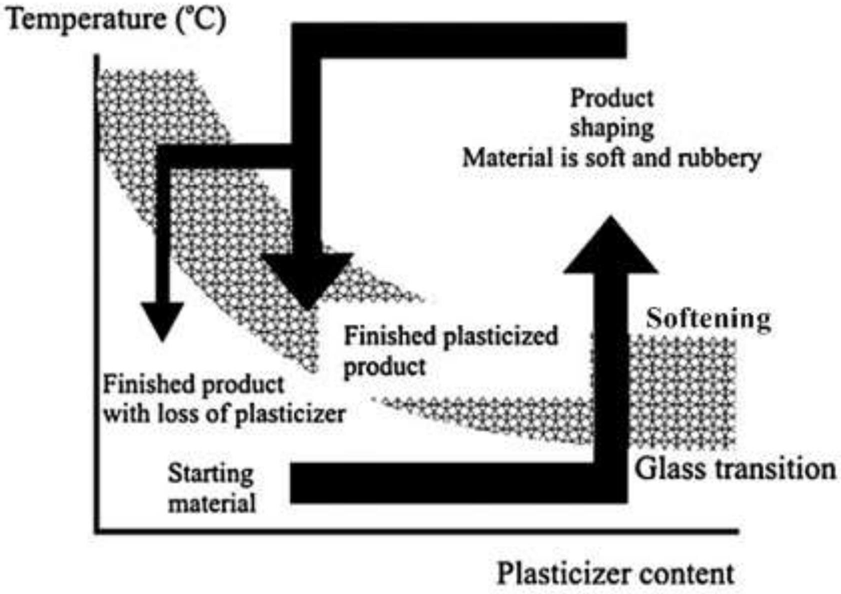


Figure 2.3 Schematic representation of the thermoplastic process applied to protein-based materials [4].

The plasticizing effect of small polar molecules such as glycerol and water has been described in terms of insertion and positioning within the 3-dimensional protein network. The following plasticizing mechanisms have been proposed to describe the effect of plasticizers on the protein network

1. The lubricity theory, where the plasticizer is seen as acting as a lubricant to facilitate mobility of the chain molecules past one another.
2. The gel theory, which considers the disruption of polymer–polymer interactions (hydrogen bonds and van der Waals or ionic forces).
3. The free volume theory, which considers that the plasticizer increases the free volume and mobility of polymer chains. This theory has been used to understand the effect of plasticizers in lowering the glass transition temperature.
4. The coiled spring theory, which explains plasticizing effects from the point of view of tangled macromolecules [2].

2.2.3 Processing technique

Production of protein-based material can be divided into two types, wet processing and dry processing as shown processing in Figure 2.4. The wet processing, or casting, involves dispersing and solubilizing the protein in large quantities of solvent followed by solvent removal by drying. In some of the dry processing, it is based on thermoplastic properties of some biopolymers in low water content conditions and had been applied with success to produce biodegradable materials by using common melt processing technologies. For example, batch mixing devices were employed, where the mixing time and shear rate could be controlled. After mixing, products could be shaped by various techniques such as, injection molding and compression molding. Formed products were subsequently infused with formaldehyde or an equivalent. It was found that the induced cross-links improved mechanical properties and water resistance.

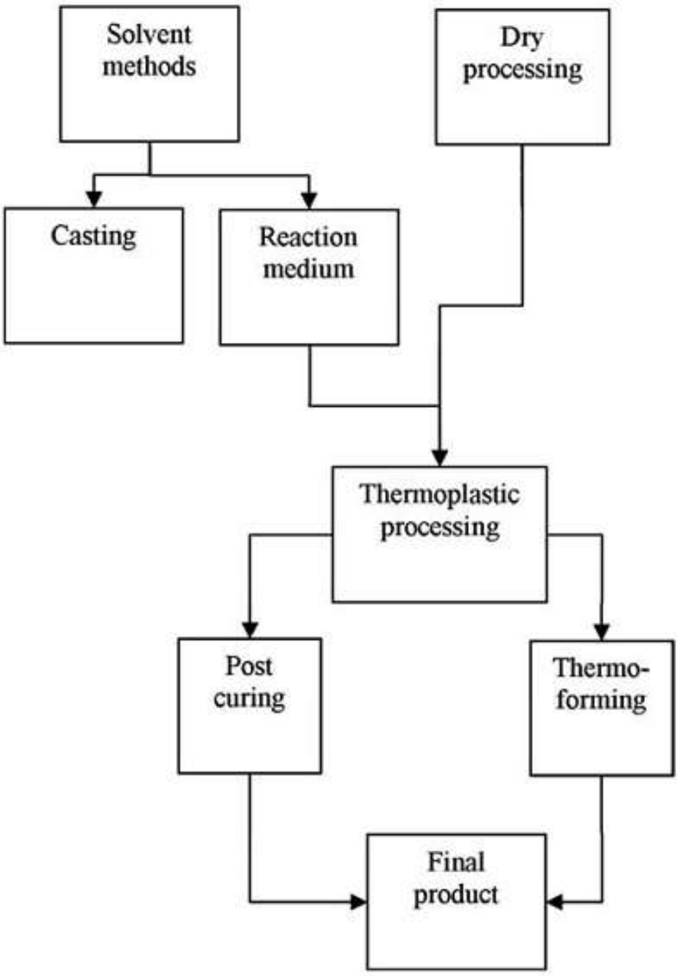


Figure 2.4 Processing paths leading to thermoplastic processing [4].

Polymer processing is concerned with the mixing and shaping of polymeric materials to form them into useful products. Processing usually involves the application of heat and pressure. The method used to form a specific polymer depends on whether the material is thermoplastic or thermoset. If thermoplastic, the softening temperature, atmospheric stability, as well as the geometry and size of the finished product is important when considering processing means.

Thermoplastic processing involves melting a polymer, flowed by shaping and finally cooling the material in its new form. The heat required for melting can be supplied by radiation, conduction or mechanical work. The most important thermoplastic processing techniques can be categorized as follows: extrusion, post-die processing, thermo-forming and injection molding. Molding is the most common method for forming plastic polymers. Several techniques are used such as, compression, extrusion, and injection molding.

2.2.3.1 Compression Molding

During compression molding, the mixed polymer and required additives are placed between heated male and female mold members. Heat and pressure are applied and the material becomes viscous, conforming to the mould's shape as shown in Figure 2.5. Thermosetting materials take less time to process in this way, because once formed they can be removed, where as thermoplastics need to be cooled under pressure.

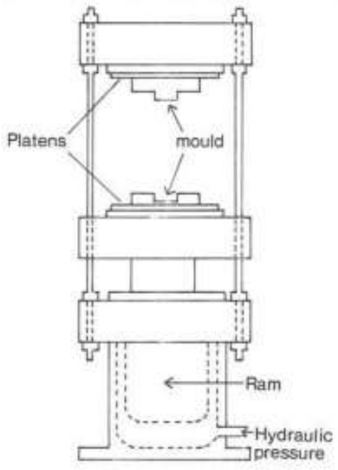


Figure 2.5 Compression molding press [27].

2.2.3.2 Extrusion

Extrusion is one of the most important polymer processing techniques in use today. It is used to melt and pump thermoplastics through the shaping device called a die. A mechanical screw pumps the palletized material, which becomes compacted, melted and formed into a continuous charge of viscous fluid. This technique can be used to produce continuous lengths of tubes, sheets and hose channels. The final shape of the extrudate may be further modified by stretching before final cooling and solidification. The two most common types of extruders are single and twin-screw extruders. The single screw extruder utilizes the frictional forces between the screw and barrel to force the material towards the die as shown in Figure 2.6. The twin-screw extruder uses intermeshing screws that compound the material, and acts as a positive displacement pump (low friction), providing better mixing conditions.

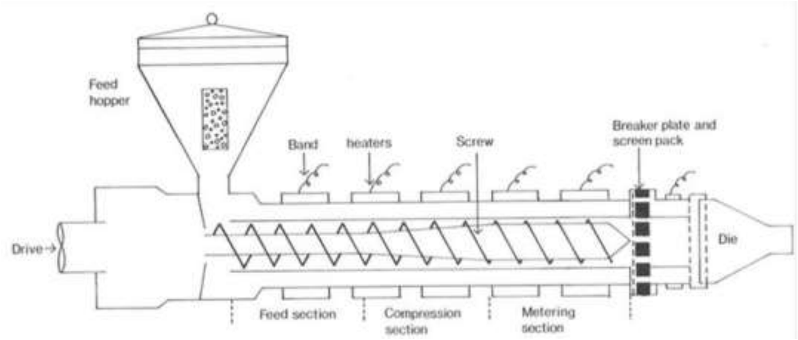


Figure 2.6 Main features of a single screw extruder [27].

Three zones can be identified within the extruder: the feed zone, compression zone and the metering zone. The feed zone is where granular material is introduced to the barrel and pre-warmed. The compression zone involves melting of the material and removal of air between the original grains. Finally the metering zone is the region where the highest temperatures and pressures are applied, in order to force the material out of the shaped die.

2.2.3.3 Injection Molding

During injection molding molten polymer is forced under high pressure into a closed mold of the required shape (Figure 2.7). It is the most widely used technique for fabricating thermoplastic materials. Thermosetting polymers may also be injection molded, where curing takes place while the material is under pressure in a heated mold, often termed reaction injection molding.

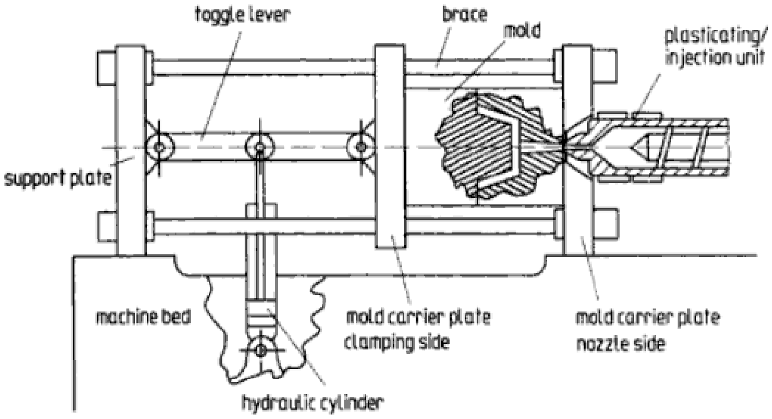


Figure 2.7 Main features of injection molding [28].

2.3 Protein-based biomaterial

2.3.1 Cross-linking of protein based material [29-31]

Proteins are heteropolymers. The different amino acids which constitute the polymer offer a large spectrum of chemical functionalities, which may give rise to a big variety of polymer network structures. Those polymers in which two linear chains are joined together by covalent bonds, the chains are crosslinks. Network polymers have three dimension structures in which each chain is connected to all others, and are characterized by their crosslinking density or degree of crosslink. Cross-linking of protein may give rise to a big variety of polymer network structures. One of the outstanding features of proteins is its unique viscoelastic properties, which in the past have been mainly investigated. Its viscoelastic and flow properties have already been subject to large investigations. In addition, cross-linking leads to the formation of larger aggregates, accompanied by an increase in molecular weight and reduction solubility. The cross-linking reactions can result in a high glass transition temperature and high melt viscosity, which require addition of plasticizers to increase free volume and mobility of the molecules. The first step in producing plastic is heating and plasticization, the plasticized proteins turn into a soft, rubbery material that can be shaped into desired forms. In second step when temperature reach activity zone, disulfide-bond cross-linking will be formed as follow equation 2.1.

$$2\ RSH \rightarrow RS\text{-}SR + 2\ H^+ + 2\ e^- \qquad (2.1)$$

The third step, the proteins are then formed upon cooling, the matrix network gets fixed into the desired structure through hydrogen, ionic, hydrophobic, and covalent interactions.

Denaturation → Aggregation → Crosslinking

Proteins require denaturation by unfolding and alignment before a new three-dimensional network can be formed and stabilized by new inter- and intra-molecular interactions that is followed three sequential events. Protein-based bioplastics are manufactured using the process of denaturation, induced by thermal or chemical means. The denaturation temperature of proteins depends on the amino acid sequence, the type of chemical additives used and processing method employed. Due to the diverse building blocks of proteins and its unique structure, a large variety of biodegradable materials can be produced offering a wide range of functional properties [4].

Processing of protein based material requires three main steps: breaking of intermolecular bonds (non covalent and covalent, if necessary) that stabilize polymers in their native forms by using chemical or physical rupturing agents; arranging and orienting mobile polymer chain in the desired shape; and, finally, allowing the formation of new intermolecular bonds and interaction to stabilize the three-dimensional network.

Another way of processing protein based biomaterials is the mechanical method, or thermoplastic processing, which consists of solid-like material. Bioplastics can be processed using existing plastics processing machinery, including thermoforming, and various types of injection molding, compression molding and extrusion [29].

2.3.2 Rheological properties

Biomaterial formation from proteins requires denaturation and unfolding using thermal and chemical means, allowing new interactions to form between chains. Example, thermoplastic extrusion also requires sufficient chain mobilization, enabling flow through the barrel. The proteins physiochemical characteristics, plasticizer content and chemical additives will govern its processing behavior, structural and material properties. Chemical additives were essential, breaking covalent bonding which allowed chain extension. The plasticizer content also strongly influenced the processability were essential for improved processing. Successful processing with required increased chain mobilization through the reduction of inter- molecular and intra-molecular interactions which led to less ordered structures.

2.3.2.1 Effect of shear rate on viscosity

Most polymer melts exhibit non- Newtonian behavior with the apparent viscosity decreasing with increasing shear rate (shear thinning). Viscosity of polymer at high shear rate may be several orders of magnitude smaller than the viscosity at low shear rates. The reduction of viscosity with increasing rate of shear is taken advantage of in achieving desirable and optimum viscosity for polymer processing machine and equipment without raising the temperature to detrimental levels, simply by raising the shear rate to as high a level as economically and otherwise possible. A reduction in viscosity with increasing shear rate is also taken advantage of in brushing and spraying of paints which are polymer solution/suspension containing pigments.

2.3.2.2 Effect of temperature on viscosity

The viscosity of most polymers changes with temperature. An Arrhenius equation of the form equation 2.2

$$\eta = Ae^{E/RT} \tag{2.2}$$

Where A is a constant

E is the activation energy

η is a viscosity

T is a temperature

In addition, while temperature raises at constant pressure cause a decrease in viscosity, pressure rises at constant temperature cause an increase in viscosity since this cause a decrease in free volume.

In processing, the shear rate depends principally on the nature of the shaping as shown in Table 2.3 [32].

Table 2.3 shear rate depends principally on the nature of the shaping [32].

Process	**Shear rate (s^{-1})**
Compressing molding	1-10
Calendering	10-100
Extrusion	100-1000
Injection molding	$1000\text{-}10^5$
Reverse roll coation	3×10^3

In plastics processing, viscosity and viscoelasticity data can also provide some information about changes in molecular structure.

In 2008, Ralston et al. studied viscosity of soy protein plastics determined by screw-driven capillary rheometry. Soy protein plastics behaved as shear thinning fluids that displayed power-law behavior. The viscosities fit a power-law model and were found power-law indices, n, of 0.18–0.46 and consistency indices, m, of 1.1×10^4–1.0×10^5. Consistency indices increased and power-law indices decreased with increasing soy protein: corn starch [33].

2.3.3 Viscoelastic properties

The viscoelastic behavior of amorphous or semi-crystalline polymers can be divided into five regions; these are the glassy, leathery, rubbery rubbery flow and viscous states as showed in Figure 2.8. The transformation from one to the other region is dependent on temperature. While the temperature at which each transition occurs is dependent on the polymer structure. Excellent viscoelastic behavior of polymers make them ideally suited for use as damping materials, because of their ability to dissipate mechanical energy in the transition region from the glassy to rubbery state during dynamic mechanical analysis. The glass transition temperature (T_g) is becoming recognised as an important parameter in defining the properties of polymer. The glass transition serves as corresponding state for viscoelastic response. That is temperature at maximum of tan δ peak which changes from glassy to rubbery consistency. Above the T_g, further heating to above the softening point, results in a material with lower viscosity, which can easily be processed.

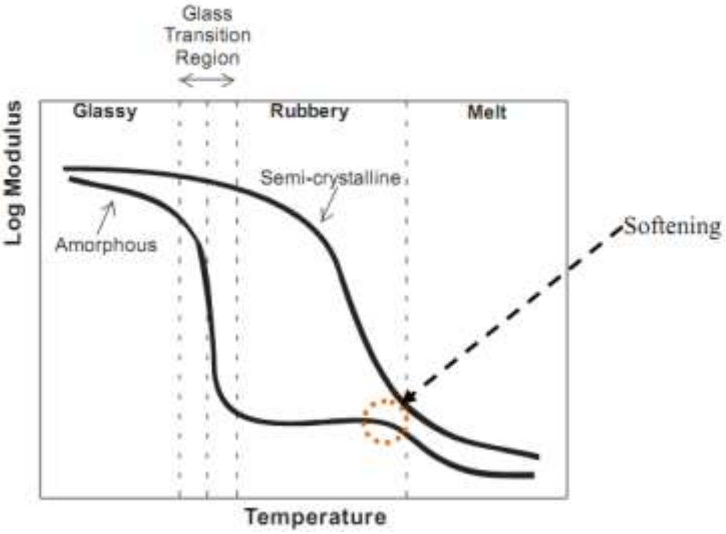


Figure 2.8 General change in modulus with temperature of amorphous and semi-crystalline polymer materials, showing the different regions of viscoelastic behavior [34].

DMA measures the change in viscoelastic properties of the polymer with changing temperature, frequency or deformation. Viscoelastic properties of materials are measured: the storage modulus (E') and the loss modulus (E"). E' is a measure of the energy stored and subsequently released and is related to material elasticity; E" is a measure of energy dissipated per cycle of deformation and is related to material viscosity. The ratio (tan δ =E"/E') is a measure of the dynamic character of the protein-protein bonds in the material network; a higher tan delta value means that a material reacts to a stress in a relatively more viscous and less elastic. In addition, the glass transition temperature is interpreted from the storage and loss modulus data obtained from a DMA run. Table 2.4 summarizes some storage modulus of various materials.

Table 2.4 Storage modulus of material and composite [35-37]

Material	***Storage modulus (MPa)***
Fish protein/Kraft lignin/glycerol	3-29.7
DRBP/ Kraft lignin/glycerol	3.3-9.4
AESO[1]/styrene/PKL[2]	0-10
Vinyl ester thermoset	10-30
Gluten/glycerol	0.4-1
Wheat gliadin/glycerol	30-60
Nylon	300-400
polypropylene	100-500
Polylactic Acid	3500
Glass	72000
Flax	45000-100000
Thermoplastic starch	2-2.5
Thermoplastic polyurethanes	10^3-10^5

[1] AESO: Acrylated epoxidized soybean oil

[2] PKL: Pine Kraft lignin

2.3.4 Mechanical Properties

Structural characteristics of material are known to influence its mechanical properties. The mechanical properties of polymers are largely associated with distribution and concentration of inter- molecular interactions and intra-molecular interactions allowed by the amino acid sequence of the polypeptide chain [4]. A single protein chain may contain up to 20 different monomer units leading to large differences in properties between protein-based materials. Heat, pressure, shear and chemical additives used for processing protein-based materials will affect molecular interactions and thereby its mechanical properties.

Extrusion and other thermal processing techniques led to structural rearrangements and new interactions, which can be adjusted with the use of plasticizers and chemical additives. In general, true plasticizers will increase the flexibility of a molded product, imparting greater extensibility. On the other hand, increasing molecular interactions will result in a material with higher tensile strength and stiffness. Furthermore, harsh processing conditions can lead to degradation, adversely effecting mechanical properties. Table 2.5 summarizes some mechanical properties of various thermo-mechanical processed protein.

Table 2.5 Mechanical properties of material and composite [36][15]

	Tensile properties		
	Tensile strength (MPa)	**Young's modulus (MPa)**	**Elongation (%)**
Soy protein isolate/Corn starch	2.9-3.9	28-46	69.4-94.7
Sunflower/Sodium sulfide	10.6-16.1	500-2000	0.58-1.8
Starch/Zein	20 – 25		4.7-5.3
Whey protein/water/glycerol	3.1-4.1	30.6-46.5	121-132
Zein/water	17.3-22.4	186.1-299.8	11.4-12.8
DRBP/ Kraft lignin/glycerol	1.16-2.24	10.62-24.48	4.74-24.21
Soy protein/ Alkaline lignin/ glycerol	0.8-1.98	4-55	45-135

Protein plastics manufactured through processing technique, produced materials with reasonable mechanical properties. Compared to synthetic plastics, they were found to be of similar tensile strength, but generally more brittle. Further research into processing technique of proteins is required to produce bioplastics with high tensile strength and good ductility [34].

Tensile strength, Young’s modulus and percent elongation at break are indicators of protein-protein interactions in materials. Tensile strength is the maximum amount of force applied to a material per unit original cross- sectional area before material breakage. Elongation is the distance the material will stretch before breaking divided by the original material length. Young's modulus is a measure of the stiffness of an elastic material and is a quantity used to characterize materials. The tensile properties can be adjusted to make more flexible, stretchable material by changing the state of the protein or by the addition of plasticizers. Increased cross-linking that occurs during denaturation leads to stronger and stiffer materials.

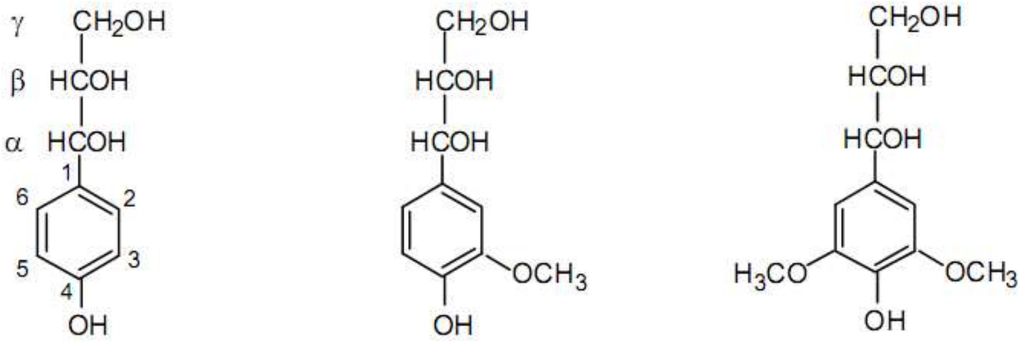
2.3.5 Water Absorption

Proteins materials are highly water absorption. Further research into processing technique of proteins is required to produce bioplastics with less water sensitivity. Polymer blending or chemical addition through material results in both a better processability and a reduce water absorption of the final material. In addition, water absorption is a qualitative test for analyzing the cross-link of protein bioplastics, which can be related to mechanical properties. A material with increased water absorption is assumed to have less covalent cross-linking, resulting in an increased ability for the cross-linked network to swell. However, in some cases water absorption need to be controlled irrespective of cross-link density, depending on the intended use of the biomaterial.

2.4 Lignin

2.4.1 Lignin structure [38]

Lignin is one of the most abundant renewable organic materials on earth, found in higher plants; wood and other vascular tissues generally contain 20–30% lignin (Figure 2.10). Lignin is a three-dimensional amorphous macromolecule made of phenylpropane units that arise from the copolymerization of three primary precursors: p-hydroxyphenylpropane (p-hydroxyphenyl), 3-methoxy-4-hydroxyphenylpropane (guaiacyl) and 3,4-dimethoxy-4-hydroxyphenylpropane (syringyl), Figure 2.9. The propane chain of the monomers, is substituted by hydroxyl and carbonyl groups in various amounts. They are complex heteropolymers that contain aromatic moieties and ether linkages [39]. Typical molecular masses of isolated lignin are in the range 1000–20,000 g/mol [40].



p-hydroxyphenyl guaiacyl syringyl

Figure 2.9 Fundamental type-structures of the monomers in lignin [40].

Lignin from different sources can be oxidized to obtain phenolic compounds: spent pulp liquor from paper industry, native lignin, sugar cane bagasse, Klason lignin, among others [38].

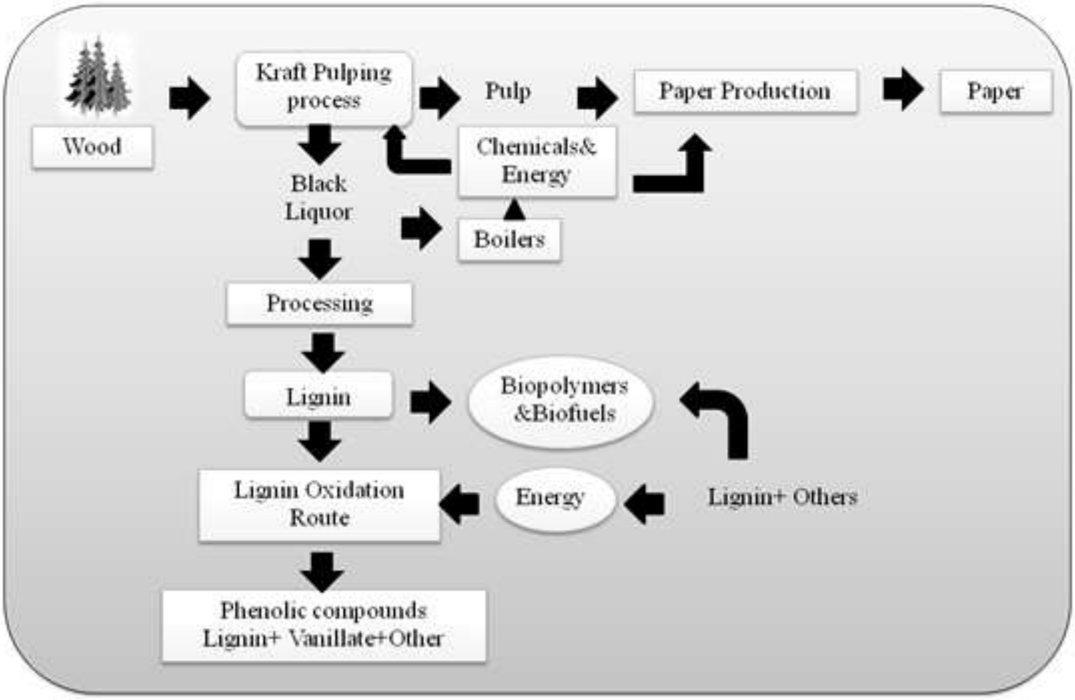


Figure 2.10 Valorisation of Kraft lignin by a biorefinery concept: the proposed integrated process for producing biopolymers [38].

Lignin can be divided in to two types. Normal softwood lignins are usually referred to as guaiacyl lignins because the structural elements are derived principally from coniferyl alcohol (>90%), with the remainder consisting mainly of p-coumaryl alcohol-type units. Normal hardwood lignins, termed guaiacyl–syringyl lignins, are composed of coniferyl alcohol and sinapyl alcohol-type units in varying ratios. In hardwood lignins, the methoxyl content per phenylpropanoid unit is typically in the range of 1.2–1.5. Grass lignins are also classified as guaiacyl–syringyl lignins. However, unlike hardwood lignins, grass lignins additionally contain small but significant amounts of structural elements derived fromp-coumaryl alcohol. Grass lignins also containp-coumaric, hydroxycinnamic, and ferulic acid residues attached to the lignin through ester and ether linkages.

The lignin macromolecule also contains a variety of functional groups that have an impact on its reactivity. Lignin mostly contains methoxyl groups, phenolic hydroxyl groups, and few terminal aldehyde groups. Only a small proportion of the phenolic hydroxyl groups are free since most are occupied in linkages to neighboring phenylpropane linkages. Carbonyl and alcoholic hydroxyl groups are incorporated into the lignin structure during enzymatic dehydrogenation. Table 2.6 illustrates the frequency of some common functional groups found in lignin.

Table 2.6 Functional groups in softwood lignin per 100 phenyl propane units.

Functional Group	**Abundance per 100, C_9 units**
Carbonyl	10-15
Benzyl alcohol	15-20
Phenolic hydroxyl (free)	15-30
Methoxyl	92-96

The nature of the lignin polymerization reactions results in the formation of a three-dimensional, highly-branched, interlocking network of essentially infinite molecular weight. Figure 2.11 is a schematic representation of a softwood lignin. This model was constructed based on the analysis of various linkages and functional groups.

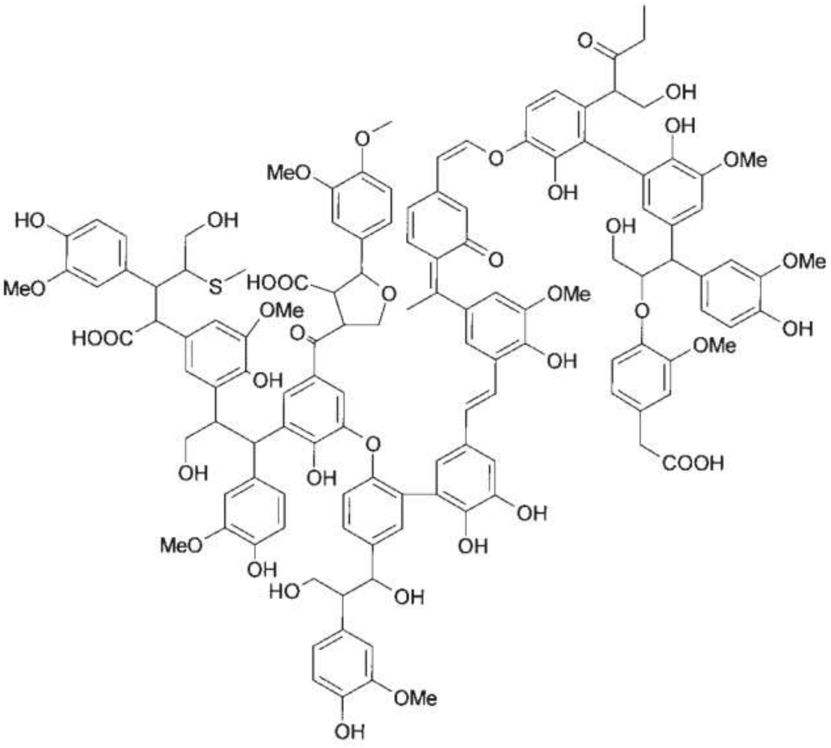


Figure 2.11 Structural model Kraft lignin [41].

There are a number of physicochemical factors which suggest a bright future for lignin-based products:

1. Compatibility with a wide range of industrial chemicals;
2. Presence of aromatic rings providing stability, good mechanical properties, and the possibility of a broad range of chemical transformations;
3. Presence of other reactive functional groups allowing facile preparation of graft copolymers;
4. Good rheological and viscoelastic properties for a structuralmaterial;
5. Good film-forming ability;
6. Small particle size; and
7. Hydrophilic or hydrophobic character depending on origin, allowing a wide range of blends to be produced [40].

Lignins are hindered phenolic polymers which can exert antioxidant properties. However, lignins are polar polymer (on average 1–2 hydroxyl groups per monomer and thus exhibit a very poor solubility in a polar media. This may limit their reactivity with the radicals responsible for the oxidation and subsequently limit their protecting effect compared to that of synthetic antioxidants. Some factors have importance on stabilisation properties of classical antioxidants: good solubility, mobility and low volatility [42].

2.4.2 Kraft lignin

Lignin obtained from the alkaline Kraft pulping process (Kraft lignins) is relatively hydrophobic and poorly soluble in water at acid or neutral pH. They are heterogeneous polymers consisting of highly cross-linked aromatic skeletons carrying some key functional groups [43]. Kraft lignin is highly reactive thermoplastic polymer due to strongly interact with proteins.

2.4.3 The physical properties of lignin

The physical properties for softwood Kraft lignins can be found elsewhere. Kraft lignins have low molecular weight (MW 2000–3000) [40], and high content in free phenolic hydroxyl groups as show in Table 2.7. The content in free phenolic hydroxyl groups of Kraft lignin is especially rich with values around 3.1 meq g^{-1}. This functional group plays an important role in the dissolution of macromolecules.

Table 2.7 Molecular weight and functional groups of lignin [40].

Lignin type	**M_n ($gmol^{-1}$)**	**COOH (%)**	**OH phenolic (%)**	**Methoxy(%)**
Soda (bagasse)	2160	13.6	5.1	10.0
Organosolv (bagasse)	2000	7.7	3.4	15.1
Soda (wheat straw)	1700	7.2	2.6	16
Organosolv (hardwood)	800	3.6	3.7	19
Kraft (softwood)	3000	4.1	2.6	14

Table 2.8 T_g of different lignin types [40].

Types of lignin	**T_g (◦C)**
Milled wood lignin	
Hardwood	110–130
Softwood	138–160
Kraft lignin	124–174
Organosolv lignin	91–97

Another important parameter is the glass transition temperature, T_g, which is an indirect measure of crystallinity and degree of crosslinking and directly indicates the rubbery region of the material (Table 2.8).

Lignin Tg will depend on the amount of water and polysaccharides, protein, as well as molecular weight and chemical functionalisation, but in general the Tg will be lower the greater the mobility of the lignin molecules. While Tg generally increases with increasing molecular weight, the impact of structural variation based on the degree of polymerisation has only recently been established. It is showed using a series of transgenic poplars that the variations in Tg were closely related to the degree of polymerisation of lignin as determined by thioacidolysis.

2.4.3.1 Molecular Weight

Because it is not possible to isolate lignin from wood without degradation, the true molecular weight of lignin in wood is not known. Different methods for measuring the molecular weight of isolated lignins give various results, and aggregation of lignin molecules may prevent determination of real molecular weight. Light scattering and vapor phase pressure osmometry are the traditional methods of analysis. By using these methods, the weight-average molecular weight, MW of softwood milled wood lignin is estimated to be 20,000; lower values have been reported for hardwoods.

2.4.3.2 Solution Properties

Lignin in wood behaves as an insoluble, three-dimensional network. Solvents meeting these requirements include dioxane, acetone, methyl cellosolve, pyridine, and dimethyl sulfoxide (DMSO).

2.4.3.3 Thermomechanical properties [44]

As an amorphous polymer, lignin behaves as a thermoplastic material undergoing a glass-transition at temperatures that vary widely depending on the method of isolation, sorbed water, and heat treatment.

2.4.3.4 Free radical scavenging

Kraft lignin is thus basically a polyphenolic compound, containing mainly aromatic groups with different functions ranging from hydroxyl (phenolic or alkyl), conjugated double bond, methoxyl, sulfonate, etc. Such phenolic structures are known for their radical scavenging properties: the conjugation of the aromatic nucleus in their structure induces the resonance stabilization of the phenoxy radical. Kraft lignin act as antioxidants against the biological, chemical, and mechanical stresses to which plant are subjected. Hindered pheonolic hydroxyl groups of lignin can act as stabilizer of reactions induced by oxygen and its radical species. It was revealed that the antioxidant activity of lignins varies with their genetic origin, isolation method, and molecular characteristics. Lignin's ability to scavenge the radicals responsible for the oxidation is influenced by limited diffusion into polymer matrix. It has been shown that lignin possess multiple properties such as antioxidant and antimicrobial activity.

Electron Spin Resonance (ESR) spectroscopy are essential tools for the study of structure and dynamics of molecular systems containing one or more unpaired electrons. These methods have found application as a highly sensitive tool for the detection and identification of free-radical species in lignin and lignin model compounds. Milled wood lignin generally exhibits a singlet ESR signal with a g-value of 2.0023 and a line width of 1.6 mT (16 G), typical of a phenoxy radical.

2.4.3.5 Fluorescence

Fluorescence is an intrinsic property of lignin. The structural complexity of lignin makes its fluorescence spectra difficult to interpret. In order to interpret the results, fluorescence spectra of a variety of lignin model compounds were examined. Fluorescence spectroscopy was used as a sensitive analytical tool in the studies of lignin constituents in waters and soils, as well as in photochemistry of wood fibres and paper.

Lignin exhibits a strong UV-B-excited UV-A fluorescence and a much weaker visible fluorescence either UV-A-excited or as a tail of the UV-B-excited fluorescence. The UV-A-emitting lignin fluorophore has received much attention and putative candidates have been periodically proposed: anion of coniferyl alcohol, stilbene structures, phenylcoumarone structures. The UV-A-excited visible emitting lignin fluorophores remain more elusive. According to the published literature, it is unclear whether lignin fluorescence is produced by a charge-transfer mechanism or distinct molecular species within lignin polymer. Therefore, the real origin of lignin fluorescence is still ambiguous [45]. Lignin units fluoresce when excited at shorter wavelengths

2.5 Protein–lignin blends [40]

Proteins have been used for the production of plastics. However, the main drawbacks of protein-based materials are high water absorption and the difficulty of separating the proteins from naturally occurring colourants without denaturation, but these obstacles are gradually being overcome. As a crosslinked material with a largely aromatic structure, lignin has the capacity to increase the tensile strength, Young’s modulus, thermal stability, elongation at break and high water resistant of protein materials.

Kunanopparat et al. [13] studied effect of Kraft lignin on wheat gluten network formation during biomaterial processing and reported that Kraft lignin in gluten-based material resulted in a strong wheat gluten protein depolymerization. In addition, addition of Kraft lignin results in a strong interact and cross-linking during mixing with protein. Most processing of gluten protein increased the degree of crosslinking, incorporation of Kraft lignin in gluten reduced protein/protein interactions and prevented loss of solubility. This has obvious implications for processibility of gluten-based materials, suggesting Kraft lignin is a promising additive for such materials. It was suggested that Kraft lignin had a radical scavenging activity, reacting with the sulfur centred radicals responsible for gluten crosslinking.

Huang et al.[15, 46] studied the structure and thermal and mechanical properties of soy protein plastics blended with lignosulfonate and alkaline lignin. They found that addition lignosulfonate and alkaline lignin can able to cross-link with soy protein, thus enhancing tensile strength and elongation, and also improving water resistance of soy protein plastics. Huang and co-workers also proposed a theoretical model for the physical cross-linking interactions between soy plastic lignosulfonate. They proposed that during the heat processing of the blends, the soy protein isolate molecules undergo a conformational change from a compact coil to an expanded chain and interact with the lignosulfonate center through hydrogen bonding, dipole–dipole, charge–charge, and hydrophobic interactions to form a complete cross-linked network. These reactions resulted in blends with improved mechanical properties and water resistance.

Huang et al. [14] study the effect of soy protein isolate/Kraft lignin composites compatibilized with methylene diphenyl diisocyanate. Kraft lignin were blended with soy protein using methylene diphenyl diisocyanate (MDI) as a compatibiliser. Methylene diphenyl diisocyanate will form urethane links between hydroxy groups on lignin molecules and in the protein. Only a slight reduction in water absorption was observed, but the addition of 2 wt% methylene diphenyl diisocyanate caused a simultaneous enhancement of modulus, strength, and elongation at break of the polymer blends, which was attributed to graft copolymerisation and crosslinking.

Some factors of lignin have importance on stabilisation properties of classical antioxidants: good solubility, mobility and low volatility [42].