EFFECT OF KRAFT LIGNIN ON RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF FISH PROTEIN-BASED BIOMATERIAL

MISS YOTSAVIMON SAKUNKITTIYUT

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
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Abstract

The objective of this research was to investigate the effect of Kraft lignin on rheological and functional properties of fish protein-based biomaterial. Fish protein powder plasticized with 30% glycerol was blended with 0-70% Kraft lignin; the whole content was then thermally molded. The rheological properties were determined by a capillary rheometer as well as a dynamic mechanical analyzer (DMA). Addition of Kraft lignin resulted in a decrease in the viscosity and storage modulus of the biomaterial. The rheology of plasticized fish protein/Kraft lignin biomaterial followed a shear thinning behavior and the presence of Kraft lignin in the mixture seemed to improve the rheological properties. However, Fourier transform infrared spectra revealed no new chemical bonds. The addition of Kraft lignin resulted in an increase in the protein solubility in sodium dodecyl sulfate buffer. However, the changes of the protein molecular weight of plasticized fish protein/Kraft lignin biomaterial as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were not observed. In addition, radical scavenging effect between fish protein and Kraft lignin, which was measured using electron spin resonance (ESR) spectroscopy, was not observed. Concerning the functional properties introduction of Kraft lignin into fish protein blend improved the functional properties of the biomaterial by improving the mechanical properties and by decreasing the water absorption of the biomaterial. Therefore, addition of Kraft lignin is a good way to improve the processability as well as properties of fish protein-based biomaterial.

Keywords: Biomaterial/ Fish Protein/ Free Radical/ Kraft Lignin/ Polymer Blend

หัวข้อวิทยานิพนธ์	อิทธิพลของคราฟท์ลิกนินที่มีต่อสมบัติเชิงการไหลและสมบัติเชิงหน้าที่ของวัสดุย่อยสลายได้จากโปรตีนปลา
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บทคัดย่อ

วัตถุประสงค์ของงานวิจัยนี้คือการศึกษาผลของคราฟท์ลิกนินที่มีต่อสมบัติเชิงการไหลและสมบัติเชิงหน้าที่ของวัสดุย่อยสลายได้จากโปรตีนปลา ทั้งนี้เตรียมวัสดุผสมโดยการผสมโปรตีนปลากับกลีเซอร์รอลซึ่งเป็นพลาสติไซเซอร์ในปริมาณร้อยละ 30 และคราฟท์ลิกนินตั้งแต่ร้อยละ 0-70 จากนั้นขึ้นรูปส่วนผสมทั้งหมดด้วยความร้อนโดยวิธีการอัดด้วยแรงดันและวิเคราะห์สมบัติเชิงการไหลโดยอาศัยเครื่องคาปิลารี่รีโอมิเตอร์และเครื่องวิเคราะห์ทางกลศาสตร์เชิงพลศาสตร์ จากผลการทดลองพบว่าการเติมคราฟท์ลิกนินช่วยลดความหนืดและค่ามอดูลัสสะสม และพบว่าวัสดุผสมโปรตีนปลา-คราฟท์ลิกนินมีพฤติกรรมการไหลเป็นแบบเชียร์ทินนิ่ง ดังนั้นการเติมคราฟท์ลิกนินจึงช่วยปรับปรุงสมบัติเชิงการไหลได้ อย่างไรก็ตามผลการวิเคราะห์อินฟราเรดสเปกตรัมไม่แสดงให้เห็นการเกิดพันธะเคมีใหม่ นอกจากนี้พบว่าการเติมคราฟท์ลิกนินช่วยเพิ่มการละลายของโปรตีนในโซเดียมโดเด็กซิลซัลเฟตบัฟเฟอร์ แต่จากการวิเคราะห์ด้วยเทคนิคโซเดียมโดเด็กซิลซัลเฟต พอลิอะคริลาไมด์เจลอิเล็กโตรโฟริซิส ไม่พบการเปลี่ยนแปลงน้ำหนักโมเลกุลของโปรตีน และจากการวิเคราะห์ด้วย

เครื่องอิเล็คตรอนสปินเรโซแนน ไม่พบผลของการจับอนุมูลอิสระระหว่างโปรตีนปลาและคราฟท์ลิกนิน นอกจากนี้การเติมคราฟท์ลิกนินยังช่วยปรับปรุงสมบัติเชิงหน้าที่ของวัสดุย่อยสลายได้ โดยพบการเปลี่ยนแปลงของสมบัติเชิงกลและการลดลงของค่าการดูดซึมน้ำของวัสดุย่อยสลาย ดังนั้นการเติมคราฟท์ลิกนินจึงน่าจะช่วยปรับปรุงสมบัติของวัสดุย่อยสลายได้จากโปรตีนปลาให้ดีขึ้นได้

คำสำคัญ: โปรตีนปลา/โพลีเมอร์ผสม/ ลิกนินคราฟท์/ วัสดุย่อยสลายได้/ อนุมูลอิสระ

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LIST OF ABBREVIATIONS

A	Constant
AOAC	Association of Official Agricultural Chemists
°C	Degree Celsius
ΔCp	Molar excess heat capacity
Da	Dalton
DMA	Dynamic Mechanical Analyzer
DPPH	2, 2’- diphenyl-β-picryl hydrazyl
DSC	Differential Scanning Calorimetry
DTE	Dithioerythriol
E	Activation energy
E'	Storage modulus
E"	Loss modulus
ESR	Electron Spin resonance
FP	Fish protein
FTIR	Fourier Transform Infrared Spectrometer
×g	Relative centrifugal force
kDa	Kilodalton
KL	Kraft lignin
M	Molar
MHC	Myosin heavy chain
MW	Molecular Weight
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SH	Sulfhydryl
T	Temperature
TB	Threadfin bream
Td	Thermal degradation
T_g	Glass transition temperature
TGA	Thermogravimetric Analysis
UV	Ultraviolet
η	Viscosity

CHAPTER 1 INTRODUCTION

1.1 Rational

Most of plastics are petroleum-based plastic. It’s non renewable. The pollution of these plastic leads to serious environmental problem. Therefore, the development of bioplastics to substitute synthetic polymer has become an important challenge [3]. In addition, bioplastics are environmental friendly products. It can be degraded in a relatively short period of time. Its by-products are water, carbon dioxide and biomass. Biodegradable plastics from agricultural resources such as carbohydrates, starch, and proteins have become the potential replacements for petroleum plastic [2-4].

Polymer blend is one of the effective methods for providing new desirable polymeric materials for a variety of applications. Polymer blend contains mixture of polymer at least two components in order to produce materials with better properties compared to similar materials made from the respective pure polymers [5].

Thailand is one of the largest surimi producers in Southeast Asia [6]. Surimi production was estimated to be 140,000 metric tons and generates 2.8 million liters of wastewater. Protein contents in waste water are 4.8-5.4% [7]. In addition, fish processing operations produce waste in a solid (fish carcasses, viscera, skin, heads, dark muscle) [8]. Therefore, protein from fish waste led to one of the most attractive for bioplastic production as a result to value-added industrial wastes. Also the reduced amounts of waste in water help improve the environment.

Proteins are natural, complex hetero-polymers offering a variety of functional properties. Protein-based bioplastic could be defined as a three-dimensional macromolecular network stabilized and strengthened by hydrogen bonds, hydrophobic interactions and disulfide bonds [4]. Interaction of proteins can be formed and stabilized by new inter-molecular and intra-molecular interactions.

A main drawback of protein-based bioplastics is a narrow window processing on using conventional methods such as extrusion and injection molding due to its high viscosity. The problem of protein-based in extrusion process when using high screw speed caused extrudate rupture was reported [9, 10]. During thermal processing, protein has aggregation via sulfhydryl-disulfide interchange reactions, leading to an increase of the covalent bonds and high viscosity. Ullsten et al. [10] reported that salicylic acid allowed an enlargement of protein extrusion window. To delay protein aggregation, free radical scavenger actions of salicylic acid were proposed and were confirmed by electron spin resonance analysis. However, in terms of functional properties, protein-based bioplastic has low water resistance and moderate mechanical properties [9], therefore requiring much research to fully understand how to manipulate final material properties.

Lignin is a natural polymer and a main component in plants [11]. Beside, Kraft lignin is the byproduct of the alkaline pulping process from pulp and paper industry. It is a polyphenolic thermoplastic compound which is known for its radical scavenging properties [12, 13]. Kraft lignin may interfere the protein aggregation and may decrease viscosity of protein due to its free radical scavenging properties. In addition, Kraft lignin is relatively hydrophobic and high water resistant [14]. The use of lignin as a filler in materials have been reported [15] [16]. Therefore, blending fish protein (FP) and Kraft lignin (KL) may be an effective method to produce a new value-added product with better properties. Therefore, this research was aimed to study the effect of Kraft lignin on rheological properties and functional properties of fish protein-based biomaterials.

1.2 Objective

1. To study the effect of Kraft lignin on rheological and functional properties of fish protein based-biomaterial.

1.3 Scopes

1. Fish protein powder from threadfin bream was prepared by drying method. Then, chemical compositions of fish protein were characterized.
2. Materials containing a mixture of protein: Kraft lignin: glycerol in a weight ratio ranging from 70:0:30 to 0:70:30 were prepared by mixing and compression molding methods.
3. Rheological properties in term of viscosity, viscoelastic properties of plasticized fish protein/ Kraft lignin blends were determined by capillary rheometer and Dynamic Mechanical Analyzer (DMA), respectively.
4. Chemical bonds, protein aggregation, protein molecular weight and free radical of plasticized fish protein/ Kraft lignin blends were determined by Fourier Transform Infrared Spectrometer (FTIR), protein solubility in Sodium dodecyl sulfate, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electron spin resonance (ESR) in order to study the radical scavenging properties of Kraft lignin on protein aggregation.
5. Functional properties in term of mechanical properties and water absorption of plasticized fish protein/ Kraft lignin biomaterials were determined by texture analyzer and water immersion.

1.4 Expected Benefit

1. To reduce wastewater treatment and added-value of fish protein on surimi industry.
2. To improve processability of protein blend and functional properties of bioplastics.
3. To understand influence of Kraft lignin on protein aggregation during processing.

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.

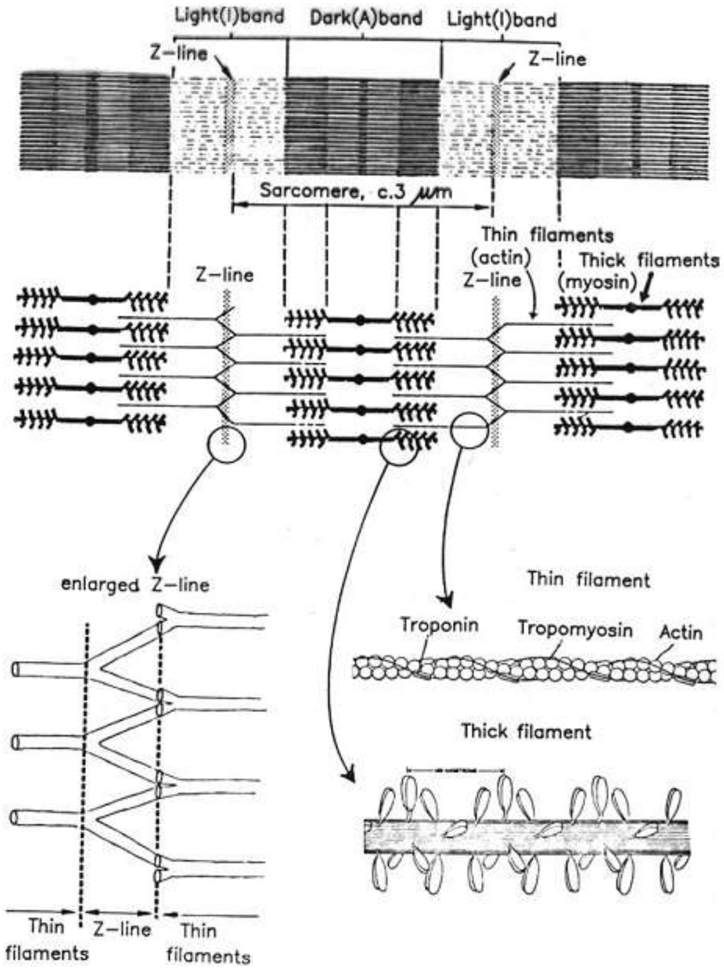


Figure 2.1 Muscle cell and components of protein [19].

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.

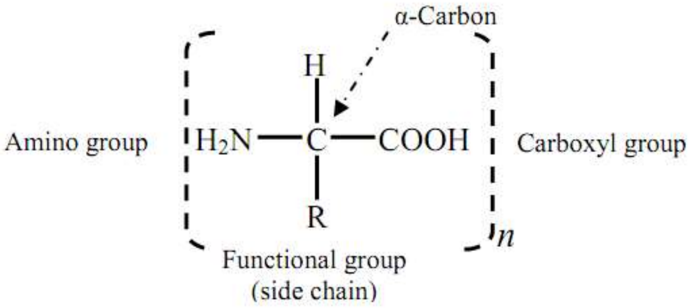


Figure 2.2 General structure of an amino acid [4].

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.

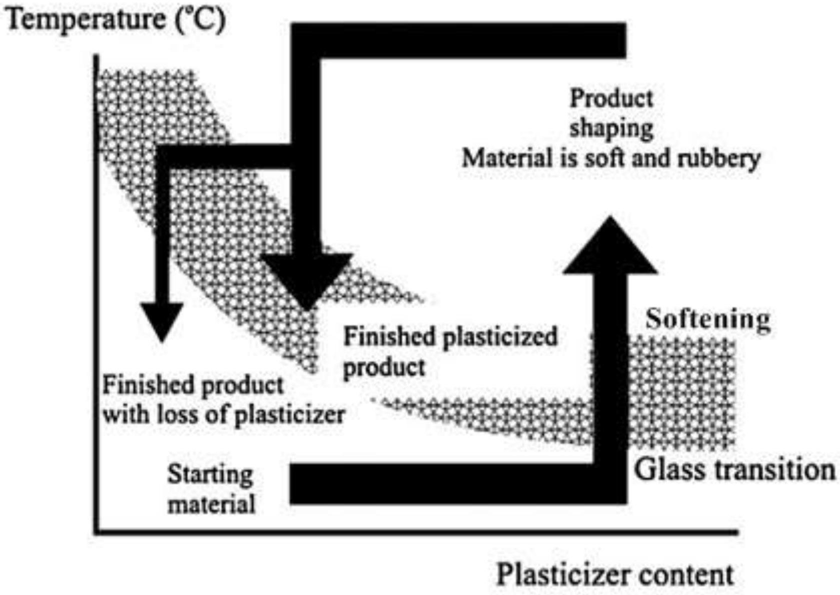


Figure 2.3 Schematic representation of the thermoplastic process applied to protein-based materials [4].

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.

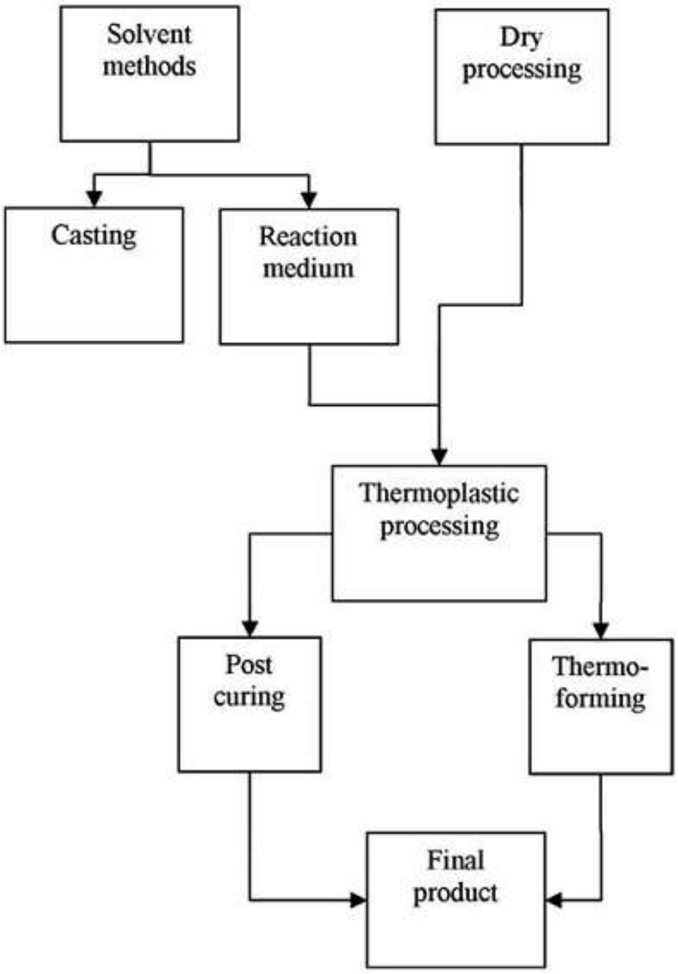


Figure 2.4 Processing paths leading to thermoplastic processing [4].

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.

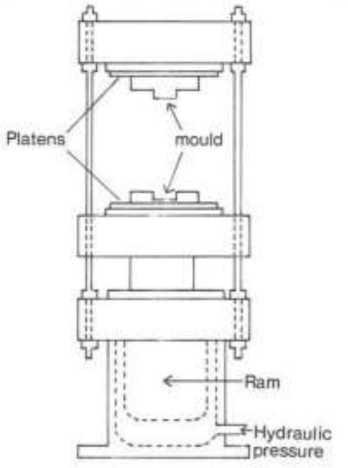


Figure 2.5 Compression molding press [27].

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.

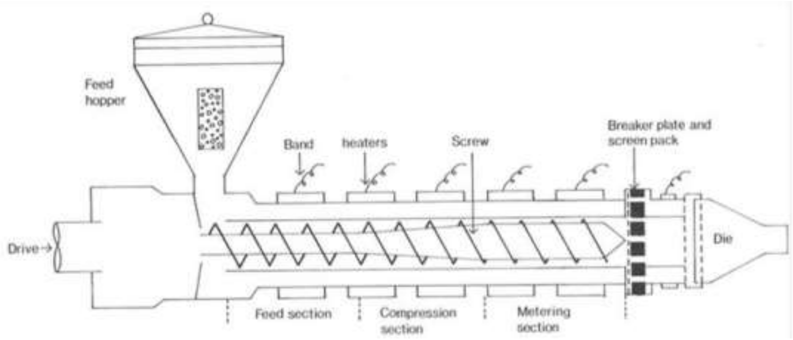


Figure 2.6 Main features of a single screw extruder [27].

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.

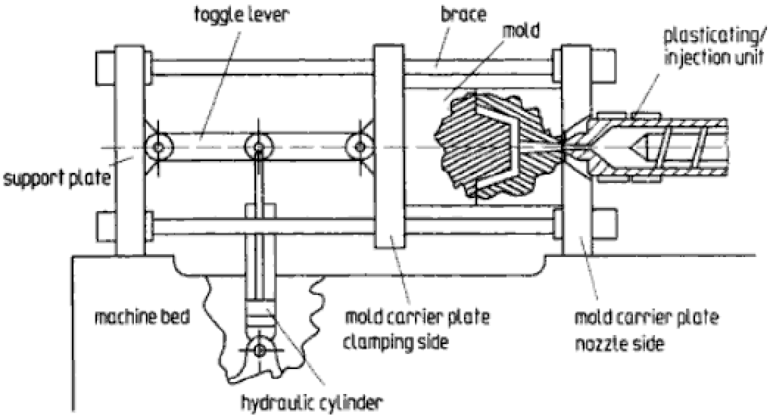


Figure 2.7 Main features of injection molding [28].

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.

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

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	1000-10^5
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.

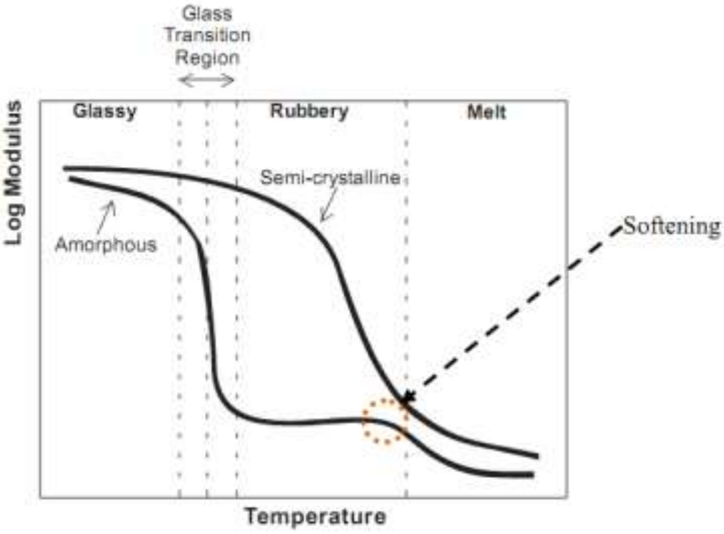


Figure 2.8 General change in modulus with temperature of amorphous and semi-crystalline polymer materials, showing the different regions of viscoelastic behavior [34].

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 ($\tan \delta = E''/E'$) 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.

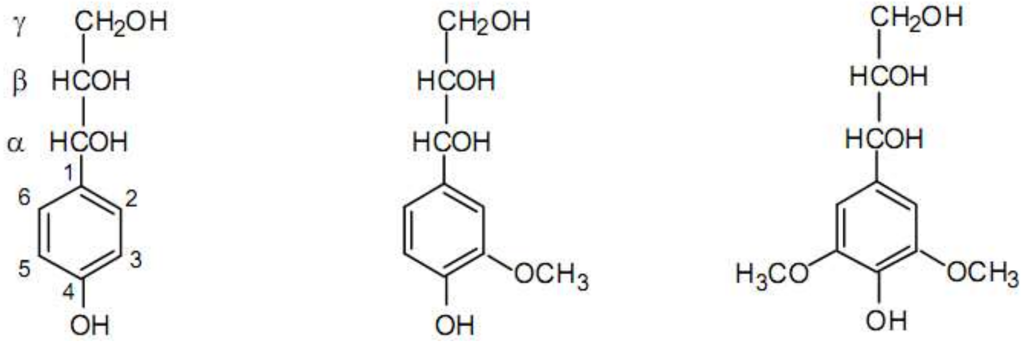
2.3.5 Water Absorption

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].

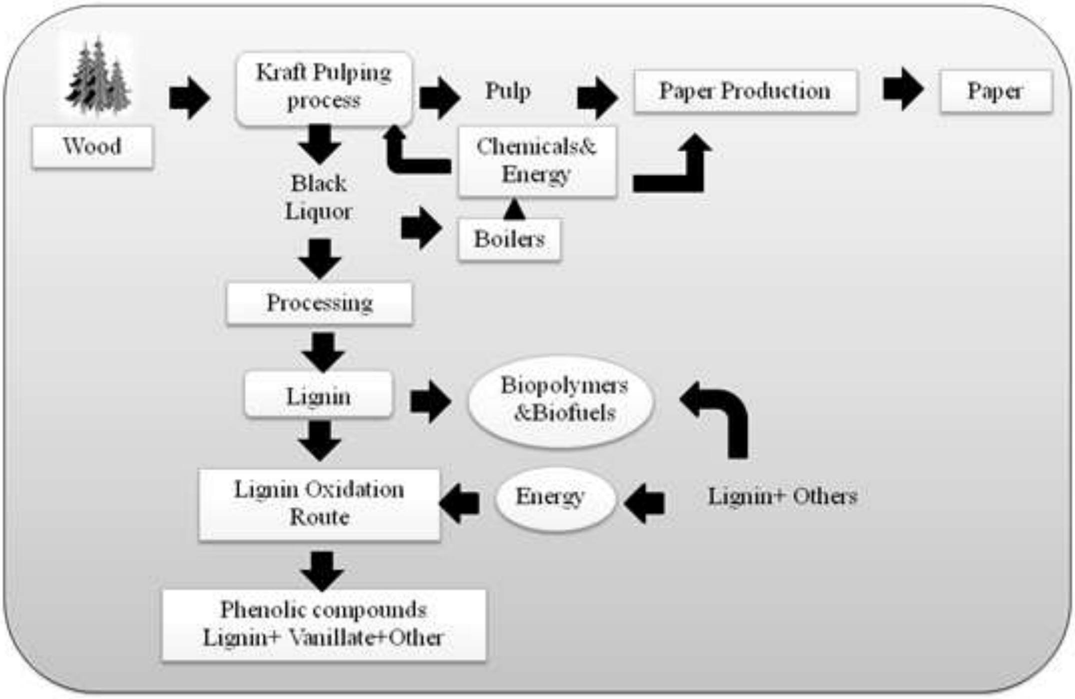


Figure 2.10 Valorisation of Kraft lignin by a biorefinery concept: the proposed integrated process for producing biopolymers [38].

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.

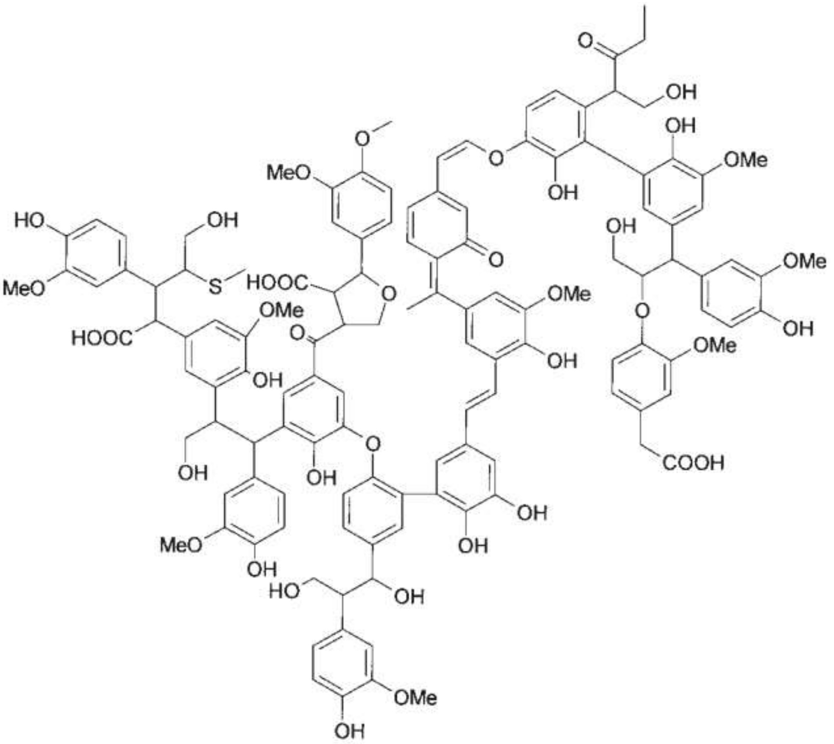


Figure 2.11 Structural model Kraft lignin [41].

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].

CHAPTER 3 MATERIALS AND METHODS

This chapter presents the overall experimental set-up as well as the techniques which are used in the study of the effect of Kraft lignin on rheological properties and functional properties of fish protein-based biomaterial.

3.1 Raw Materials and Equipments

3.1.1 Raw Materials and Chemicals

1. Threadfin bream (*Nemipterus* sp.), Prachauthid 61 market, Bangkok, Thailand as shown in Figure 3.1a.
2. Kraft lignin, Raja engineering. Co. Ltd, Bangkok, Thailand
3. Reagents
    - Acetone, AR Grade, Labscan, Bangkok, Thailand
    - Anhydrous glycerol ($C_3H_5(OH)_3$), AR Grade, Roongsub Chemical Ltd, New South Wales, Australia
    - Magnesium nitrate ($Mg(NO_3)_2$), AR Grade, Ajax Finechem Ltd, Auckland, New Zealand
    - Sodium azide (NaN_3), AR Grade, Merck
    - Phosphorus pentoxide (P_2O_5), AR Grade, Carlo Erba, Italy
    - 4% Boric acid solution, AR Grade, Riedel-de, Seelze, Germany
    - 2-propanol, AR Grade, Carlo Erba, Rodano, Italy
    - 95% Ethyl alcohol, Commercial Grade, Carlo Erba, Rodano, Italy
    - Hexane, AR Grade, J.T.Baker, Phillipsburg, USA
    - Hydrochloric acid, AR Grade, BHD, UK
    - Kjeltabs, AR Grade, Tecator, Höganäs, Sweden
    - Sodium carbonate, AR Grade, Carlo Erba, Rodano, Italy
    - Sodium hydroxide, AR Grade, Carlo Erba, Rodano, Italy
    - Sulfuric acid, AR Grade, BHD, UK
    - Trizma base, AR Grade, Carlo Erba, Rodano, Italy

3.1.2 Apparatus

- Centrifuge, Himac, Model CR21, Chiba, Japan

- Chopper, Waring, Model WCG75E, Torrington, LT, USA
- Hot air oven, Memmert, Model ULM 600 II, Germany
- Hot air oven, Termarks, Model TS8000, Bergen, Norway
- Furnance, Ney, Model 2-160, Californea, USA
- Grinder, Waring, Model HSB2WT, Torrington, CT, USA
- Incubator shaker, New Brunswick Scientific, Model G-24, New Jersey, USA
- pH meter, Schott Gerate, Model CG841, Mainz, Germany
- Protein analyzer, Tecator, Model 1002, Hoganas, Sweden
- Fat analyzer, Soxtec System-Texator, Sweden
- Shaking water bath, Heto, Model AT110, Allerod, Denmark
- Sieve shaker, Retsch, Model AS200 basic, Germany
- Stirrer hot plate, Morat-Magnetrüher, Model M21/1, Germany
- Vacuum pack, Mutivac Model A300, Germany
- Weighing machine, Sartorius, Model LA230S, Goettingen, Germany
- Internal mixer, Plasti-corder W50, Brabender, Duisburg, Germany
- Hydraulic Press Machine, 20 T., SMC TOYO METAL Co., Ltd., Thailand
- Thermal analysis instrument, Mettler Toledo TGA/SDTA 851^e, USA
- Differential Scanning Calorimeter, Mettler Toledo DSC 822^e, Mettler Toledo, USA
- Capillary rheometer, RHEO-TESTER 2000, Germany
- Dynamic mechanical thermal analyzer, NETZSCH DMA 242, Piscataway, USA
- Fourier Transform Infrared Spectrometer, Perkin Elmer instruments, Singapore
- Electron Spin Resonance spectroscopy, JES-RE2X, JEOL, Japan.
- Texture Analyzer, Stable Micro System, TA-XT.plus, Surrey, UK

3.2 Preparation of fish protein powder

Threadfin bream (*Nemipterus* sp.) were gutted, headed fish and passed through a meat bone separator. After that, the fish mince were washed twice with tab water, stained in hydraulic machine, chopped in a cutter and dried in hot air oven at 50 ^oC for 5 hours and 40 ^oC for 24 hours or until their weight was constant. Finally, the dried fish protein (FP) was ground less than 425 µm as shown in Figure 3.1b. The fish protein powder was vacuum packed in polyethylene bags.

(a) (b)

Figure 3.1 Threadfin bream (a), Fish protein powder (b)

3.3 Preparation of plasticized fish protein/ Kraft lignin biomaterial

Materials in this study contain a mixture of fish protein powder: Kraft lignin: glycerol in a weight ratio ranging from 70:0:30 to 0:70:30 as shown in Table 3.1.

Table 3.1 Composition of biomaterial with 0-70% Kraft lignin

Composition (%wt)	**Sample**							
	0% KL	**10% KL**	**20% KL**	**30% KL**	**40% KL**	**50% KL**	**60% KL**	**70% KL**
Fish protein (FP)	70	60	50	40	30	20	10	0
Kraft lignin (KL)	0	10	20	30	40	50	60	70
Glycerol	30	30	30	30	30	30	30	30

3.3.1 Mixing process

Fifty gram of Kraft lignin/fish protein and glycerol were mixed in a mixer (Plasti-corder W50, Brabender, Duisburg, Germany) at temperature 80 °C, 100 rpm for 15 minute. The torque and product temperature were continuously recorded during mixing. The torque rheometer was used to measure the melt flow behavior of biomaterials. Quantitative information for the melt flow of the samples could be obtained by recording the torque and temperature as functions of time during processing. When sufficiently blended, the solid powders turn into a melt fluid state, and the obtained. Equilibrium torque and temperature were related to the apparent viscosity of the material under process conditions, thus indirectly reflecting the flow behavior.

3.3.2 Compression molding process

Ten or twenty gram of the blend was placed in a squared mould (9 × 9 cm^2) and thermomolded at 100 °C for 15 min. Samples were preheated for 5 min and beginning of the experiments for 10 min in a Hydraulic Press Machine (20 T., SMC TOYO METAL Co., Ltd., Thailand). A pressure of 1 ton was directly applied to the sample in the mold. The thickness of material is approximately about 1 or 2 mm depending on initial weight of blend. Process of fish protein and Kraft lignin specimen preparation is explained below, Figure 3.2.

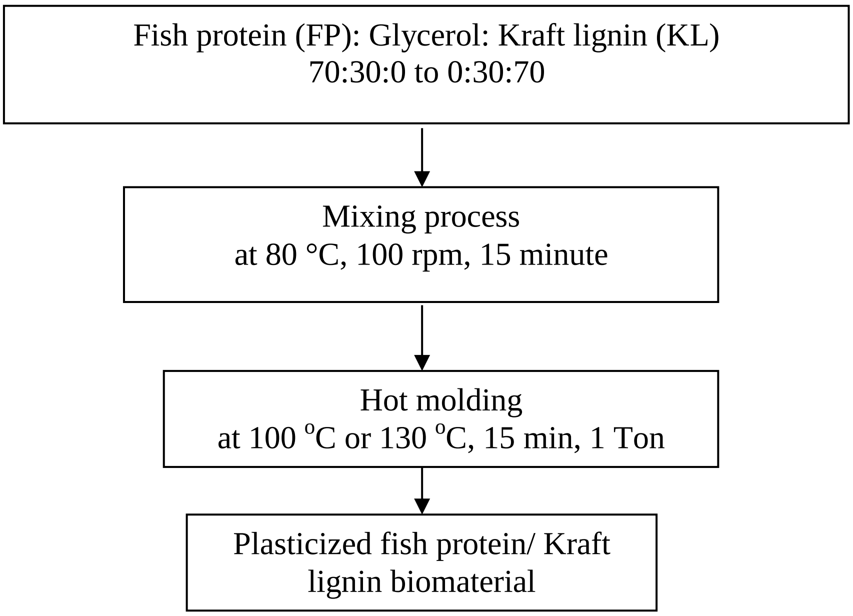


Figure 3.2 Process of plasticized fish protein/ Kraft lignin biomaterial specimen preparation

3.4 Characterization of materials

3.4.1 Proximate composition

The proximate composition was determined according to AOAC methods [47]. Crude protein content was determined using the Kjeldahl method (Kjeltex System-Texator, Sweden). Crude lipid content was determined by the Soxhlet method (Soxtec System-Texator, Sweden). Ash content was determined by burning samples overnight at 550 °C. Moisture content was determined by drying samples overnight at 105 °C until constant weight was achieved. Each sample was analyzed in 3 replicated, standard deviation was calculated and the data was expressed in mean ± SD.

3.4.2 Thermogravimetric analysis (TGA)

Fish protein powders and Kraft lignin were tested by thermal analysis instrument (Mettler Toledo TGA/SDTA 851^e, USA). The experiments were carried out in the air. Samples were equilibrated at 0% RH over P_2O_5. The samples were about 5–10 mg, the scope of testing temperature was from the room temperature 30°C to 600 °C and the heating rate was 10 °C/min. Purge the sample chamber with dry nitrogen (or other inert gas) to avoid thermo-oxidative reactions. and record the thermal curve [43]. Select a point on the mass loss thermal curve from before and another on the mass loss plateau immediately after the first mass loss [48]. These temperature points were identified.

3.4.3 Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter was used to measure the glass transition temperature and enthalpy of material melting in the sample. Calorimetric measurements were performed using a Mettler Toledo DSC 822^e (Mettler Toledo, USA). An empty stainless steel pan was used in the reference holder. Fish protein powder and Kraft lignin were equilibrated over P_2O_5, 0%RH. The samples were heated first time at 10 ºC/min from 25 to 250 ºC. It was then cooled to 25 ºC at 10 ºC/min and heated a second time at 10 ºC/min to 250 ºC. Finally the samples were cooled to 25 ºC at 10 ºC/min. The glass transition temperature (T_g), was determined as the temperature midpoint of the heat capacity change observed during the second run [49].

3.5 Characterization of plasticized fish protein/Kraft lignin biomaterial

3.5.1 Rheological Analysis

After mixing, the melt flow behavior of one hundred gram of plasticized fish protein/ Kraft lignin blends were determined by a one barrel capillary rheometer (capillary rheometer RHEO-TESTER 2000, Germany) with a 2-mm capillary die and a length-to-diameter ratio of 15. Measurements were carried out at 140 °C under a shear rate ranging from 10 to 1000 s^{-1}(with step change of 50 s^{-1}).

The power law model was used to determine the rheological behavior that describe relationship between viscosity and shear rate is

$$\tau_w = K \dot{\gamma}_w^n \tag{3.1}$$

where the consistency (K) corresponds to the viscosity value for a shear rate γ of s^{-1} and the power-law index (n) characterizes the deviation from the Newtonian behavior.

The apparent viscosity of samples (η), at constant shear rate, was calculated as follows:

$$\eta = \frac{\tau_w}{\dot{\gamma}_w} = K \dot{\gamma}^{n-1} \tag{3.2}$$

Where: η correspond to apparent viscosity. The values of n are between 0 and 1 for thermoplastic materials, which explains their shear thinning behavior [50-52].

3.5.2 Glass transition temperature (T_g)

Thermomechanical properties were conducted with a dynamic mechanical thermal analyzer (NETZSCH DMA 242, Piscataway, USA). Rectangular samples (15 × 3 × 1 mm^3) were preconditioned at 25 ^oC and 0 % relative humidity over P_2O_5. After that they were analyzed with equipped with a cryogenic system fed with liquid nitrogen. A tensile test was performed with a temperature ramp from –100 to 200°C at a heating rate of 3°C.min^{-1}. A variable sinusoidal mechanical stress was applied to the sample (frequency = 1 Hz) to produce a sinusoidal strain amplitude of 0.05% and the displacement amplitude of 2 μm, which ensure measurements in the linear domain of viscoelasticity [53]. During analysis, the storage modulus (E'), the loss modulus (E") and tan δ (=E"/E') were recorded and plotted against temperature for further evaluation of thermal transition. T_g was identified as the temperature of the tan δ maximum.

3.5.3 FTIR spectra

The dry samples were sprinkled into a matrix of KBr, and ground in an agate mortar (KBr pellet technique). The samples are tested by using a Fourier Transform Infrared Spectrometer (FTIR, Perkin Elmer instruments, Singapore). Investigations have been performed in the transmission mode at the resolution of 4 cm^{-1}. Each sample recording consisted of 64 scans recorded in % Transmittance from 400–4000 cm^{-1} [11, 13]. Prior to recording, the spectra were transformed against a KBr background. Fourier transform infrared spectroscopy (FTIR) was employed to study the structural organization of biomaterials.

3.5.4 Protein solubility in SDS

Protein solubility was determined according to the method of Kunanopparat et al. [13]. Briefly, the fish protein powder (26.67 mg) was stirred for 80 min at 60 ºC in the presence of 20 ml of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% sodium dodecyl sulfate (SDS). SDS is known to be an efficient dissociating detergent able to disrupt hydrophobic, hydrogen and ionic bonds. The addition of strong anionic surfactant like SDS allows the suppression of the intermolecular interactions between proteins and brings them into solution. The SDS-soluble protein extract was recovered by centrifugation (50 min at 15,000g and 20 ºC), and 1000 µl was used to determine protein content. The remaining SDS-insoluble protein fraction can be further extracted in the same SDS buffer, after the cleavage of the disulfide bridges, which are responsible for the protein interchain cross-linking. The pellet was suspended in 5 ml of SDS-phosphate buffer containing 20 mM dithioerythritol (DTE) as reducing agent that is often used to break the disulfide bonds and decrease the polymer size. After it was shaken for 60 min at 60 ºC, the extract was sonicated for 3 min. These treatments brought insoluble protein from the pellet into the solution. After centrifugation (50 min, 15,000 g, 20 ºC), a part of the supernatant was determined protein content using the Kjeldahl method (Kjeltex System-Texator, Sweden).

3.5.5 SDS-PAGE

SDS-soluble protein fraction and SDS-insoluble protein fraction were solubilized in an SDS–solution containing 10 ml of 10% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.5 M Tris–HCl (pH 6.8) and Trace bromophenol blue 0.0003 g. The supernatant was used for gel electrophoresis as described by Laemmli [54]. The amount of protein loaded onto the polyacrylamide gel was 30 µl. A total of 100 µl of each protein were mixed with 400 µL of SDS buffer then, used for gel electrophoresis. Stacking gel and separating gel were made of 4% (w/v) and 15% (w/v) polyacrylamide, respectively. The amount of protein loaded onto the polyacrylamide gel was 15 mg. The Precision Plus Protein^{TM} standard (10-250 kDa) as a marker was also injected at the loading of 20 µl in the left lanes of the gel and the electrophoresis was carried out at 200 V for about 60 min. After electrophoresis, gels were stained and detained with silver staining because of the nature of this protein (high stability and hydrophobicity), it is difficult to solubilize and to digest for electrophoretic analysis. In addition, its low protein content difficult ability prevents it from being stained with simple staining procedures, such as the use of Coomassie Blue.

3.5.6 Electron Spin Resonance (ESR) Spectroscopy

The radicals formed on material after treatments were identified according to the method of Ullsten et al. [10] and quantified by electron spin resonance spectroscopy. Firstly, samples were collected immediately after mixing and molding, were put into liquid nitrogen, and were preconditioned at 0 % relative humidity over P_2O_5. In testing, the samples (8-10 mg) were placed into ESR glass containers. These were immediately transferred to a liquid nitrogen container, in order to inhibit further reaction before the spectromagnetic investigation. The ESR spectra were recorded at liquid-nitrogen (77 K) temperature to detect thiyl and di-sulfide radicals that often cannot be detected at room temperature [55]. ESR spectra (first derivatives) were recorded for the frozen samples using a electron spin resonance spectroscopy (JES-RE2X, JEOL, Japan). ESR spectra using the following instrumental parameters: scan range, 324.0 mT, width 5.0×10 mT at 1 mW microwave power, sweep time 2 min amplitude 1.6×100 and 0.5 mT modulation amplitude. The g values were determined by standardization with α, α'- diphenyl-β-picryl hydrazyl (DPPH). The g value is calculated from the relationship

$$h\nu = g\beta B \tag{3.7}$$

where h is Plank's constant (6.63×10^{-34} J s),

ν is the microwave frequency (9.4 GHz, measured by a frequency counter),

β is the Bohr magneton (9.27×10^{-24}A m^2),

B is the magnetic field (G), Spectra were recorded during runs.

3.5.7 Mechanical properties

Tensile tests were performed on a Texture Analyzer (Stable Micro System, TA-XT.plus, Surrey, UK). Samples were cut into dumb-bell-shaped specimens of 75 mm overall length and 5 mm width for the elongating part and preconditioned at 25 °C, 53% RH over a saturated salt solution of $Mg(NO_3)_2$. Specimen thickness was measured with a caliper. The initial grip separation was 50 mm and elongation speed was 1 $mm\cdot s^{-1}$.

The tensile strength, elongation at break and Young's modulus was determined. The elastic constants in The Stress values (Pa), Strain values and Young's modulus are calculated according to equations 3.8, 3.9 and 3.10 respectively.

$$\text{Stress (Pa)} = \text{force values (N)} / \text{cross-sectional area (mm}^2) \tag{3.8}$$

$$\text{Strain (Pa)} = \text{elongation length } (\Delta L) / \text{initial length } (L_0 = 20 \text{ mm}) \tag{3.9}$$

$$\text{Young's modulus (\%)} = \text{Stress} / \text{Strain} \tag{3.10}$$

Tensile properties-tensile strength, elastic modulus and percent elongation and are indicators of mechanical properties in biomaterial [56].

Tensile strength is the maximum stress that a material can withstand while being stretched or pulled before necking, which is when the specimen's cross-section starts to significantly contract. Tensile strength is the opposite of compressive strength and the values can be quite different.

Elongation at break of material is the percentage increase in length that occurs before it breaks under tension.

Young's modulus is a measure of the stiffness of an elastic material and is a quantity used to characterize materials. A high Young's modulus means that the material is rigid.

More stress is required to produce a given amount of strain. In polymers, the modulus and compressive modulus can be close or may vary widely.

3.5.8 Water absorption

Samples (20 mm in diameter) were dried in hot air oven at 50 ^{o}C 48 h. or until their weight was constant (W_i). Then, they were immersed in 50 ml distilled water containing 0.05% NaN_3 (to avoid the microbial growth) at 25 ^{o}C. The swollen samples were wiped and weighed (W_w) after 1 week. Then, they were dried in hot air oven at 50 ^{o}C 48 h. or until their weight was constant (W_f). Each sample was analyzed in four replicate. Water absorption was calculated by the following equations (3.6):

$$\text{Water absorption (\%)} = 100 \times (W_w - W_f) / (W_i) \qquad (3.11)$$

3.6 Statistical analysis

The experimental data were analyzed and presented as mean values with standard deviations. The one-way ANOVA was used to compare means at 95% confidence ($p \leq 0.05$). The statistical program SPSS (version 11) was used to perform all statistical calculations.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Characterization of materials

4.1.1 Proximate composition of fish protein powder

Fish powder was prepared from fish mince by chopping and drying in hot air oven until weight constant. The proximate compositions of fish protein powder were determined as described by AOAC method [47]. Fat, protein moisture and ash content were analyzed as shown in Table 4.1

Table 4.1 Chemical composition of fish protein powder

Chemical composition	Percent (%)
Fat	4.28±0.07
Protein	84.93±0.45
Moisture	6.42±0.15
Ash	2.78±0.01

Values are mean ± SD, Protein conversion factor is 6.25 [57].

4.1.2 Thermogravimetric analysis (TGA)

Thermal decomposition of fish protein and Kraft lignin were studied. Table 4.2 shows T_d (thermal decomposition) of fish protein and Kraft lignin. The initial weight loss around 100 ºC is due to the evaporation of water [16]. This result was shown in appendix B.1. Thermal degradation temperatures were determined from T_d curves that samples start to degrade at around 190°C for fish protein and 150 °C for Kraft lignin. For Kraft lignin, it could not be degraded due to the presence of strong carbon-carbon linkages and other chemical groups such as aromatic groups, which are very resistant to treatment [58]. In addition, the initial stage in thermal degradation of lignin is concerned with hydroxyl groups [59]. Therefore, the temperature of 140 °C which is lower than degradation temperature was taken to study rheological properties of plasticized fish protein/Kraft lignin blend during the heating in order to avoid degradation.

Table 4.2 T_d (thermal decomposition) and T_g (glass transition temperature) of fish protein and Kraft lignin.

Sample	T_d (°C)	T_g (°C)
Fish protein	192	167.2±1.0
Kraft lignin	165	151.7±0.7

4.1.3 Differential Scanning Calorimetry (DSC)

Table 4.2 displays the DSC results which are the second DSC scan of fish proteins and Kraft lignin respectively. This sample shows the glass transition temperature. Fish protein has glass transition temperature at 167.2 ºC. For Kraft lignin, it melted has a glass transition temperature at 151.7 ºC. Glass transition temperature of protein and Kraft lignin are very close to those reported by Dean et al. [60]. In this work, the first DSC heating curve is often affected by desorption of moisture and by enthalpy relaxation effects. Thus, the results from the second heating curve were used for the various calculations.

4.2 Rheological properties of plasticized fish protein/ Kraft lignin blend

Rheological behavior represents the most important property of biomaterial during processing

4.2.1 Torque rheology

Mixing of fish protein/Kraft lignin-plasticizer blends has an influence on the properties of thermo mechanically processed biomaterial. In processing plastics, the material is melted, transferred and then solidified. The transfer or flow process is critical to processing. An understanding of the flow properties of the material is important for manufacturing of biomaterial.

Figure 4.1 shows the equilibrium torque and equilibrium temperature of plasticized fish protein/ Kraft lignin blend at 0–70% Kraft lignin content during mixing at 80 °C 100 rpm for 15 min. Equilibrium temperature of polymer blend increased more than initial process temperature because shear induces heat during mixing [61], which is attributed to the thermal energy dissipation due to the high medium viscosity. In addition, increasing % of Kraft lignin content can decrease equilibrium torque and temperature. Therefore, addition of Kraft lignin decreased viscosity of polymer blend.

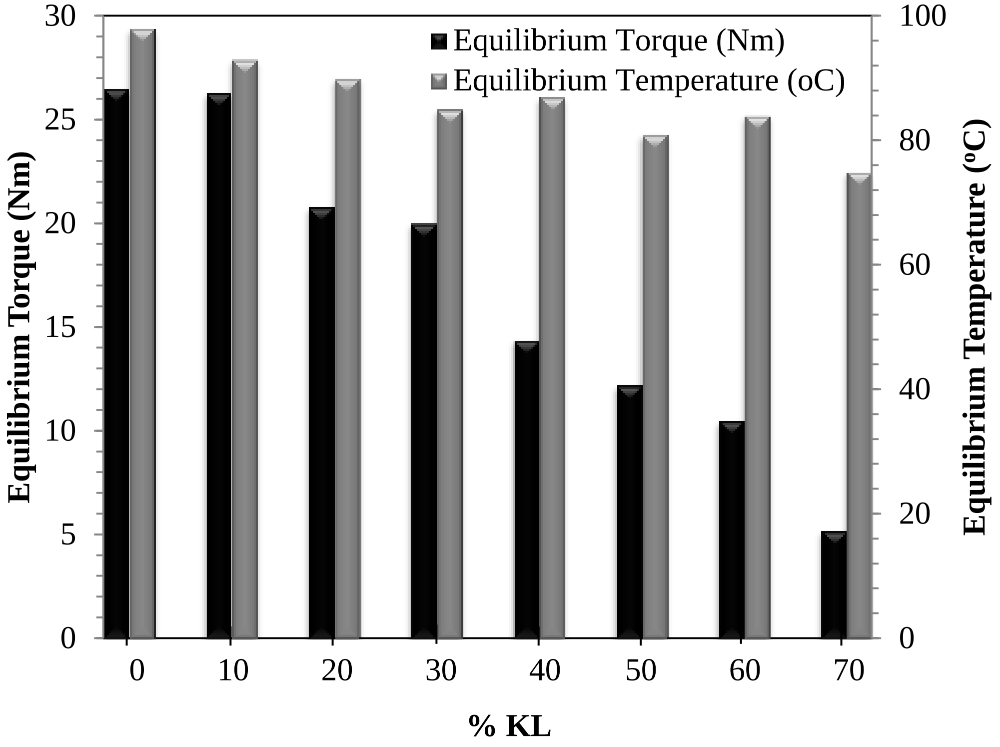


Figure 4.1 Equilibrium torque and equilibrium temperature of plasticized fish protein/ Kraft lignin blend with 0-70%Kraft lignin content at 80 °C 100 rpm for 15 min.

4.2.2 Capillary rheology

In all plastics conversion processes the flow characteristics are important in defining process parameters. For this reason, it is not surprising that the measurement of polymer melt flow has been considered as a critical factor in determining the processability of the polymer. Therefore, this study was to investigate the effect of Kraft lignin addition on flow properties of plasticized fish protein blend.

Viscosity data can also provide some information about changes in molecular structure [33]. Viscosity of fish protein blends with 0-70% Kraft lignin were determined by using capillary rheometer which is a technique whereby a sample undergoes extrusion through a die of defined dimensions. The test sample was pushed by a piston driven at temperature of 140 ^{o}C through a capillary die and measured its rheological properties like shear rate and shear viscosity. The data was obtained from steady shear measurement as shown in Figure 4.2.

The shear viscosity of plasticized fish protein/ Kraft lignin blend decreased when shear rate increased. The shear viscosity of the blends was recorded in the shear rate range of 10-1000 s^{-1}. However, the testing region for fish protein blend with 0% Kraft lignin could not exceed 500 s^{-1} with the same amount of sample. Thus, the melt of the blend exhibited more stability under high shear. The addition of Kraft lignin resulted in a decrease of shear viscosity of mixed blend.

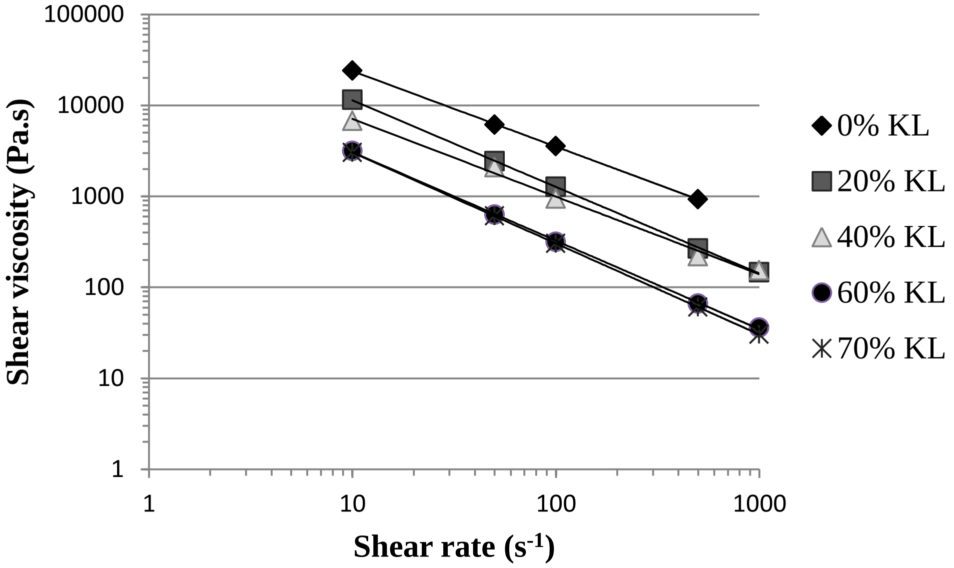


Figure 4.2 Viscosity of Fish protein blends with 0-70% Kraft lignin content determined by capillary rheometer at 140 ^{o}C die temperature

The relationship between the shear viscosity and shear rate can be described by power law equation according eq. (3.6). Power-law index and flow behavior consistency were calculated as shown in Table 4.3. K is often known as the flow behavior consistency. This describes the overall range of viscosities across the part of the flow curve that is being modeled. Also, if the Power law region includes $1s^{-1}$ shear rate then K is the viscosity or stress at that point. The exponent n is known as the Power Law Index (or sometimes the rate Index). For a shear thinning fluid: $0<n<1$, the more shear-thinning the product, the closer n is to zero. For experiment, the power-law index of fish protein-based material is 0.17. Values for the power index for protein ranging from 0.12 to 0.78 have been reported in the literatures [33, 49, 62]. All samples represented shear thinning behavior and their ability to flow. Power-law index and the consistency coefficient decreased when Kraft lignin content increased. This may be explained by a low molecular weight of Kraft lignin (MW= 2000-5000 Da) [11, 38, 42] compared to fish protein (MW= 8-600 kDa) [2, 63]. In addition, addition of Kraft lignin may increase chain-mobility at high temperature and high shear rate. Therefore, the presence of Kraft lignin in the mixture seemed to improve the flow properties substantially. The rheological properties of fish protein-based biomaterial strongly affected by the behavior of Kraft lignin and were expected to play an important role in any process developed to produce bioplastics from fish protein.

Table 4.3 Power-law model parameters of plasticized fish protein/ Kraft lignin blends

Sample	Consistency (Pa.s^n)	n
0% KL	145693^a±21442	0.17^a ±0.01
20% KL	105266^b± 4981	0.02^b ± 0.03
40% KL	47727^c± 3976	0.14^a ± 0.01
60% KL	28459^c± 428	0.03^b ± 0.00
70% KL	30235^c± 117	0.00^b ± 0.00

4.3 Thermomechanical properties

In order to investigate the effect of Kraft lignin as a filler on viscoelastic properties of fish protein material, fish protein materials with 0-70% Kraft lignin were determined by using Dynamic mechanical analysis (DMA).

Figure 4.3 shows storage modulus (E') and tan δ of plasticized fish protein/Kraft lignin materials. As a function of temperature, storage modulus decreased down to minimum values, showing a rubbery-like plateau region about 120ºC. For tan δ, it was clearly found that two peaks appeared for all of the fish protein-based bioplastics. The lower temperature peak was attributed to the glass transition temperature (T_g) of the free glycerol [36]. The higher temperature peak corresponded to the T_g of the plasticized materials.

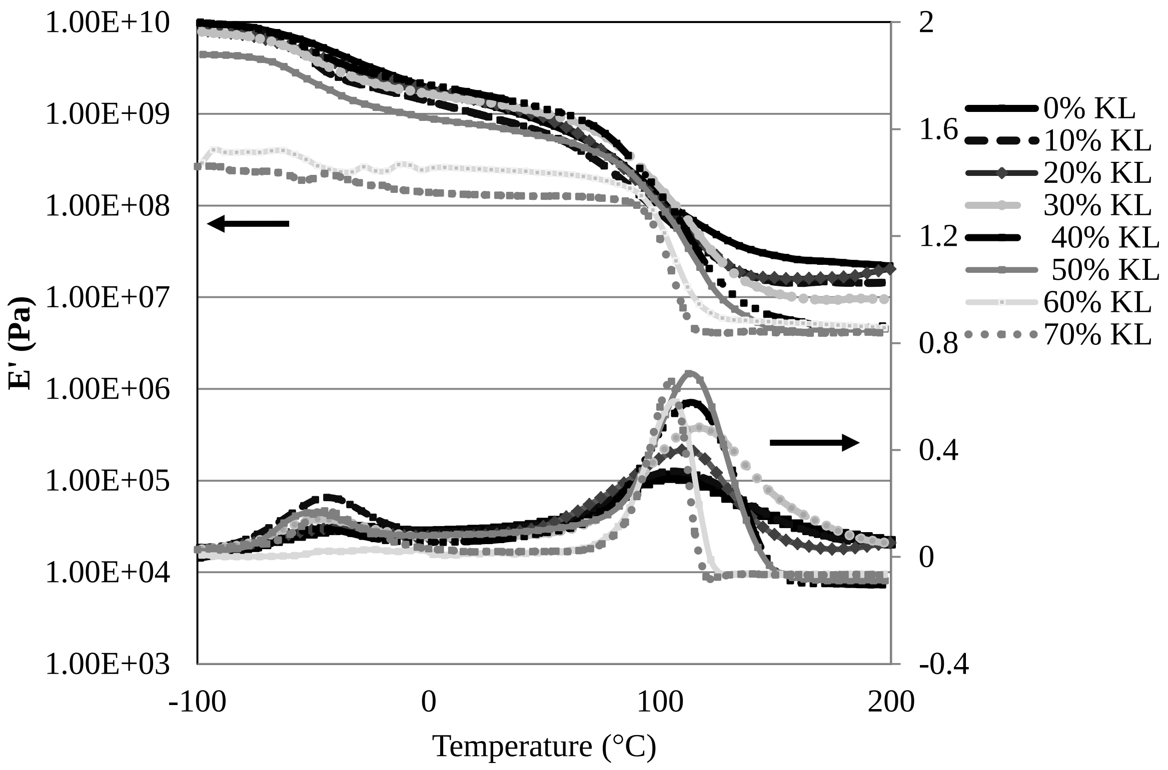


Figure 4.3 Storage modulus (E') and tan *δ* of plasticized fish protein/ Kraft lignin biomaterial

Table 4.4 shows the values of storage modulus E' in rubbery regions, tan *δ* peak height (E"/E') and the T_g which was determined by the temperature at maximum of tan *δ* peak height. Addition of Kraft lignin decreased storage modulus in rubbery state. Moreover, tan *δ* peak height increased with Kraft lignin content, suggesting an increase in liquidlike nature of materials. This may be associated with a low molecular weight of Kraft lignin and plasticization properties of Kraft lignin which has a number of hydroxyl groups [4]. The glycerol plasticized protein from liquidlike viscosity network structure. When the content of Kraft lignin increased, protein becomes more viscous and the resistance to small deformation decrease.

Processing of protein-based bioplastics on extrusion and injection molding is limited because protein blend has high viscosity due to protein aggregation during processing. Addition of Kraft lignin decrease viscosity of protein blend. Therefore, Kraft lignin is an alternative additive to enlarge the protein thermal processing window by decreasing the viscosity at high processing temperature.

As shown in Table 4.4, T_g of 0% Kraft lignin content is 105.5 ºC and T_g of 70% Kraft lignin content is 109.6 ºC. However, T_g of the material increased with Kraft lignin content increase and the maximum enhance was observed when Kraft lignin content reached to 30 wt%. Because, it may be associated with cross linking of plasticized fish protein/Kraft lignin biomaterial which might be due to the intra-molecular interactions of the hydroxyl and carboxyl groups in the fish protein and the methoxyl and carboxyl groups in Kraft lignin including hydrogen bonding and polar interactions [64, 65]. However, T_g of the materials are lower than pure fish protein and Kraft lignin because glycerol may have reduced the T_g by mechanisms other than those commonly associated with plasticization [66].

Table 4.4 Storage modulus (E') at 140^{o}C, glass transition temperature (T_g) and tan *δ* peak height of plasticized fish protein/ Kraft lignin biomaterial

KL (%)	**E' at 140°C (MPa)**	**Tan *δ* peak height (E''/E')**	**T_g (°C)**
0%	$29.7^{a}±4.1$	$0.32^{e}±0.03$	$105.5^{c}±1.9$
10%	$16.9^{b}±0.03$	$0.34^{e}±0.03$	$107.8^{abc}±2.4$
20%	$15.2^{bc}±2.1$	$0.43^{de}±0.05$	$113.1^{ab}±2.5$
30%	$10.7^{cd}±4.2$	$0.49^{cd}±0.01$	$115.1^{a}±2.7$
40%	$8.1^{de}±0.2$	$0.59^{bc}±0.01$	$113.0^{ab}±0.5$
50%	$7.1^{de}±1.9$	$0.64^{ab}±0.07$	$111.3^{abc}±2.9$
60%	$5.9^{de}±0.6$	$0.59^{bc}±0.01$	$106.6^{bc}±0.7$
70%	$3.4^{e}±1.2$	$0.74^{a}±0.09$	$109.6^{abc}±5.0$

4.4 FTIR spectra

The cross linking of plasticized fish protein/ Kraft lignin biomaterial were supported by the FTIR spectrum. FTIR was used to investigate functional group and the interaction between fish protein and Kraft lignin because addition of Kraft lignin increases T_g of materials until the content of lignin reached to 30 wt%, so new chemical bond may occur.

Figure 4.4 shows the FTIR spectra of fish protein materials with 0-70% lignin content. The characteristic bands of fish protein at 3399 cm^{-1} represented O-H stretching vibration called Amide III. The bending vibration of N-H group and stretching vibration of C-N group called Amide II. That is attributed to an out-of-phase combination of CN stretch and in-plane NH deformation modes of the peptide group and also showed the peak at 1649 cm^{-1} representing carbonyl group called Amide I. The absorption in amide I region is the most useful for infrared spectroscopic analysis of the secondary structure of proteins [67]. For the FTIR spectra of material with 70% Kraft lignin showed various structural units that the products contain hydroxyl groups, indicated by the broad O–H stretching absorption 3390 cm^{-1}, C–H stretching bands of aromatic at 2936 cm^{-1}, aldehyde group indicated by H–CO stretching band at 2882 cm^{-1}, carbonyl group indicated by C=O stretching band at 1598 cm^{-1} and aromatic stretching band at 1462 cm^{-1} in agreement with literature [11, 68].

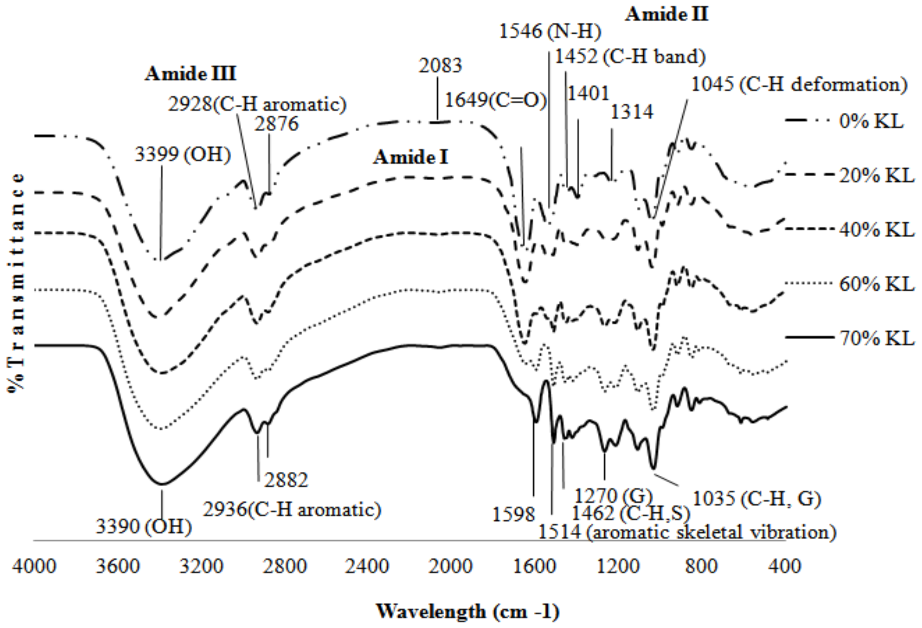


Figure 4.4 shows the FTIR spectra (top to bottom) of biomaterial with 0% Kraft lignin, 20% Kraft lignin, 40% Kraft lignin, 60% Kraft lignin and 70% Kraft lignin

However, cross linking of plasticized fish protein/Kraft lignin based biomaterial showed no new chemical bond between fish protein and Kraft lignin was observed. This suggests that these samples have the same main structure. In other words, this means that the biomaterial did not change the main structure of Kraft lignin and protein. This may be due to low cross-linking between fish protein and Kraft lignin [69].

The assignments of these intense peaks of lignin samples based on literature are summarized in Table 4.5

Table 4.5 IR peaks assignments for lignin [11, 59, 70]

Peak positions (cm^{-1})	**Peak assignments (Lignin)**
3420	O–H stretching
2960	C–H stretching
1590	C=C aromatic skeletal vibration
1510	C–C ring skeletal vibration
1460	C–H vibration
1420	C–O stretching
1393	Phenolic O-H- band
1271	aryl-O of aryl-OH and aryl-O-CH$_3$; guaiacyl ring (C=O group)
1182	C–O stretching
1102	Out of phase C-C-O stretch of phenol
1050	C–O stretching
969	C-CH and –HC=CH- deformation

Table 4.6 IR peaks assignments for protein [71, 72]

Type	***Peak positions (cm^{-1})***	***Peak assignments***
Amide I	1600-1614	Side-chain vibrations
	1616-1639	Intemolecular β-sheet
	1640-1650	Random coi1
	1651-1655	α-Helix (C=O stretching band C-N stretching and CCN deformation)
	1680-1688	Intermolecular β –sheet
Amide II	1550	NH bending and CN stretching C=O in-plane bending and NC stretching.
Amide III	1300	N-H bending and CN stretching CC stretching and CO bending

4.5 Protein aggregation of plasticized fish protein/ Kraft lignin biomaterial

4.5.1 Protein solubility

Solubility of protein biomaterial before and after processing is often regarded as a good indicator of cross-link formation during processing [4]. In order to study the effect of Kraft lignin on protein aggregation, changes in solubility of protein in SDS phosphate buffer were investigated. In this test, protein was dissolved in the SDS buffer. This protein fraction is called SDS-soluble protein. Then, DTE as a reducing agent was added to the remaining protein in order to cleavage disulfide bonds [66]. This SDS-insoluble protein was then dissolved in SDS-buffer. SDS-insoluble protein was a well established quantity, which reflects the covalent cross-linking degree of the protein network [31].

Protein content of SDS-soluble and SDS-insoluble fraction of Kraft lignin /fish protein molded at 100°C is shown in Table 4.7. After mixing and compression molding process, SDS soluble fraction of fish protein powder decreased dramatically from 64 to 18% compared to fish protein-based material with 0% Kraft lignin. This was attributed to heat treatment induced cross-linking during the molding process that led to an increase in covalent cross linking of protein by disulfide bonds, hydrogen bonds and hydrophobic interactions [4, 13, 73]. Total protein recovery was less than 100% in all samples. This may be explained by the original high protein of fish protein powder and the high processing temperature. The addition of Kraft lignin resulted in an increase of the SDS-soluble protein and total protein recovery compared to fish protein material without Kraft lignin. These results indicated a decrease of protein molecular weight of samples**,** suggesting the effect of Kraft lignin on protein aggregation.

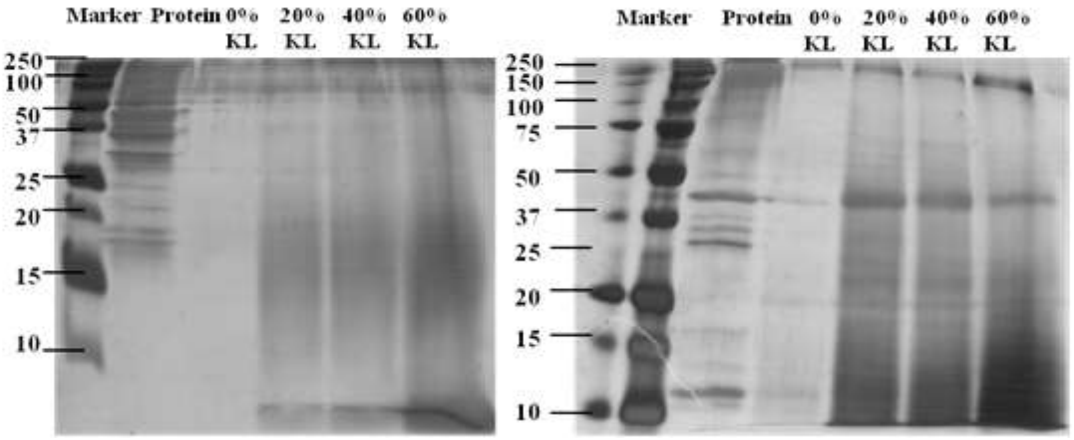
Table 4.7 Protein content (%) of SDS-soluble, SDS-insoluble fraction and total protein recovery of fish protein powder and plasticized fish protein/ Kraft lignin biomaterial molded at 100°C.

Sample	SDS-soluble protein fraction	SDS-insoluble protein fraction	Total protein recovery (%)
Fish protein	$64^{a}\pm8$	$24^{a}\pm4$	88
0% KL	$18^{c}\pm2$	$8^{b}\pm3$	26
20% KL	$21^{bc}\pm1$	$11^{b}\pm2$	33
40% KL	$23^{bc}\pm4$	$12^{b}\pm3$	35
60% KL	$28^{b}\pm5$	$9^{b}\pm3$	37

4.5.2 Protein molecular weight

As Kraft lignin is a free radical scavenger, it may interfere protein aggregation. The effect of Kraft lignin on protein aggregation was studied by measuring protein molecular weight of material using SDS-PAGE. 15% SDS-PAGE gel allows the separation of the majority of fish protein. The molecular weight of soluble fish protein and insoluble fish protein after molding with different Kraft lignin content were measured and showed in Figure 4.5.

Protein molecular weights of plasticized fish protein/ Kraft lignin molded at 100 °C were measured using 15% SDS-PAGE gel. Figure 4.5 (a) and (b) show respectively molecular weight of SDS-soluble and SDS-insoluble protein of plasticized fish protein/Kraft lignin materials. Molecular weight of fish protein powder ranges from 18 to 250 kDa in both SDS-soluble and insoluble. After compression molding, fish protein-based material presented only the low intensity band at 50-75 kDa for SDS-soluble and at 37-50 kDa for SDS-insoluble. Band of materials with 20-60% Kraft lignin contents presents the same trend as materials with 0% Kraft lignin. Therefore, no significant change of molecular weight of protein was observed in the studied molecular weight range (10-250 kDa) when Kraft lignin was added in the fish protein-based materials.



(a) **(b)**

Figure 4.5 Protein molecular weight of SDS-soluble (a) SDS-insoluble (b) of fish protein powder and fish protein biomaterial with 0-70% Kraft lignin contents.

4.6 Free radicals

As Kraft lignin is known as free radical scavenger, it may interfere the protein aggregation by capturing free radicals during processing fish protein -based material. Therefore, electron spin resonance (ESR) signals of plasticized fish protein/ Kraft lignin biomaterial were investigated at 77 K.

ESR spectra corresponded to relatively stable free radicals of plasticized fish protein/ Kraft lignin biomaterial after mixing at 80 °C and compression molding at 100 °C are shown in Figure 4.6 (a) and (b), respectively. Plasticized fish protein/ Kraft lignin biomaterial after mixing presented ESR spectra clearly similar to that of after compression molding. The signal intensity of Fish protein-based biomaterial (0% Kraft lignin) indicated the nitrogen or nitroxyl radicals ($NO^{\cdot}$) at g = 2.00 [74, 75]. However, sulfur centered radicals such as thiyl and disulfide radicals were either absent or their signal was hidden in the nitrogen or nitroxyl signal. For Kraft lignin-based material (70% Kraft lignin), high intensity is observed at g = 2.00 corresponded aromatic radicals [76]. Therefore, ESR results indicated that no radical scavenging effect between Kraft lignin and fish protein during mixing and molding process was observed.

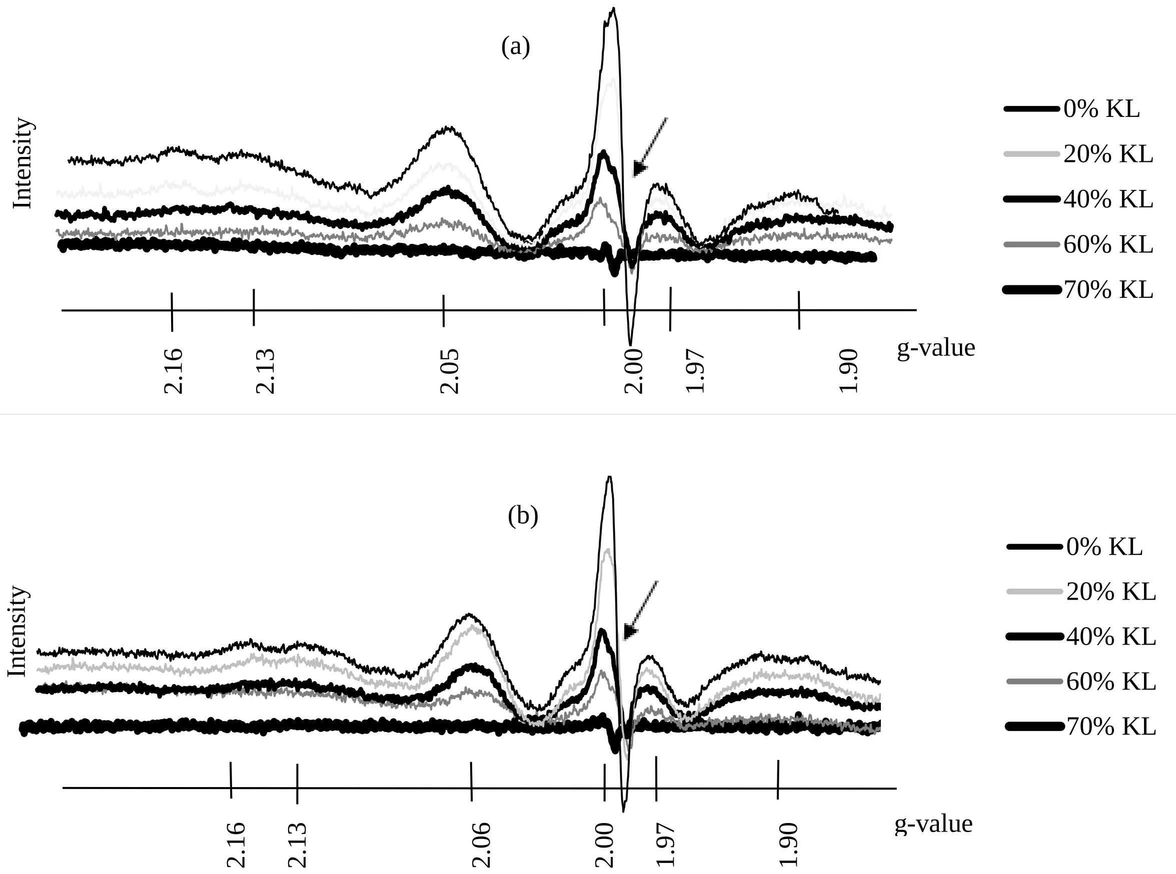


Figure 4.6 Free radicals of plasticized fish protein/ Kraft lignin biomaterial after mixing at 80°C (a) and molding at 100°C (b)

4.7 Mechanical properties of plasticized fish protein/Kraft lignin biomaterial

The functional properties of Kraft lignin-filled material are generally affected by the properties of their components. The mechanical properties of polymers blend was largely associated with distribution and concentration of inter- molecular interactions and intra-molecular interactions allowed by the sequence of the polypeptide chain [4]. Therefore, mechanical properties of Kraft lignin as a filler on fish protein material were investigated.

The effect of Kraft lignin on mechanical properties was investigated and shown in Figure 4.7. This showed that the elongation at break of the materials decreases with an increasing Kraft lignin content. Young's modulus and tensile strength of the materials increase when Kraft lignin content increased. The maximum enhancement is observed when the content of lignin reached to 30 wt% because it has mainly protein component in material. Because, protein is consists of polar and non-polar side chains. There are strong intra- molecular interactions and inter-molecular interactions. Therefore, the strong polar interactions between side chains of protein molecules restrict segment rotation and molecular mobility, which lead to increase in modulus, and tensile strength. However, tensile strength of materials decreased at more than 30 % Kraft lignin may be due to the effect of natural properties of Kraft lignin which is brittleness [38]. The effect of Kraft lignin on mechanical properties can shown data in Table 4.8. The results revealed that blend ratio of 40:30:30 (fish protein: Kraft lignin: glycerol) showed maximum value in Young's modulus and tensile strength. It was also concluded that polymer blends of this ratio had a high stress resistance and elasticity.

Mechanical properties of plasticized fish protein/ Kraft lignin biomaterial were low when compared to other protein in literatures. Sun et al., 2008 reported that Thermo-molded at 105ºC of wheat gluten plastics plasticized with glycerol presented the tensile strength of 3.03 MPa, elongation at break of 288.5 % and Young's modulus of 20.0 MPa. In addition, Zhang et al., 2000 studied mechanical properties of extruded soy protein sheets plasticized with glycerol that showed, tensile strength of 15.6 MPa, and 133% elongation at break of 112% and Young's modulus of 374 MPa.

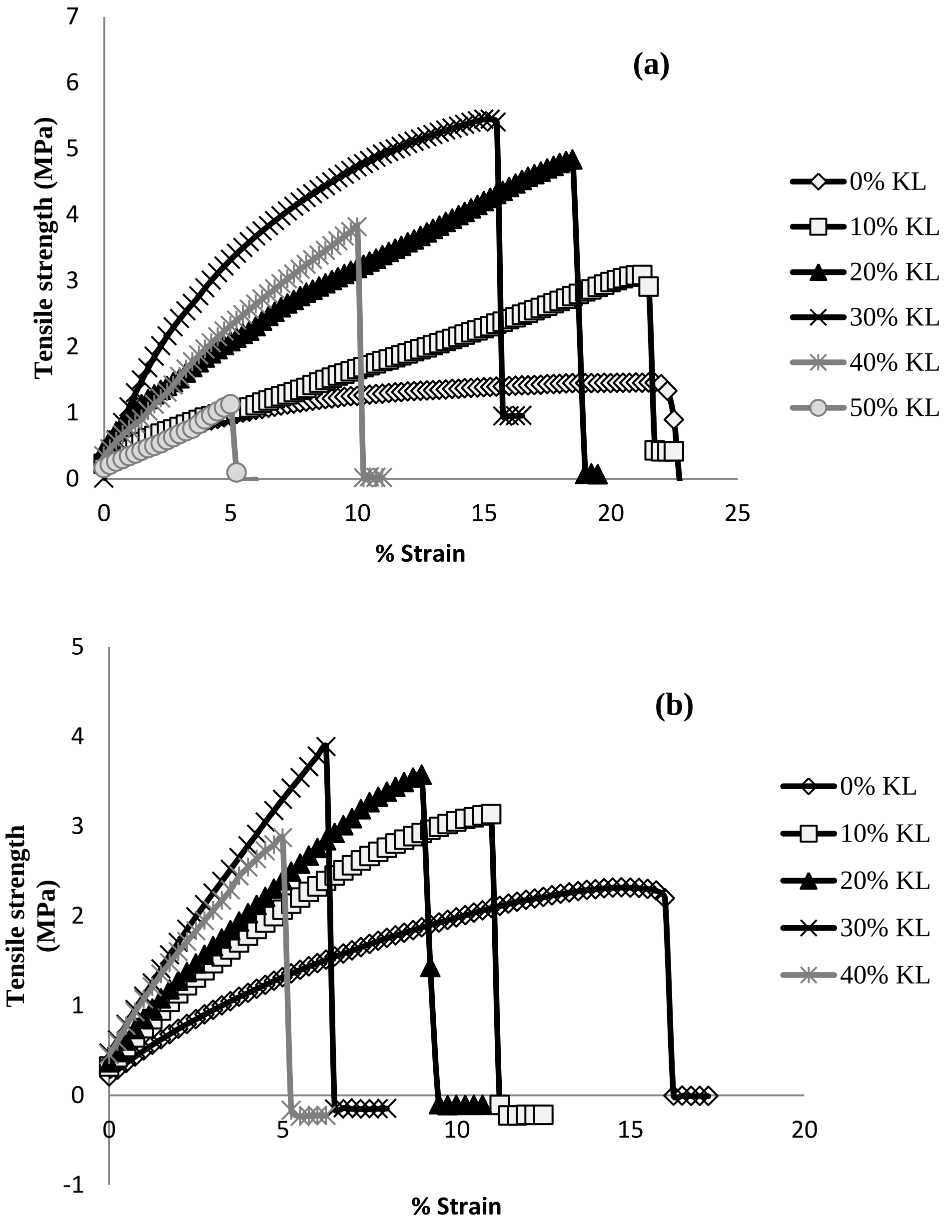


Figure 4.7 Mechanical properties of plasticized fish protein/ Kraft lignin biomaterial molded at 100 ºC (a) and 130ºC (b)

The effect of molding temperature on mechanical properties of plasticized fish protein/Kraft lignin biomaterial at molded 100 ºC and 130ºC was investigated and shown in Table 4.8. The tensile strength and Young's modulus of material increased as molding temperature increased because at high temperature induced cross-linking between protein and Kraft lignin result in protein aggregation as high strength.

Table 4.8 Mechanical properties of plasticized fish protein/ Kraft lignin biomaterial molded at 100ºC and 130ºC

Kraft lignin (%)	**Molded at 100ºC**			**Molded at 130ºC**		
	Young's modulus (MPa)	**Tensile strength (MPa)**	**Elongation at break (%)**	**Young's modulus (MPa)**	**Tensile strength (MPa)**	**Elongation at break (%)**
0%	$26.5^{cd}\pm8.0$	$1.6^{c}\pm0.6$	$22.5^{a}\pm9.3$	$27.9^{c}\pm2.6$	$1.8^{d}\pm0.5$	$10.9^{a}\pm3.1$
10%	$29.3^{c}\pm4.5$	$3.0^{b}\pm0.4$	$21.3^{ab}\pm5.6$	$40.2^{b}\pm2.6$	$3.3^{c}\pm0.3$	$10.9^{a}\pm0.5$
20%	$39.1^{ab}\pm7.8$	$4.4^{a}\pm0.6$	$20.9^{ab}\pm4.2$	$66.1^{a}\pm14.8$	$4.4^{b}\pm0.8$	$8.6^{ab}\pm2.0$
30%	$42.2^{a}\pm4.4$	$4.9^{a}\pm1.0$	$19.5^{ab}\pm4.1$	$67.1^{a}\pm5.8$	$5.3^{a}\pm0.3$	$8.5^{ab}\pm2.0$
40%	$30.2^{bc}\pm6.0$	$3.6^{b}\pm0.2$	$12.2^{bc}\pm2.0$	$64.6^{a}\pm3.7$	$3.7^{bc}\pm0.7$	$6.5^{b}\pm1.7$
50%	$18.3^{d}\pm0.5$	$1.2^{c}\pm0.1$	$6.1^{c}\pm1.3$	$5.8^{d}\pm8.5$	$0.2^{e}\pm0.1$	$0.9^{c}\pm0.8$

4.8 Water absorption of plasticized fish protein/Kraft lignin biomaterial

Water absorption of plasticized fish protein/ Kraft lignin biomaterial after immersion in water containing 0.05% NaN_3 to avoid the microbial growth for 1 week was determined. Figure 4.8 shows water absorption of fish protein-based bioplastics with 0-70% Kraft lignin content. Addition of Kraft lignin content decreased water absorption of the plasticized fish protein/ Kraft lignin biomaterial because Kraft lignin is relatively hydrophobic and aromatic in nature [11, 43, 77]. Therefore, the high lignin content limits water absorption of biomaterial. Meanwhile, studying the effect of alkaline lignin on the water absorption of the soy protein plastics showed as same trend by Huang et al. [15]. Tests of water absorption proved that the introduction of hydrophobic alkaline lignin effectively reduced water absorption with an increase of alkaline lignin content from 240 to 85%.

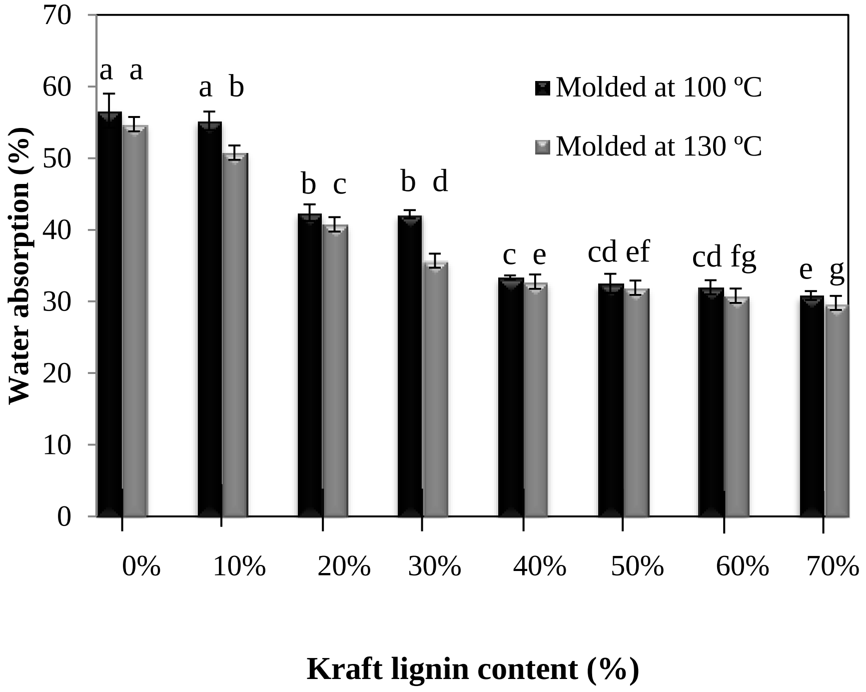


Figure 4.8 Water absorption of fish protein-based biomaterial with 0-70% Kraft lignin content at mold 100ºC and 130 ºC

The effect of molding temperature on water absorption of plasticized fish protein/ Kraft lignin biomaterial with difference Kraft lignin content at molded 100ºC and 130 ºC was investigated and shown in Figure 4.8. Water absorption decreased as molding temperature increasing. Since, when exposed to higher temperatures, more protein molecules unfold resulting in more entanglement (aggregation) and packing tighter during the molding process, which could inhibit water absorption. Water absorption results suggest that the cross-link of the polymer is increased by the presence of the molding temperature.

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The effect of Kraft lignin on rheological and functional properties of fish protein-based biomaterial was studied. Fish protein powder plasticized with 30% glycerol was blended with 0-70% Kraft lignin and thermal molded. Then, properties of plasticized fish protein/ Kraft lignin biomaterial were determined.

Viscosity of plasticized fish protein/ Kraft lignin blends with 0-70% Kraft lignin was determined by capillary rheometer. The addition of Kraft lignin resulted in a decrease of shear viscosity. In addition, storage modulus in rubbery state of biomaterials determined by DMA showed the same the trend as the shear viscosity. The addition of Kraft lignin decreased the storage modulus in rubbery state at processing temperature. Moreover, tan δ peak height increased with Kraft lignin content which showed liquidlike nature of biomaterial. This might be because Kraft lignin has low molecular weight compared to protein or Kraft lignin interfered protein aggregation. The addition of Kraft lignin increased T_g of materials until the content of lignin reached to 30 wt%. It may be associated with cross linking of plasticized fish protein/ Kraft lignin biomaterial. However, FTIR spectra showed no new chemical bond between fish protein and Kraft lignin.

The effect of radical scavenging properties of Kraft lignin on protein aggregation was investigated. Addition of Kraft lignin increased protein solubility in SDS. This might be because Kraft lignin interfered protein aggregation. However, the no changes in protein molecular weight in studied range investigated by SDS-PAGE was observed. In addition, free radical scavenging effect between fish protein and Kraft lignin measured by ESR spectroscopy was not observed.

For functional properties, the addition of Kraft lignin in range of 10-40% Kraft lignin can improve mechanical properties of biomaterial. When Kraft lignin was the major component in biomaterial (50-70% Kraft lignin) Young’s modulus and tensile strength of biomaterial decreased because of hard brittle property of Kraft lignin. Moreover, the hydrophobic nature of Kraft lignin effectively reduced the water absorption of the biomaterial.

From this study, it may be concluded that Kraft lignin is an alternative to enlarge the protein thermal processing window. Kraft lignin not only improved the rheological properties of protein by decreasing the viscosity at high processing temperature, but also improved functional properties in terms of mechanical properties and water absorption of fish protein materials. These fish protein-based biomaterial may be used as packaging materials (sheet, container, flower pot) to substitute petroleum-based materials.

5.2 Recommendation

Plasticized fish protein/ Kraft lignin biomaterial prepared by the extrusion or injection should be studied in order to confirm the possibility of Kraft lignin on the processability improvement of protein-based materials.

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APPENDIX A

Proximate analysis

Table A.1 Protein content in fish protein powder

Sample	Replication	*Protein (%)
Protein powder	1	85.2019
Protein powder	2	84.4076
Protein powder	3	85.1792

*N× 6.25

Table A.2 Moisture content in fish protein powder

Sample	Replication	Moisture (%wb)	Moisture (%db)
Protein powder	1	6.4801	6.9292
Protein powder	2	6.5316	6.9881
Protein powder	3	6.2478	6.6642

Table A.3 Ash in fish protein powder

Sample	Replication	Ash (%)
Protein powder	1	2.7700
Protein powder	2	2.7792
Protein powder	3	2.7917

Table A.4 Fat content in fish protein powder

Sample	Replication	Fat (%)
Protein powder	1	4.2510
Protein powder	2	4.2329
Protein powder	3	4.3665

Table A.5 sieve analysis of fish protein powder for preparation sample

Maximum size present in substantial proportions(μm)	Minimum weight of sample dispatched for testing(g)	% of sample dispatched for testing
1000	0.1	0.20
710	0.09	0.18
500	0.16	0.32
425	0.66	1.32
300	4.18	8.35
180	9.83	19.65
75	15.02	30.02
<75	19.99	39.96

APPENDIX B

Properties of fish protein and Kraft Lignin

Table B.1 TGA of fish protein and Kraft lignin which test temperature from the room temperature (30°C) to 600 °C and the heating rate was 10 °C/min.

Temperature	Fish protein		Kraft lignin	
	% weight loss	DTG (mg/min)	% weight loss	DTG (mg/min)
30	99.98	1.15E-05	99.95	-1.87E-04
40	99.93	6.13E-05	99.63	-3.65E-04
50	99.79	-2.59E-04	99.12	-6.79E-04
60	99.54	-2.80E-04	98.49	-5.95E-04
70	99.20	-3.81E-04	97.95	-4.98E-04
80	98.77	-4.77E-04	97.49	-3.43E-04
90	98.25	-5.62E-04	97.24	-1.63E-04
100	97.68	-5.55E-04	97.09	-2.32E-04
110	97.17	-4.48E-04	96.90	-1.19E-04
120	96.77	-3.37E-04	96.75	-1.42E-04
130	96.48	-2.49E-04	96.60	-1.66E-04
140	96.27	-1.81E-04	96.37	-2.56E-04
150	96.11	-1.66E-04	96.12	-3.00E-04
160	95.94	-1.68E-04	95.82	-2.98E-04
170	95.79	-1.44E-04	95.48	-3.14E-04
180	95.61	-2.32E-04	95.17	-4.13E-04
190	95.33	-3.84E-04	94.74	-4.50E-04
200	94.89	-5.90E-04	94.20	-6.75E-04
210	94.24	-6.49E-04	93.55	-5.12E-04
220	93.47	-8.57E-04	92.95	-7.72E-04
230	92.58	-8.69E-04	92.22	-7.11E-04
240	91.61	-1.03E-03	91.45	-8.31E-04
250	90.48	-1.26E-03	90.61	-8.72E-04
260	89.05	-1.65E-03	89.64	-1.11E-03
270	87.28	-1.92E-03	88.50	-1.10E-03
280	85.21	-2.30E-03	87.38	-1.15E-03
290	82.68	-2.84E-03	86.24	-1.21E-03
300	79.48	-3.60E-03	85.00	-1.28E-03
310	75.56	-4.14E-03	83.63	-1.41E-03
320	70.95	-5.28E-03	82.15	-1.56E-03
330	65.17	-5.91E-03	80.54	-1.76E-03
340	59.64	-5.21E-03	78.61	-2.16E-03
350	54.92	-4.32E-03	76.30	-2.36E-03
360	50.88	-3.92E-03	73.81	-2.62E-03
370	47.16	-3.51E-03	71.14	-2.68E-03
380	43.95	-2.99E-03	68.55	-2.49E-03
390	41.24	-2.53E-03	66.27	-2.13E-03
400	38.88	-2.16E-03	64.24	-1.82E-03

Table B.1 TGA of fish protein and Kraft lignin which test temperature from the room temperature (30°C) to 600 °C and the heating rate was 10 °C/min (Continues)

	Fish protein		**Kraft lignin**	
Temperature	**% weight loss**	**DTG (mg/min)**	**% weight loss**	**DTG (mg/min)**
410	36.99	-1.70E-03	62.67	-1.41E-03
420	35.48	-1.35E-03	61.44	-1.14E-03
430	34.20	-1.29E-03	60.33	-1.20E-03
440	33.00	-1.08E-03	59.11	-1.10E-03
450	31.98	-9.36E-04	58.03	-1.00E-03
460	31.12	-7.79E-04	57.10	-9.11E-04
470	30.41	-6.57E-04	56.22	-8.61E-04
480	29.82	-5.54E-04	55.43	-8.22E-04
490	29.30	-4.57E-04	54.60	-8.27E-04
500	28.89	-3.46E-04	53.82	-6.97E-04
510	28.54	-3.54E-04	53.06	-8.02E-04
520	28.19	-3.08E-04	52.24	-7.72E-04
530	27.89	-2.94E-04	51.50	-7.29E-04
540	27.65	-1.67E-04	50.87	-6.34E-04
550	27.40	-2.65E-04	50.16	-7.50E-04
560	27.18	-1.90E-04	49.53	-5.70E-04
570	26.92	-2.29E-04	48.78	-6.28E-04
580	26.71	-2.31E-04	48.16	-6.45E-04
590	26.53	-1.93E-04	47.62	-5.62E-04
600	26.31	-2.43E-04	47.00	-7.54E-04

Table B.2 DSC of fish protein was heated first time at 10 ºC/min from 25 to 250 ºC and heated a second time at 10 ºC/min to 250 ºC. Finally the sample was cooled to 25 ºC at 10 ºC/min.

	Replication 1		**Replication 2**	
Temperature(ºC)	**Heating (W/g)**	**Reheating (W/g)**	**Heating (W/g)**	**Reheating (W/g)**
25.83	0.10	-0.05	0.11	-0.04
30.50	0.20	0.13	0.18	0.13
35.17	0.21	0.14	0.20	0.15
39.83	0.22	0.14	0.20	0.14
44.50	0.23	0.14	0.21	0.14
49.17	0.23	0.13	0.21	0.13
53.83	0.23	0.13	0.21	0.13

Table B.2 DSC of fish protein was heated first time at 10 ºC/min from 25 to 250 ºC and heated a second time at 10 ºC/min to 250 ºC. Finally the sample was cooled to 25 ºC at 10 ºC/min (Continues)

Temperature(ºC)	**Replication 1**		**Replication 2**	
	Heating (W/g)	**Reheating (W/g)**	**Heating (W/g)**	**Reheating (W/g)**
58.50	0.24	0.13	0.22	0.13
63.17	0.25	0.13	0.23	0.13
67.83	0.26	0.13	0.24	0.13
72.50	0.28	0.13	0.26	0.12
77.17	0.30	0.13	0.29	0.12
81.83	0.32	0.14	0.31	0.12
86.50	0.34	0.14	0.33	0.12
91.17	0.36	0.14	0.35	0.12
95.83	0.39	0.14	0.39	0.12
100.50	0.42	0.14	0.41	0.12
105.17	0.45	0.14	0.44	0.12
109.83	0.47	0.14	0.46	0.12
111.00	0.47	0.14	0.46	0.13
115.67	0.49	0.15	0.48	0.13
120.33	0.50	0.15	0.47	0.12
125.00	0.49	0.15	0.45	0.13
129.67	0.48	0.15	0.44	0.13
134.33	0.46	0.15	0.41	0.13
139.00	0.44	0.16	0.39	0.13
143.67	0.41	0.16	0.36	0.13
148.33	0.39	0.16	0.33	0.13
153.00	0.36	0.16	0.30	0.13
157.67	0.33	0.16	0.27	0.13
162.33	0.31	0.17	0.25	0.13
167.00	0.29	0.17	0.24	0.14
171.67	0.27	0.18	0.22	0.14
176.33	0.26	0.19	0.21	0.15
181.00	0.26	0.20	0.21	0.16
185.67	0.27	0.21	0.22	0.16
190.33	0.28	0.21	0.25	0.17
195.00	0.31	0.22	0.27	0.17
199.67	0.36	0.22	0.31	0.18
204.33	0.40	0.22	0.36	0.18
209.00	0.43	0.23	0.38	0.18
213.67	0.45	0.23	0.40	0.18
218.33	0.47	0.24	0.42	0.18
223.00	0.47	0.24	0.42	0.19

Table B.2 DSC of fish protein was heated first time at 10 ºC/min from 25 to 250 ºC and heated a second time at 10 ºC/min to 250 ºC. Finally the sample was cooled to 25 ºC at 10 ºC/min (Continues)

Temperature(ºC)	Replication 1		Replication 2	
	Heating (W/g)	Reheating (W/g)	Heating (W/g)	Reheating (W/g)
227.67	0.47	0.25	0.41	0.20
232.33	0.46	0.26	0.39	0.21
237.00	0.45	0.28	0.38	0.22
241.67	0.45	0.29	0.39	0.24
246.33	0.47	0.32	0.41	0.26
247.50	0.48	0.32	0.42	0.27

Table B.3 DSC of Kraft lignin was heated first time at 10 ºC/min from 25 to 250 ºC and heated a second time at 10 ºC/min to 250 ºC. Finally the sample was cooled to 25 ºC at 10 ºC/min (Continues)

Temperature(ºC)	Replication 1		Replication 2	
	Heating (W/g)	Reheating (W/g)	Heating (W/g)	Reheating (W/g)
25.83	0.11	0.00	0.08	0.01
30.50	0.19	0.13	0.19	0.13
35.17	0.20	0.14	0.20	0.15
39.83	0.20	0.13	0.20	0.15
44.50	0.21	0.13	0.21	0.14
49.17	0.21	0.13	0.21	0.14
53.83	0.22	0.13	0.22	0.14
58.50	0.24	0.13	0.23	0.14
63.17	0.25	0.13	0.25	0.14
67.83	0.27	0.13	0.26	0.14
72.50	0.28	0.14	0.28	0.14
77.17	0.31	0.14	0.30	0.14
81.83	0.33	0.14	0.33	0.14
86.50	0.36	0.14	0.36	0.14
91.17	0.39	0.14	0.38	0.14
95.83	0.41	0.14	0.40	0.14
100.50	0.42	0.14	0.41	0.14
105.17	0.42	0.14	0.40	0.14
109.83	0.40	0.14	0.39	0.14
111.00	0.40	0.14	0.38	0.14
115.67	0.37	0.14	0.35	0.14

Table B.3 DSC of Kraft lignin was heated first time at 10 ºC/min from 25 to 250 ºC and heated a second time at 10 ºC/min to 250 ºC. Finally the sample was cooled to 25 ºC at 10 ºC/min (Continues)

Temperature(ºC)	Replication 1		Replication 2	
	Heating (W/g)	Reheating (W/g)	Heating (W/g)	Reheating (W/g)
120.33	0.34	0.14	0.32	0.14
125.00	0.31	0.15	0.29	0.14
129.67	0.28	0.15	0.27	0.14
134.33	0.26	0.15	0.25	0.14
139.00	0.25	0.15	0.24	0.14
143.67	0.25	0.15	0.24	0.14
148.33	0.26	0.16	0.25	0.14
153.00	0.26	0.16	0.25	0.15
157.67	0.27	0.17	0.26	0.15
162.33	0.27	0.17	0.26	0.16
167.00	0.26	0.18	0.25	0.16
171.67	0.26	0.18	0.25	0.17
176.33	0.25	0.19	0.24	0.17
181.00	0.24	0.19	0.23	0.18
185.67	0.23	0.20	0.22	0.18
190.33	0.22	0.20	0.21	0.18
195.00	0.21	0.20	0.20	0.18
199.67	0.20	0.20	0.19	0.18
204.33	0.19	0.20	0.19	0.18
209.00	0.19	0.20	0.19	0.18
213.67	0.18	0.20	0.18	0.18
218.33	0.19	0.20	0.18	0.20
223.00	0.19	0.19	0.18	0.18
227.67	0.19	0.19	0.18	0.17
232.33	0.18	0.19	0.17	0.17
237.00	0.18	0.19	0.15	0.17
241.67	0.16	0.18	0.14	0.17
246.33	0.15	0.18	0.13	0.16
247.50	0.14	0.17	0.12	0.16

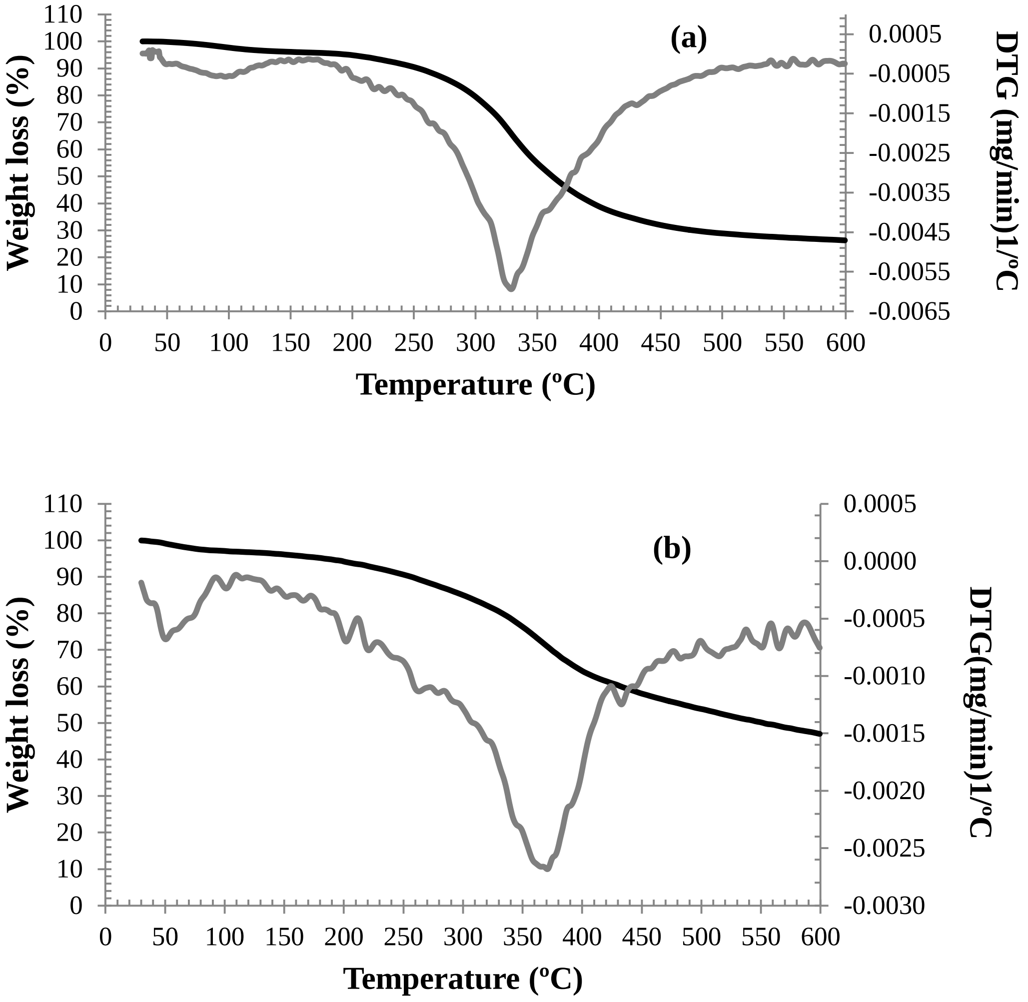

Figure B.1 (—) TG and (—) derivative TG (DTG) curves of Fish protein (a) and Kraft lignin (b) measured at 10°C /min in nitrogen

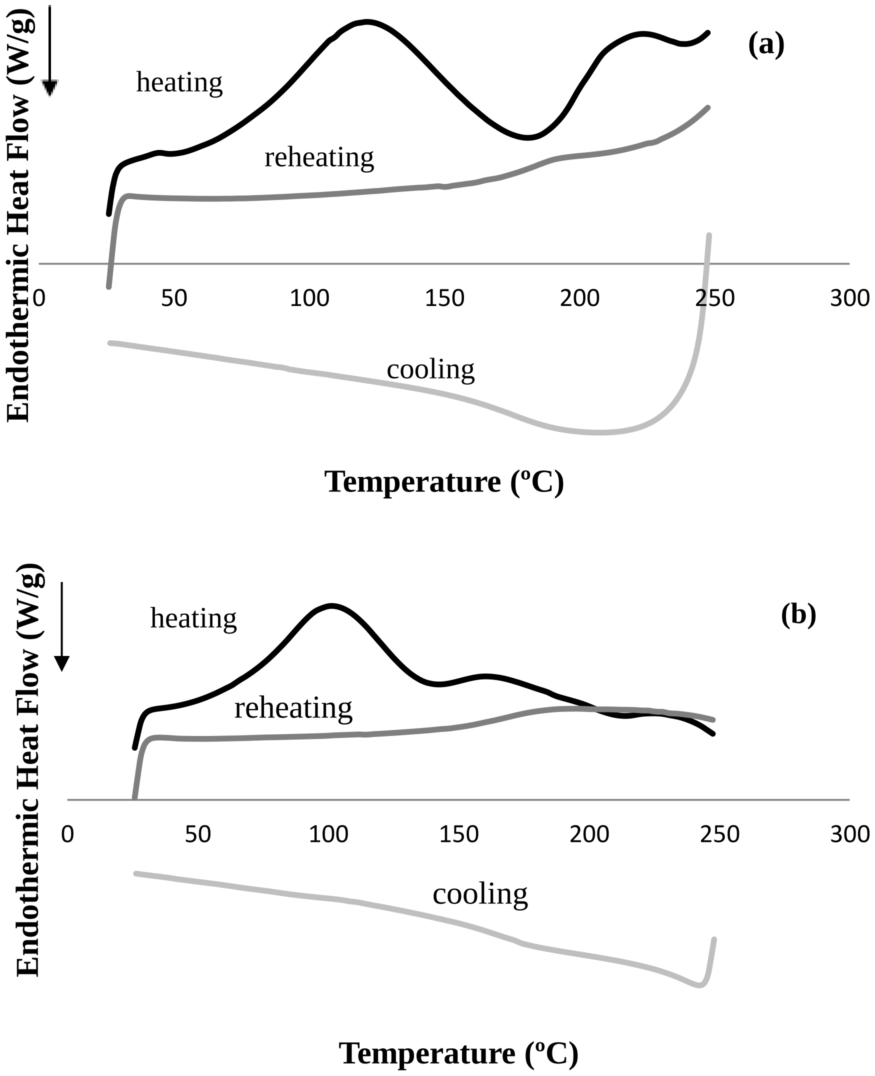

Figure B.2 DSC scan of Fish protein (a), Kraft lignin (b) of biomaterial at 0% RH

APPENDIX C

Rheological Properties of Plasticized fish protein/Kraft Lignin Blend

Table C.1 Shear rate and appearance viscosity of plasticized fish protein/ Kraft lignin mixed blend and die temperature is 140 °C

Sample	Replication	Shear rate (1/s)	Shear viscosity (Pa.s)
0% KL	1	9.9936	23987
		49.997	6114
		99.994	3568.4
		500	926.21
		999.99	23987
	2	9.9936	20986
		49.997	4564.7
		99.994	2307.3
		500	797.27
		999.99	
20% KL	1	9.9936	11485
		49.997	2435.6
		99.994	1272.8
		500	267.2
		999.99	145.76
	2	9.9936	10902
		49.997	2189
		99.994	1092.8
		500	218.89
		999.99	
40% KL	1	9.9936	6751
		49.997	2085.8
		99.994	941.26
		500	218.89
		999.99	153.09
	2	9.9936	6534.2
		49.997	1645.9
		99.994	671.38
		500	205.9
		999.99	125.6

Table C.1 Shear rate and appearance viscosity of plasticized fish protein/ Kraft lignin mixed blend and die temperature is 140 ^{o}C (Continues)

Sample	**Replication**	**Shear rate (1/s)**	**Shear viscosity (Pa.s)**
60% KL	1	9.9936	3150.5
		49.997	629.74
		99.994	316.54
		500	66.3
		999.99	36.149
	2	9.9936	3067.1
		49.997	613.07
		99.994	306.54
		500	61.637
		999.99	35.649
70% KL	1	9.9936	3033.8
		49.997	606.41
		99.994	303.2
		500	60.637
		999.99	30.319
	2	9.9936	3017.1
		49.997	603.07
		99.994	301.54
		500	60.304
		999.99	30.152

APPENDIX D

Plasticized fish protein/Kraft Lignin Biomaterial Experiment Data

TableD.1 Dynamic mechanical analysis of 0% KL

Replication 1				Replication 2			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-100.5711	407.9459	10704.7931	0.0248	-97.3281	229.5245	6150.4178	0.0311
-95.5711	181.4832	9784.7381	0.0217	-92.3281	194.9366	5896.2823	0.0346
-90.5711	152.7085	9546.0147	0.0219	-87.3281	210.7801	5785.8813	0.0391
-85.5711	232.5351	9257.8490	0.0258	-82.3281	268.3915	5651.8771	0.0450
-80.5711	320.1492	8991.9235	0.0324	-77.3281	295.1092	5455.9933	0.0520
-75.5711	304.0294	8722.5805	0.0410	-72.3281	292.5711	5169.8154	0.0604
-70.5711	438.3701	8141.2384	0.0517	-67.3281	311.6215	4844.9788	0.0718
-65.5711	516.8680	7639.3604	0.0629	-62.3281	390.5231	4527.1866	0.0860
-60.5711	496.3176	7064.6471	0.0723	-57.3281	440.5451	4212.5139	0.0988
-55.5711	526.7814	6513.8580	0.0818	-52.3281	439.7339	3894.5740	0.1080
-50.5711	534.8727	5897.2287	0.0905	-47.3281	401.7409	3556.8531	0.1134
-45.5711	510.4629	5254.2321	0.0981	-42.3281	368.8408	3185.6327	0.1159
-40.5711	481.5853	4666.2130	0.1035	-37.3281	330.5680	2822.8159	0.1147
-35.5711	457.1857	4137.8303	0.1067	-32.3281	286.4351	2541.4131	0.1104
-30.5711	399.9154	3643.8498	0.1064	-27.3281	239.4494	2301.2523	0.1042
-25.5711	332.7921	3248.8627	0.1033	-22.3281	197.5509	2073.3035	0.0978
-20.5711	285.4162	2929.5557	0.0990	-17.3281	167.1647	1878.9314	0.0928
-15.5711	245.2736	2620.4050	0.0947	-12.3281	150.0901	1703.6899	0.0906
-10.5711	214.0372	2365.8330	0.0915	-7.3281	142.9408	1549.5392	0.0904
-5.5711	188.7284	2135.7659	0.0898	-2.3281	135.4391	1447.2237	0.0910
-0.5711	173.5306	1934.1528	0.0896	2.6720	122.2449	1315.1380	0.0922
4.4289	162.0116	1769.6717	0.0904	7.6720	112.1190	1209.1387	0.0937
9.4289	149.9451	1637.1570	0.0916	12.6720	106.2732	1118.0062	0.0961
14.4289	141.1743	1511.7149	0.0931	17.6720	102.7132	1030.0615	0.0992
19.4289	132.5779	1390.5799	0.0951	22.6720	98.1146	943.7028	0.1030
24.4289	125.3597	1282.0020	0.0973	27.6720	93.0046	863.2882	0.1074
29.4289	117.7422	1180.9270	0.1001	32.6720	87.9383	783.4686	0.1127
34.4289	112.4729	1092.4680	0.1034	37.6720	84.0919	713.3656	0.1186
39.4289	108.3397	999.4241	0.1082	42.6720	80.5815	644.8761	0.1259
44.4289	103.3450	912.8543	0.1137	47.6720	77.6289	578.5179	0.1340
49.4289	99.8927	816.0746	0.1214	52.6720	76.0457	524.7561	0.1431
54.4289	95.7209	733.3632	0.1295	57.6720	74.5942	481.4929	0.1534
59.4289	90.4078	649.5503	0.1388	62.6720	72.8519	434.3378	0.1670
64.4289	85.7180	568.7202	0.1507	67.6720	70.6165	391.4052	0.1824
69.4289	80.1376	490.1195	0.1657	72.6720	67.6373	342.1990	0.2012
74.4289	74.7848	413.4467	0.1846	77.6720	64.6053	293.3266	0.2236
79.4289	69.3779	340.4069	0.2071	82.6720	60.2580	242.7431	0.2503
84.4289	63.0770	273.2602	0.2323	87.6720	54.7051	197.0709	0.2779
89.4289	55.8697	215.5729	0.2576	92.6720	48.1818	158.0107	0.3035
94.4289	47.9345	170.4404	0.2788	97.6720	41.1717	126.2042	0.3237
99.4289	40.0199	134.8352	0.2935	102.6720	33.6661	99.1419	0.3367
104.4289	30.6993	101.7945	0.2995	107.6720	27.0011	78.5467	0.3402

TableD.1 Dynamic mechanical analysis of 0% KL (Continues)

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
109.4289	24.4645	82.4362	0.2955	112.6720	21.1224	62.6158	0.3343
114.4289	19.7040	68.3759	0.2857	117.6720	16.2778	50.6300	0.3197
119.4289	15.4642	57.1959	0.2698	122.6720	12.5256	41.7897	0.2983
124.4289	11.9111	48.3163	0.2475	127.6720	9.6709	35.4736	0.2728
129.4289	9.3332	41.5229	0.2243	132.6720	7.5996	31.1036	0.2462
134.4289	7.2380	36.4254	0.2006	137.6720	6.0875	27.9857	0.2205
139.4289	5.7718	32.9293	0.1785	142.6720	4.9956	25.6816	0.1968
144.4289	4.7703	30.4344	0.1603	147.6720	4.1817	24.0641	0.1766
149.4289	4.1361	28.6372	0.1448	152.6720	3.6644	23.0386	0.1609
154.4289	3.5972	27.0847	0.1300	157.6720	3.3325	22.3771	0.1485
159.4289	3.0440	25.7868	0.1158	162.6720	3.0846	21.8547	0.1385
164.4289	2.5980	25.1746	0.1034	167.6720	2.7951	21.5987	0.1296
169.4289	2.1994	24.8220	0.0932	172.6720	2.5964	21.5409	0.1219
174.4289	2.0501	24.4045	0.0856	177.6720	2.5168	21.5794	0.1156
179.4289	1.9600	23.8404	0.0801	182.6720	2.3856	21.7810	0.1093
184.4289	1.7444	23.2489	0.0744	187.6720	2.2855	22.2257	0.1031
189.4289	1.5966	22.8945	0.0685	192.6720	2.2375	22.7081	0.0967
194.4289	1.3853	22.5202	0.0613	197.6720	2.1100	23.2983	0.0896
199.4289	1.2290	21.8687	0.0533	202.6720	1.8592	23.9200	0.0823
204.4289	0.9453	21.2253	0.0448	207.6720	1.7540	23.9205	0.0757
209.4289	0.6285	19.7136	0.0361	212.6720	1.6595	23.1438	0.0688
214.4289	0.4654	17.0273	0.0276	217.6720	1.3668	21.3614	0.0600
219.4289	0.3010	13.9083	0.0193	222.6720	0.9447	18.7686	0.0487
				227.6720	0.5406	15.1328	0.0359

TableD.2 Dynamic mechanical analysis of 10% KL

Replication 1				Replication 2			
Temp (^oC)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^oC)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-99.0091	454.2947	9754.7669	0.0353	-99.6350	209.6732	6241.1351	0.0309
-94.0091	332.3091	9642.3505	0.0367	-94.6350	225.1035	6157.6951	0.0334
-89.0091	355.7725	9435.2462	0.0409	-89.6350	220.1715	6079.4290	0.0368
-84.0091	448.0455	9063.3591	0.0507	-84.6350	251.6157	5875.8238	0.0439
-79.0091	550.5172	8517.9840	0.0654	-79.6350	310.6484	5685.7527	0.0560
-74.0091	656.4737	7931.5092	0.0846	-74.6350	374.9220	5373.2080	0.0762
-69.0091	790.2771	7194.9244	0.1084	-69.6350	487.6694	4965.7581	0.1038
-64.0091	845.3071	6358.2436	0.1347	-64.6350	602.8599	4419.4865	0.1386
-59.0091	838.5710	5412.4438	0.1640	-59.6350	651.8562	3824.6150	0.1708
-54.0091	833.5751	4456.9464	0.1914	-54.6350	645.8496	3197.3952	0.1962
-49.0091	793.2042	3550.2283	0.2136	-49.6350	584.1481	2659.0461	0.2085
-44.0091	689.5928	2865.6797	0.2217	-44.6350	498.9028	2297.9222	0.2052
-39.0091	563.4193	2515.2705	0.2158	-39.6350	400.6664	2092.3766	0.1896
-34.0091	435.6279	2252.7854	0.1963	-34.6350	301.7423	1939.7944	0.1636
-29.0091	345.4806	2083.3426	0.1719	-29.6350	237.5919	1786.5130	0.1383
-24.0091	276.1923	1935.5517	0.1479	-24.6350	191.0444	1653.8586	0.1191
-19.0091	223.5137	1805.8734	0.1270	-19.6350	158.6938	1530.8011	0.1055
-14.0091	186.2569	1685.1679	0.1126	-14.6350	138.1582	1420.8244	0.0972
-9.0091	161.0902	1572.6028	0.1027	-9.6350	122.7264	1309.9166	0.0931
-4.0091	143.0129	1461.1847	0.0972	-4.6350	111.0538	1202.5527	0.0916
0.9909	129.9788	1356.7756	0.0948	0.3651	102.7323	1101.5058	0.0916
5.9909	119.0168	1252.7600	0.0940	5.3651	94.1634	1014.8085	0.0922
10.9909	109.2968	1163.0200	0.0946	10.3651	86.8349	932.1029	0.0934
15.9909	103.7645	1079.0631	0.0964	15.3651	80.9881	855.9754	0.0955
20.9909	100.0521	999.7913	0.0992	20.3651	77.7510	787.4499	0.0986
25.9909	95.2358	926.2386	0.1022	25.3651	74.4766	729.0898	0.1025
30.9909	90.3677	854.4089	0.1057	30.3651	72.1076	673.7867	0.1068
35.9909	86.4955	794.8201	0.1094	35.3651	70.1253	627.3407	0.1115
40.9909	83.7383	733.0931	0.1141	40.3651	69.2458	588.6794	0.1168
45.9909	80.5177	669.1387	0.1199	45.3651	67.7810	549.5096	0.1217
50.9909	76.8999	604.7469	0.1268	50.3651	65.7849	515.4861	0.1279
55.9909	73.1803	538.0433	0.1354	55.3651	64.0246	473.8450	0.1359
60.9909	69.0237	470.3691	0.1466	60.3651	62.9888	428.1127	0.1473
65.9909	63.8277	391.4524	0.1639	65.3651	61.5393	378.5554	0.1630
70.9909	59.4060	329.6369	0.1822	70.3651	59.7443	328.6807	0.1835
75.9909	54.6064	270.2136	0.2052	75.3651	57.3147	276.9214	0.2094
80.9909	49.3437	215.1236	0.2318	80.3651	54.2781	227.8456	0.2399
85.9909	43.9372	169.9952	0.2579	85.3651	50.3579	184.2310	0.2723
90.9909	37.6692	131.9491	0.2831	90.3651	44.8512	147.4807	0.3035
95.9909	31.2677	102.2591	0.3033	95.3651	38.4548	116.1417	0.3312
100.9909	25.2376	79.1667	0.3164	100.3651	32.1636	90.9793	0.3520
105.9909	19.9450	61.7722	0.3209	105.3651	25.9414	70.4911	0.3644

TableD.2 Dynamic mechanical analysis of 10% KL (Continues)

Replication 1				**Replication 2**			
Temp (^oC)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^oC)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
110.9909	15.3138	48.1698	0.3170	110.3651	20.7459	56.1115	0.3672
115.9909	11.6306	38.1307	0.3058	115.3651	15.9092	44.1290	0.3616
120.9909	8.8239	30.5844	0.2889	120.3651	11.4887	33.1622	0.3454
125.9909	6.7746	25.2913	0.2674	125.3651	8.6466	26.6316	0.3244
130.9909	5.1980	21.1491	0.2416	130.3651	6.6350	22.2381	0.2985
135.9909	3.9362	18.4623	0.2127	135.3651	5.0654	19.1221	0.2680
140.9909	2.9267	16.6576	0.1845	140.3651	3.9600	16.8075	0.2348
145.9909	2.4326	15.2833	0.1606	145.3651	3.0969	14.9210	0.1998
150.9909	2.0595	14.6480	0.1397	150.3651	2.1675	13.4741	0.1607
155.9909	1.7466	14.3209	0.1216	155.3651	1.4471	12.4267	0.1243
160.9909	1.5281	14.2246	0.1056	160.3651	1.0494	11.2347	0.0941
165.9909	1.3426	14.6115	0.0912	165.3651	0.6721	10.2601	0.0715
170.9909	1.1128	14.7739	0.0785	170.3651	0.4620	9.5982	0.0573
175.9909	0.9857	14.5097	0.0682	175.3651	0.4437	9.0275	0.0507
180.9909	0.8644	14.3644	0.0600	180.3651	0.4634	8.8238	0.0462
185.9909	0.7574	14.3612	0.0542	185.3651	0.4392	8.7690	0.0389
190.9909	0.7228	14.2960	0.0499	190.3651	0.2881	8.7070	0.0250
195.9909	0.6899	14.4098	0.0465	195.3651	-0.0321	9.6708	0.0050

TableD.3 Dynamic mechanical analysis of 20% KL

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-100.4342	209.8395	8108.8158	0.0218	-100.1311	31.0523	6236.2296	0.0062
-95.4342	201.9851	7961.8970	0.0246	-95.1311	82.1664	6193.7561	0.0120
-90.4342	203.0507	7789.1998	0.0283	-90.1311	99.7236	6237.1708	0.0176
-85.4342	249.0162	7537.7694	0.0343	-85.1311	158.5169	6116.0547	0.0245
-80.4342	310.8197	7289.2706	0.0423	-80.1311	184.0893	5877.6354	0.0330
-75.4342	358.2434	6952.3010	0.0520	-75.1311	243.3107	5607.9993	0.0440
-70.4342	413.0913	6492.3134	0.0631	-70.1311	289.0564	5297.6542	0.0580
-65.4342	442.7074	5973.9507	0.0736	-65.1311	350.8239	5030.3666	0.0752
-60.4342	453.3164	5535.0254	0.0834	-60.1311	425.9393	4580.0729	0.0923
-55.4342	471.0708	5040.2736	0.0931	-55.1311	480.8361	4169.3335	0.1073
-50.4342	454.8984	4521.1214	0.1006	-50.1311	439.3588	3735.4292	0.1154
-45.4342	430.3207	4035.5594	0.1051	-45.1311	380.8575	3248.5875	0.1170
-40.4342	391.6319	3598.9725	0.1062	-40.1311	328.3535	2890.0043	0.1136
-35.4342	330.0125	3232.6052	0.1037	-35.1311	283.8005	2620.8348	0.1073
-30.4342	285.8311	2886.9487	0.0992	-30.1311	230.2059	2359.3426	0.0985
-25.4342	250.5471	2634.7002	0.0936	-25.1311	185.8942	2129.5086	0.0887
-20.4342	211.6464	2438.3686	0.0879	-20.1311	157.1020	1971.7090	0.0808
-15.4342	183.2868	2259.4546	0.0825	-15.1311	134.7950	1835.6376	0.0748
-10.4342	165.6630	2110.3529	0.0788	-10.1311	122.1063	1719.2081	0.0712
-5.4342	148.7680	1959.1055	0.0761	-5.1311	114.5919	1612.4285	0.0692
-0.4342	138.4812	1838.0009	0.0747	-0.1311	100.9125	1507.9997	0.0682
4.5658	127.7545	1719.8924	0.0741	4.8689	97.1844	1408.7045	0.0685
9.5658	118.7762	1619.7581	0.0746	9.8689	91.6377	1319.4684	0.0699
14.5658	116.2423	1525.9832	0.0761	14.8689	89.3203	1230.4478	0.0724
19.5658	114.0577	1433.1287	0.0786	19.8689	87.8748	1143.0250	0.0756
24.5658	110.0908	1344.5986	0.0816	24.8689	84.5318	1069.3936	0.0792
29.5658	106.8410	1260.5607	0.0849	29.8689	81.8373	985.2487	0.0841
34.5658	105.5446	1177.8126	0.0893	34.8689	82.4555	914.1320	0.0904
39.5658	104.9616	1089.9710	0.0954	39.8689	82.4381	838.8389	0.0978
44.5658	102.8600	1007.5222	0.1026	44.8689	81.7541	767.9137	0.1062
49.5658	103.3081	923.7793	0.1133	49.8689	82.0246	706.3051	0.1167
54.5658	106.8509	817.7927	0.1306	54.8689	82.2961	641.5993	0.1281
59.5658	105.1690	713.0300	0.1492	59.8689	82.5855	583.7935	0.1393
64.5658	104.0351	608.0662	0.1712	64.8689	82.1077	524.8770	0.1549
69.5658	99.7294	504.2086	0.1956	69.8689	79.5952	461.6895	0.1727
74.5658	90.3216	407.1562	0.2209	74.8689	76.0944	396.1130	0.1948
79.5658	79.8362	323.4729	0.2475	79.8689	72.7891	330.1319	0.2239
84.5658	68.2906	251.8064	0.2765	84.8689	68.2540	262.7007	0.2620
89.5658	58.8020	191.6753	0.3073	89.8689	61.3958	201.7807	0.3056
94.5658	49.7202	147.0101	0.3372	94.8689	52.9177	150.7063	0.3512
99.5658	40.5816	111.9333	0.3658	99.8689	43.8023	111.3095	0.3948
104.5658	32.4866	84.7199	0.3881	104.8689	35.4332	82.5008	0.4310

TableD.3 Dynamic mechanical analysis of 20% KL (Continues)

Replication 1				Replication 2			
Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ	Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ
109.5658	25.9351	65.4887	0.3990	109.8689	27.8209	60.6097	0.4581
114.5658	19.9347	48.0875	0.3945	114.8689	20.9117	43.5004	0.4700
119.5658	14.3293	36.4051	0.3654	119.8689	15.3635	32.4608	0.4591
124.5658	8.8930	28.6339	0.3141	124.8689	10.6871	24.4757	0.4275
129.5658	5.4464	22.4074	0.2551	129.8689	7.3341	19.2298	0.3811
134.5658	3.4447	18.7564	0.1984	134.8689	5.1192	15.9804	0.3280
139.5658	2.3258	16.8753	0.1500	139.8689	3.6759	13.7933	0.2748
144.5658	1.8164	16.3989	0.1113	144.8689	2.7363	12.4200	0.2266
149.5658	1.3382	16.3200	0.0830	149.8689	2.1537	11.7575	0.1885
154.5658	0.9450	15.9397	0.0625	154.8689	1.7648	11.3453	0.1598
159.5658	0.8327	15.8728	0.0485	159.8689	1.4986	11.1101	0.1390
164.5658	0.6995	16.0337	0.0390	164.8689	1.4066	11.1414	0.1236
169.5658	0.5453	16.2222	0.0325	169.8689	1.3379	11.2413	0.1089
174.5658	0.4531	16.3497	0.0290	174.8689	1.2026	11.5955	0.0911
179.5658	0.4121	16.6132	0.0293	179.8689	0.7503	12.6191	0.0703
184.5658	0.5224	17.0340	0.0332	184.8689	0.5806	13.1874	0.0538
189.5658	0.8072	18.1940	0.0398	189.8689	0.8216	14.3482	0.0446
194.5658	0.9555	19.3457	0.0465	194.8689	0.6073	14.1174	0.0383
199.5658	1.0224	20.2995	0.0529	199.8689	0.5164	14.1438	0.0332

TableD.4 Dynamic mechanical analysis of 30% KL

Replication 1				Replication 2			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-97.8487	226.0312	7793.7108	0.0278	-100.5068	120.6208	3612.9450	0.0341
-92.8487	218.9929	7513.8952	0.0310	-95.5068	137.6622	3597.0439	0.0378
-87.8487	310.5820	7354.3381	0.0358	-90.5068	165.8441	3569.3214	0.0471
-82.8487	267.3531	7215.2479	0.0407	-85.5068	181.4504	3511.9761	0.0512
-77.8487	358.4414	6942.4267	0.0488	-80.5068	209.2151	3436.3508	0.0613
-72.8487	359.1851	6544.7025	0.0617	-75.5068	239.6068	3329.3944	0.0715
-67.8487	466.5868	6134.9739	0.0798	-70.5068	295.1724	3211.7060	0.0916
-62.8487	573.5058	5571.4156	0.1008	-65.5068	365.0344	2994.0020	0.1219
-57.8487	597.4722	4973.7032	0.1199	-60.5068	417.4380	2655.1444	0.1584
-52.8487	594.5879	4280.5755	0.1337	-55.5068	421.2553	2254.1120	0.1871
-47.8487	532.4984	3693.2824	0.1398	-50.5068	385.0662	1890.9158	0.2033
-42.8487	439.2100	3208.4725	0.1386	-45.5068	329.5194	1593.6977	0.2064
-37.8487	376.1036	2824.1932	0.1325	-40.5068	275.4854	1396.0514	0.1973
-32.8487	308.4366	2521.8240	0.1226	-35.5068	223.1734	1244.9462	0.1788
-27.8487	252.0560	2292.1556	0.1114	-30.5068	176.7984	1120.3629	0.1578
-22.8487	211.0922	2119.8974	0.1008	-25.5068	142.2818	1020.5252	0.1394
-17.8487	177.6490	1970.3281	0.0914	-20.5068	118.8064	941.8238	0.1263
-12.8487	155.5919	1855.4039	0.0837	-15.5068	102.4851	875.7337	0.1170
-7.8487	138.2556	1769.5229	0.0782	-10.5068	91.3212	816.6153	0.1119
-2.8487	125.6021	1683.2094	0.0743	-5.5068	83.4723	760.1630	0.1098
2.1513	113.9342	1607.1525	0.0719	-0.5068	77.9188	706.7802	0.1103
7.1513	108.5260	1540.4906	0.0712	4.4932	73.5731	655.2581	0.1123
12.1513	106.8644	1473.3929	0.0714	9.4932	70.2230	608.1125	0.1155
17.1513	103.2034	1417.8228	0.0719	14.4932	67.3273	563.4619	0.1196
22.1513	98.9881	1362.3678	0.0723	19.4932	64.4040	522.1662	0.1234
27.1513	94.6162	1306.3301	0.0730	24.4932	61.7307	482.8459	0.1277
32.1513	91.6479	1244.1763	0.0742	29.4932	59.8125	448.7049	0.1333
37.1513	91.0060	1179.6487	0.0765	34.4932	57.9082	423.5437	0.1368
42.1513	88.6471	1112.3538	0.0795	39.4932	56.0339	400.4416	0.1400
47.1513	86.9629	1047.0840	0.0833	44.4932	54.4319	377.4967	0.1439
52.1513	87.3225	976.5566	0.0886	49.4932	53.5680	357.5865	0.1501
57.1513	86.7992	909.4132	0.0949	54.4932	51.8405	336.1216	0.1542
62.1513	87.5183	839.4277	0.1037	59.4932	50.1433	312.4077	0.1604
67.1513	89.1766	741.5337	0.1192	64.4932	48.5248	288.5680	0.1683
72.1513	89.3550	646.3990	0.1386	69.4932	46.5114	264.7038	0.1756
77.1513	88.5344	544.1239	0.1655	74.4932	44.7583	238.9349	0.1872
82.1513	86.5704	442.0864	0.1998	79.4932	43.7112	213.7537	0.2046
87.1513	82.2249	341.3135	0.2442	84.4932	42.6809	187.8051	0.2273
92.1513	75.3683	255.4593	0.2966	89.4932	41.3543	159.9638	0.2578
97.1513	65.8520	187.5553	0.3515	94.4932	39.8312	132.3248	0.3010
102.1513	54.7671	135.9658	0.4023	99.4932	36.9551	103.4450	0.3603
107.1513	43.4430	97.3705	0.4444	104.4932	30.7065	72.7655	0.4233

TableD.4 Dynamic mechanical analysis of 30% KL (Continues)

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
112.1513	32.6298	68.6183	0.4726	109.4932	21.7223	45.2395	0.4785
117.1513	23.1036	47.0512	0.4825	114.4932	14.3260	28.9779	0.4920
122.1513	15.6064	32.4974	0.4704	119.4932	8.6476	19.5479	0.4405
127.1513	10.4593	23.6813	0.4388	124.4932	4.2980	14.8682	0.2855
132.1513	7.1287	18.1555	0.3935	129.4932	1.7200	11.6482	0.1481
137.1513	4.9988	14.8237	0.3423	134.4932	0.3957	9.2205	0.0459
142.1513	3.7132	12.9550	0.2931	139.4932	-0.1639	7.8372	-0.0187
147.1513	2.8310	11.5657	0.2490	144.4932	-0.3404	6.9984	-0.0474
152.1513	2.2435	10.6639	0.2123	149.4932	-0.3540	6.3416	-0.0529
157.1513	1.8328	10.0858	0.1821	154.4932	-0.4138	5.8149	-0.0697
162.1513	1.4723	9.5687	0.1561	159.4932	-0.5050	5.5843	-0.0925
167.1513	1.2928	9.3498	0.1356	164.4932	-0.5135	5.4276	-0.0957
172.1513	1.1420	9.2621	0.1163	169.4932	-0.4679	5.4941	-0.0829
177.1513	0.9213	9.2773	0.0972	174.4932	-0.4996	5.5122	-0.0905
182.1513	0.6794	9.5708	0.0798	179.4932	-0.5428	5.4095	-0.1015
187.1513	0.5963	9.6065	0.0675	184.4932	-0.5330	5.4490	-0.0972
192.1513	0.5989	9.5494	0.0591	189.4932	-0.5218	5.5078	-0.0940
197.1513	0.5229	9.4047	0.0519	194.4932	-0.5135	5.5372	-0.0943
				199.4932	-0.4521	5.5669	-0.0786

TableD.5 Dynamic mechanical analysis of 40% KL

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-98.6643	-76.9132	9958.7352	-0.0035	-96.4494	224.9945	9874.0010	0.0222
-93.6643	93.6071	9522.1173	0.0033	-91.4494	269.1362	10028.2186	0.0239
-88.6643	46.3206	9413.7944	0.0077	-86.4494	225.2035	10085.2615	0.0273
-83.6643	99.6417	9376.8667	0.0129	-81.4494	335.5575	9569.2891	0.0374
-78.6643	221.1178	8826.7584	0.0219	-76.4494	544.4080	9361.4625	0.0499
-73.6643	211.7462	8507.5424	0.0341	-71.4494	526.0073	8938.6553	0.0626
-68.6643	400.1305	7748.7775	0.0508	-66.4494	614.4343	8368.0893	0.0783
-63.6643	513.7631	7092.1066	0.0684	-61.4494	692.0278	7621.5406	0.0971
-58.6643	533.0381	6303.6227	0.0856	-56.4494	773.9019	6632.2138	0.1160
-53.6643	554.9663	5446.1223	0.0980	-51.4494	797.4027	5777.6382	0.1289
-48.6643	487.4788	4710.8956	0.1040	-46.4494	696.6841	4999.3696	0.1320
-43.6643	439.1188	4099.6696	0.1039	-41.4494	534.7091	4357.7680	0.1267
-38.6643	361.0034	3613.4517	0.0980	-36.4494	440.1599	3824.4316	0.1157
-33.6643	275.4048	3217.6416	0.0885	-31.4494	343.9450	3361.9666	0.1013
-28.6643	229.4734	2960.9782	0.0782	-26.4494	253.4591	3028.7313	0.0867
-23.6643	187.1753	2743.1903	0.0691	-21.4494	199.7053	2829.9906	0.0763
-18.6643	158.2722	2559.5045	0.0624	-16.4494	190.6060	2666.0173	0.0698
-13.6643	137.0253	2413.1013	0.0582	-11.4494	166.3808	2513.9331	0.0655
-8.6643	129.2813	2284.2346	0.0562	-6.4494	149.4240	2377.5410	0.0630
-3.6643	122.1021	2167.8393	0.0556	-1.4494	140.4074	2237.3337	0.0614
1.3357	115.6395	2056.0670	0.0553	3.5506	129.5365	2103.3661	0.0603
6.3357	106.7229	1951.9412	0.0553	8.5506	116.7644	1988.4216	0.0597
11.3357	101.0593	1843.0188	0.0560	13.5506	111.0806	1892.0314	0.0604
16.3357	101.1479	1746.4017	0.0576	18.5506	114.9470	1809.9550	0.0627
21.3357	101.5787	1649.1536	0.0597	23.5506	114.6188	1722.2551	0.0657
26.3357	96.6707	1556.5991	0.0620	28.5506	113.3310	1630.8712	0.0688
31.3357	92.0406	1471.7091	0.0653	33.5506	110.3627	1540.2732	0.0718
36.3357	97.0341	1375.6464	0.0706	38.5506	107.5852	1433.3802	0.0755
41.3357	100.7822	1306.4176	0.0761	43.5506	106.3755	1337.4204	0.0801
46.3357	102.0983	1205.5310	0.0851	48.5506	107.5190	1262.7275	0.0853
51.3357	107.2244	1116.3935	0.0919	53.5506	109.6845	1173.1239	0.0933
56.3357	103.5355	1045.3230	0.0980	58.5506	111.0389	1083.7877	0.1009
61.3357	99.3638	955.3699	0.1073	63.5506	111.4571	996.6844	0.1109
66.3357	104.6158	850.1736	0.1215	68.5506	111.2328	896.3580	0.1227
71.3357	105.8829	739.7647	0.1412	73.5506	110.2163	781.4008	0.1392
76.3357	103.7450	608.7122	0.1712	78.5506	108.4187	657.2104	0.1640
81.3357	98.8965	474.1110	0.2127	83.5506	105.9908	528.7739	0.2022
86.3357	91.3647	350.6395	0.2660	88.5506	99.2559	400.6539	0.2593
91.3357	82.8290	254.2849	0.3312	93.5506	90.8342	287.2933	0.3349
96.3357	72.2440	179.8052	0.4075	98.5506	78.6371	190.8015	0.4247
101.3357	58.8412	123.1508	0.4827	103.5506	60.9812	116.0802	0.5153
106.3357	46.2250	85.1780	0.5368	108.5506	41.1497	68.9464	0.5769

TableD.5 Dynamic mechanical analysis of 40% KL (Continues)

Replication 1				**Replication 2**			
Temp (°C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (°C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
111.3357	31.1414	52.9705	0.5745	113.5506	26.0303	41.9746	0.5920
116.3357	18.8882	31.8952	0.5710	118.5506	15.4665	26.5568	0.5563
121.3357	11.0357	20.5885	0.5248	123.5506	8.3097	17.3421	0.4598
126.3357	6.3842	14.6093	0.4371	128.5506	4.3804	12.4315	0.3340
131.3357	3.6825	10.8128	0.3238	133.5506	1.7216	10.0760	0.1980
136.3357	1.7797	8.6566	0.2019	138.5506	0.1616	8.6562	0.0764
141.3357	0.3428	7.5568	0.0825	143.5506	-0.3056	7.4790	-0.0084
146.3357	-0.2417	6.5376	-0.0062	148.5506	-0.3876	6.8562	-0.0562
151.3357	-0.4738	6.0150	-0.0629	153.5506	-0.4053	6.2568	-0.0751
156.3357	-0.4773	5.6501	-0.0886	158.5506	-0.4635	6.0329	-0.0784
161.3357	-0.4348	5.3565	-0.0976	163.5506	-0.3563	6.0324	-0.0737
166.3357	-0.4748	5.0469	-0.0999	168.5506	-0.3778	6.0511	-0.0696
171.3357	-0.4669	4.8327	-0.1000	173.5506	-0.4128	6.0405	-0.0680
176.3357	-0.4491	4.8616	-0.1003	178.5506	-0.3847	5.8163	-0.0690
181.3357	-0.4944	4.7674	-0.1020	183.5506	-0.3991	5.8481	-0.0729
186.3357	-0.4965	4.6874	-0.1036	188.5506	-0.4841	5.6698	-0.0783
191.3357	-0.5011	4.6954	-0.1042	193.5506	-0.4847	5.6914	-0.0819
196.3357	-0.4993	4.8490	-0.1039	198.5506	-0.4609	5.6983	-0.0836

TableD.6 Dynamic mechanical analysis of 50% KL

Replication 1				Replication 2			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-97.5985	83.6829	4452.4631	0.0192	-101.2998	78.1847	471.2311	0.1670
-92.5985	96.5617	4387.0503	0.0210	-96.2998	73.4777	466.0081	0.1573
-87.5985	106.2159	4372.8228	0.0242	-91.2998	69.8431	464.3586	0.1498
-82.5985	130.7447	4287.1851	0.0307	-86.2998	66.9508	465.4104	0.1443
-77.5985	181.9770	4150.2441	0.0421	-81.2998	64.6414	462.3163	0.1398
-72.5985	220.1283	3930.6032	0.0616	-76.2998	63.7804	456.0631	0.1407
-67.5985	322.1618	3677.5782	0.0920	-71.2998	66.0366	448.7668	0.1471
-62.5985	423.5704	3265.1547	0.1264	-66.2998	76.8293	433.8397	0.1695
-57.5985	437.8641	2804.1294	0.1524	-61.2998	95.9613	413.2416	0.2244
-52.5985	395.5369	2405.3779	0.1654	-56.2998	101.7477	422.9709	0.3880
-47.5985	350.2243	2076.7948	0.1658	-51.2998	76.3257	494.4549	0.3815
-42.5985	281.9735	1818.1309	0.1550	-46.2998	60.5219	523.7858	0.3227
-37.5985	215.6194	1573.1257	0.1381	-41.2998	60.7630	513.3140	0.3383
-32.5985	166.3999	1400.7063	0.1201	-36.2998	62.9995	575.1753	0.3504
-27.5985	134.3051	1279.6506	0.1050	-31.2998	52.2649	490.6219	0.3216
-22.5985	109.9990	1179.9794	0.0932	-26.2998	41.6848	393.1173	0.2857
-17.5985	93.6371	1099.7031	0.0854	-21.2998	42.3817	365.7931	0.2611
-12.5985	83.4607	1038.4411	0.0810	-16.2998	43.1885	341.0240	0.2614
-7.5985	77.8865	977.2024	0.0796	-11.2998	38.4402	330.9692	0.2467
-2.5985	75.1125	920.9775	0.0799	-6.2998	35.2028	328.4898	0.2462
2.4015	70.0091	878.1721	0.0807	-1.2998	34.0512	326.8949	0.2613
7.4015	69.2249	843.4792	0.0825	3.7002	36.2078	321.5990	0.2975
12.4015	69.2671	807.4618	0.0844	8.7002	40.1103	312.0501	0.3125
17.4015	67.2175	783.2575	0.0851	13.7002	43.7591	316.2427	0.3213
22.4015	63.6003	755.5500	0.0852	18.7002	43.2666	387.6149	0.2901
27.4015	62.2950	725.3539	0.0864	23.7002	37.2902	413.4243	0.1801
32.4015	62.0919	689.2339	0.0892	28.7002	31.2472	413.0024	0.0968
37.4015	60.0651	655.5918	0.0922	33.7002	27.7736	406.5058	0.0655
42.4015	59.4591	617.3779	0.0956	38.7002	26.4739	406.4943	0.0650
47.4015	57.1775	582.1900	0.0991	43.7002	26.1403	405.8967	0.0644
52.4015	57.1927	549.1167	0.1039	48.7002	26.4539	403.7995	0.0657
57.4015	56.6623	515.0570	0.1097	53.7002	26.9053	401.4561	0.0671
62.4015	55.6331	477.7400	0.1163	58.7002	27.4080	397.7470	0.0688
67.4015	54.6228	428.2710	0.1266	63.7002	28.3660	390.4808	0.0725
72.4015	53.7395	382.1275	0.1392	68.7002	30.0550	377.0901	0.0798
77.4015	53.4409	335.7862	0.1583	73.7002	32.6381	358.6013	0.0909
82.4015	53.4423	283.0087	0.1900	78.7002	36.3091	331.2634	0.1103
87.4015	53.8634	225.4511	0.2422	83.7002	40.9989	295.4113	0.1369
92.4015	52.7862	166.3795	0.3217	88.7002	47.4196	252.0792	0.1895
97.4015	49.4774	118.3956	0.4238	93.7002	52.0429	197.7951	0.2611
102.4015	44.3680	85.1594	0.5317	98.7002	53.5442	144.8652	0.3723
107.4015	35.3720	56.4035	0.6301	103.7002	47.7802	97.5904	0.5083

TableD.6 Dynamic mechanical analysis of 50% KL (Continues)

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
112.4015	24.6419	34.1804	0.6848	108.7002	32.6512	55.0486	0.5883
117.4015	14.5922	21.2607	0.6609	113.7002	15.8193	27.8157	0.5224
122.4015	7.5078	13.2260	0.5608	118.7002	5.7234	16.9200	0.3230
127.4015	3.8381	9.3886	0.4212	123.7002	1.0886	12.4365	0.0996
132.4015	1.9099	7.3988	0.2728	128.7002	-0.4551	10.3046	-0.0281
137.4015	0.8108	6.2065	0.1370	133.7002	-0.6407	9.1076	-0.0567
142.4015	0.1598	5.3424	0.0357	138.7002	-0.5327	8.5140	-0.0571
147.4015	-0.2023	4.6781	-0.0318	143.7002	-0.4784	8.3057	-0.0576
152.4015	-0.2693	4.4365	-0.0634	148.7002	-0.4747	8.0692	-0.0598
157.4015	-0.2938	4.2935	-0.0763	153.7002	-0.4762	7.9256	-0.0607
162.4015	-0.3149	4.1380	-0.0822	158.7002	-0.4689	7.8233	-0.0596
167.4015	-0.4405	4.4918	-0.0859	163.7002	-0.4709	7.8235	-0.0600
172.4015	-0.6645	4.2864	-0.0884	168.7002	-0.4799	7.8188	-0.0617
177.4015	5.6278	-5.7572	-0.0839	173.7002	-0.4825	7.8096	-0.0619
182.4015	-6.1469	33.3415	-0.0573	178.7002	-0.4823	7.7639	-0.0624
				183.7002	-0.4816	7.8878	-0.0611
				188.7002	-0.4814	7.8701	-0.0612
				193.7002	-0.4814	7.7770	-0.0622
				198.7002	-0.4809	8.0703	-0.0596

TableD.7 Dynamic mechanical analysis of 60% KL

Replication 1				Replication 2			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-98.0074	16.0869	292.5690	0.0074	-100.1836	16.9417	255.5562	0.0324
-93.0074	7.2203	405.8817	0.0030	-95.1836	14.0251	449.2802	0.0291
-88.0074	1.9007	380.4251	0.0005	-90.1836	13.1101	702.5515	0.0163
-83.0074	1.9558	379.7830	0.0007	-85.1836	12.9245	730.0235	0.0112
-78.0074	2.3773	382.3405	0.0012	-80.1836	14.2367	763.3134	0.0131
-73.0074	2.3119	380.1314	0.0014	-75.1836	5.7274	667.2986	0.0087
-68.0074	2.4975	396.0374	0.0021	-70.1836	45.4151	727.9990	0.0077
-63.0074	2.6714	397.7680	0.0029	-65.1836	145.0843	1376.9750	0.0395
-58.0074	2.7094	361.5151	0.0048	-60.1836	60.7680	757.4496	0.0520
-53.0074	3.5687	319.3480	0.0101	-55.1836	57.9897	458.3669	0.1115
-48.0074	6.3617	271.7028	0.0200	-50.1836	94.7891	372.6322	0.2159
-43.0074	7.6243	247.9399	0.0219	-45.1836	94.4092	323.1644	0.2841
-38.0074	5.8275	231.8143	0.0206	-40.1836	80.5211	294.6190	0.3319
-33.0074	4.6764	232.5994	0.0222	-35.1836	60.5638	318.8590	0.2461
-28.0074	4.9353	264.4604	0.0259	-30.1836	44.7494	322.3895	0.1590
-23.0074	4.8295	238.1308	0.0277	-25.1836	36.9864	320.5222	0.1270
-18.0074	3.8709	238.8690	0.0222	-20.1836	33.5743	325.3858	0.1100
-13.0074	3.7836	280.3816	0.0198	-15.1836	31.0666	308.4518	0.1001
-8.0074	4.4388	274.5480	0.0236	-10.1836	27.0235	297.8288	0.0892
-3.0074	4.5932	243.2485	0.0266	-5.1836	23.3325	286.9880	0.0817
1.9926	2.7205	258.6938	0.0100	-0.1836	19.7159	278.4577	0.0704
6.9926	1.6489	260.6026	0.0061	4.8164	16.4751	269.3753	0.0625
11.9926	1.8632	256.6558	0.0068	9.8164	13.7813	263.0207	0.0520
16.9926	2.9087	252.2766	0.0124	14.8164	11.8000	257.5458	0.0463
21.9926	3.0225	249.3421	0.0113	19.8164	9.4959	253.2355	0.0382
26.9926	3.6330	245.3107	0.0150	24.8164	7.8012	249.6663	0.0291
31.9926	3.5811	242.2584	0.0154	29.8164	7.4086	245.9232	0.0317
36.9926	2.6457	239.3499	0.0101	34.8164	7.2816	242.3855	0.0303
41.9926	2.9864	235.9490	0.0131	39.8164	7.0276	238.6025	0.0307
46.9926	3.6922	229.5845	0.0159	44.8164	6.1323	235.6689	0.0236
51.9926	4.1212	225.7847	0.0188	49.8164	7.1603	233.7793	0.0317
56.9926	4.3956	222.1617	0.0193	54.8164	6.3557	232.4953	0.0230
61.9926	5.4424	215.7051	0.0255	59.8164	6.6850	230.0209	0.0289
66.9926	6.7527	207.8076	0.0323	64.8164	8.2082	227.7703	0.0360
71.9926	9.2614	197.6031	0.0468	69.8164	10.8367	224.3211	0.0476
76.9926	13.6365	185.4971	0.0738	74.8164	14.5466	218.5287	0.0656
81.9926	19.8564	171.9059	0.1138	79.8164	18.6142	209.3170	0.0904
86.9926	28.9525	154.9456	0.1841	84.8164	23.2386	198.0046	0.1152
91.9926	37.6284	128.2261	0.2986	89.8164	32.3378	181.2038	0.1787
96.9926	37.6170	89.2990	0.4323	94.8164	41.6314	153.7945	0.2678
101.9926	27.1970	50.1274	0.5422	99.8164	42.2238	105.4738	0.4140
106.9926	15.0920	24.9190	0.5787	104.8164	28.9860	51.6965	0.5700

TableD.7 Dynamic mechanical analysis of 60% KL (Continues)

Replication 1				Replication 2			
Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ	Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ
111.9926	6.3380	12.9484	0.4512	109.8164	12.3742	20.6599	0.5548
116.9926	1.6057	8.4433	0.1958	114.8164	2.7833	10.4904	0.2512
121.9926	-0.1922	6.7548	-0.0109	119.8164	-0.3009	7.9272	-0.0175
126.9926	-0.4921	5.9336	-0.0660	124.8164	-0.5756	7.1171	-0.0622
131.9926	-0.4113	5.6373	-0.0653	129.8164	-0.4649	6.6577	-0.0674
136.9926	-0.3581	5.5994	-0.0636	134.8164	-0.4395	6.5338	-0.0678
141.9926	-0.3495	5.4825	-0.0652	139.8164	-0.4445	6.3294	-0.0706
				144.8164	-0.4468	6.2448	-0.0720
				149.8164	-0.4462	6.3567	-0.0701
				154.8164	-0.4462	6.3830	-0.0704
				159.8164	-0.4463	6.3664	-0.0698
				164.8164	-0.4464	6.3074	-0.0707
				169.8164	-0.4465	6.2840	-0.0714
				174.8164	-0.4463	6.3671	-0.0703
				179.8164	-0.4461	6.4725	-0.0687
				184.8164	-0.4466	6.3509	-0.0701
				189.8164	-0.4464	6.2550	-0.0721
				194.8164	-0.4454	6.5355	-0.0674
				199.8164	-0.4458	6.3900	-0.0707

TableD.8 Dynamic mechanical analysis of 70% KL

Replication 1				**Replication 2**			
Temp (^{o}C)	**E" (MPa)**	**E' (MPa)**	**Tan δ**	**Temp (^{o}C)**	**E" (MPa)**	**E' (MPa)**	**Tan δ**
-99.9792	3.0981	266.3446	0.0270	-96.9104	2.4402	62.0470	0.0840
-94.9792	4.1763	268.4244	0.0331	-91.9104	4.3942	126.9498	0.0740
-89.9792	4.7065	263.6253	0.0329	-86.9104	1.4385	79.2069	0.0666
-84.9792	5.8546	239.6177	0.0395	-81.9104	2.2406	89.8585	0.0644
-79.9792	6.9850	238.5228	0.0452	-76.9104	2.5122	94.1701	0.0634
-74.9792	7.7779	233.3661	0.0521	-71.9104	3.3378	104.0405	0.0614
-69.9792	7.6400	236.7747	0.0484	-66.9104	2.3712	95.3206	0.0579
-64.9792	10.2823	228.3686	0.0633	-61.9104	2.0099	80.6172	0.0563
-59.9792	15.9981	211.0122	0.0845	-56.9104	2.5997	84.8410	0.0595
-54.9792	22.5518	189.3831	0.1149	-51.9104	2.9496	81.5394	0.0692
-49.9792	29.2561	196.5819	0.1600	-46.9104	5.2131	67.7361	0.0853
-44.9792	34.2183	222.7759	0.1724	-41.9104	7.3380	55.4364	0.1020
-39.9792	33.5246	211.6993	0.1625	-36.9104	7.3321	51.5959	0.1117
-34.9792	27.0386	191.3115	0.1385	-31.9104	6.5619	53.1800	0.1136
-29.9792	19.8407	176.9422	0.1096	-26.9104	5.9345	69.2983	0.1065
-24.9792	14.6438	166.0735	0.0875	-21.9104	5.4886	66.5376	0.0964
-19.9792	14.5622	166.3863	0.0868	-16.9104	4.9815	60.8835	0.0850
-14.9792	8.4859	151.2677	0.0565	-11.9104	4.1002	60.1537	0.0749
-9.9792	6.7223	146.5197	0.0460	-6.9104	3.3602	54.7848	0.0695
-4.9792	5.2273	142.2543	0.0365	-1.9104	3.7223	54.3890	0.0673
0.0208	4.2722	139.0077	0.0308	3.0896	3.3264	53.7909	0.0640
5.0208	3.6754	136.4828	0.0269	8.0896	2.9040	51.8983	0.0621
10.0208	3.2531	134.6175	0.0245	13.0896	2.7061	55.5725	0.0625
15.0208	2.7810	132.4873	0.0207	18.0896	3.1841	51.4712	0.0650
20.0208	2.5673	131.3138	0.0188	23.0896	2.6994	50.6977	0.0644
25.0208	2.5912	130.3802	0.0201	28.0896	2.3970	49.4350	0.0635
30.0208	2.5562	129.2825	0.0206	33.0896	2.5416	48.7626	0.0623
35.0208	2.4117	128.1568	0.0176	38.0896	2.2922	51.5175	0.0595
40.0208	2.5035	127.2222	0.0199	43.0896	2.2968	51.8213	0.0578
45.0208	2.5988	126.8493	0.0208	48.0896	2.2625	54.6783	0.0561
50.0208	2.6204	126.8320	0.0206	53.0896	2.2347	53.2290	0.0564
55.0208	2.6191	127.0107	0.0209	58.0896	2.4069	51.2986	0.0587
60.0208	2.6112	126.4859	0.0203	63.0896	2.7884	52.9277	0.0614
65.0208	2.9532	125.5870	0.0241	68.0896	3.1431	50.2648	0.0654
70.0208	3.8583	123.5631	0.0312	73.0896	2.6179	53.2663	0.0682
75.0208	5.8646	120.8365	0.0483	78.0896	4.3906	53.2708	0.0790
80.0208	9.6332	117.4664	0.0811	83.0896	5.4254	52.9584	0.0997
85.0208	15.5906	112.2864	0.1308	88.0896	7.5483	51.7905	0.1523
90.0208	23.0138	100.9107	0.2255	93.0896	11.7119	48.3509	0.2648
95.0208	26.6227	76.6761	0.3798	98.0896	15.6037	35.4469	0.4289
100.0208	22.3032	43.5691	0.5607	103.0896	14.9103	20.4893	0.5689
105.0208	13.6893	19.4973	0.6570	108.0896	9.3012	10.5251	0.6463

TableD.8 Dynamic mechanical analysis of 70% KL (Continues)

Replication 1				Replication 2			
Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ	Temp (^{o}C)	E" (MPa)	E' (MPa)	Tan δ
110.0208	5.2711	7.6492	0.4741	113.0896	4.7862	8.1479	0.7026
115.0208	0.6864	4.5524	0.0951	118.0896	-8.3840	42.6094	0.6685
120.0208	-0.6662	4.1752	-0.0739	123.0896	20.0548	-84.8557	0.3857
125.0208	-0.6162	4.1041	-0.0752	128.0896	455.8393	-1311.6961	0.0148
130.0208	-0.3817	4.0824	-0.0673	133.0896	1138.8753	-2229.0993	-0.1664
135.0208	-0.2686	4.1862	-0.0649	138.0896	391.8862	-1485.2643	0.0347
140.0208	-0.2542	4.2518	-0.0640	143.0896	145.8111	-929.4668	0.8926
145.0208	-0.2637	4.1780	-0.0657	148.0896	2495.2241	-973.6373	2.0998
150.0208	-0.2719	4.1230	-0.0667	153.0896	748.8276	-591.1647	1.5869
155.0208	-0.2743	4.1669	-0.0655	158.0896	2904.4193	1289.5856	0.5961
160.0208	-0.2744	4.1493	-0.0659	163.0896	3675.5161	304.3809	-0.0039
165.0208	-0.2741	4.0737	-0.0676	168.0896	387.3826	-1005.5719	-0.1446
170.0208	-0.2739	4.0735	-0.0675	173.0896	74.1700	-1179.1157	-0.1058
175.0208	-0.2738	4.0924	-0.0670	178.0896	177.4747	-1186.7553	-0.0632
180.0208	-0.2737	4.1220	-0.0665	183.0896	151.6880	-1249.3602	-0.0386
185.0208	-0.2737	4.1688	-0.0657	188.0896	264.7328	-585.2894	0.0034
190.0208	-0.2737	4.1727	-0.0656	193.0896	1347.8985	236.0133	0.0591
195.0208	-0.2738	4.0908	-0.0671	198.0896	1530.4367	-406.4425	0.0501

Table D.9 FT-IR spectrum of plasticized fish protein/ Kraft lignin biomaterial

Wave number (1/cm)	% Transmittance									
	0% KL		20% KL		40% KL		60% KL		70% KL	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
4000	65.00	64.81	67.88	64.52	69.97	69.97	69.89	69.95	69.99	69.94
3980	65.00	64.82	67.85	64.51	69.98	69.93	69.87	69.91	69.95	69.95
3960	64.98	64.83	67.87	64.50	69.99	69.95	69.93	69.96	69.98	69.97
3940	64.94	64.81	67.87	64.49	69.97	69.94	69.92	69.97	69.98	69.95
3920	64.92	64.79	67.88	64.48	69.97	69.94	69.88	69.98	69.97	69.95
3900	64.92	64.79	67.89	64.50	69.97	69.93	69.83	69.98	69.99	69.96
3880	64.90	64.77	67.87	64.50	69.97	69.97	69.91	69.96	69.99	69.97
3860	64.84	64.74	67.81	64.49	69.96	69.95	69.95	69.94	69.98	69.96
3840	64.75	64.71	67.72	64.47	69.94	69.93	69.96	69.91	69.99	69.96
3820	64.67	64.67	67.59	64.43	69.90	69.90	69.95	69.89	69.99	69.96
3800	64.58	64.59	67.41	64.36	69.82	69.83	69.90	69.87	69.99	69.96
3780	64.36	64.43	67.20	64.21	69.67	69.73	69.87	69.80	69.98	69.99
3760	63.85	64.11	66.87	63.92	69.40	69.45	69.65	69.63	69.98	69.94
3740	63.23	63.66	66.44	63.52	69.03	69.06	69.31	69.36	69.93	69.83
3720	62.68	63.22	65.91	63.10	68.62	68.70	69.00	69.05	69.75	69.65
3700	62.01	62.53	65.11	62.51	67.98	68.04	68.33	68.46	69.36	69.23
3680	60.62	61.05	63.59	61.15	66.50	66.53	66.55	66.91	68.19	68.03
3660	58.90	59.40	61.56	59.41	64.46	64.26	63.57	64.35	65.97	65.87
3640	56.23	56.74	58.44	56.53	61.16	60.72	59.14	60.38	62.41	62.38
3620	52.79	53.23	54.51	52.75	56.97	56.29	54.07	55.70	58.06	58.11
3600	48.90	49.02	49.83	48.18	51.81	51.16	48.48	50.28	52.75	52.94
3580	44.69	44.59	44.96	43.43	46.38	45.72	42.84	44.64	46.93	47.33
3560	40.54	40.36	40.33	38.97	41.20	40.52	37.79	39.40	41.22	41.81
3540	36.74	36.45	36.18	35.02	36.62	35.95	33.61	34.92	36.15	36.84
3520	33.02	32.68	32.40	31.38	32.58	32.03	30.16	31.13	31.93	32.61
3500	29.58	29.22	29.02	28.11	29.06	28.68	27.32	28.00	28.55	29.15
3480	26.61	26.22	26.09	25.31	26.10	25.93	25.01	25.45	25.91	26.36
3460	24.03	23.63	23.60	22.90	23.56	23.62	23.04	23.28	23.73	24.01
3440	21.99	21.64	21.71	21.13	21.64	21.88	21.53	21.61	22.06	22.18
3420	20.69	20.47	20.56	20.23	20.51	20.76	20.58	20.58	20.92	20.94
3400	20.18	20.12	20.13	20.17	20.12	20.20	20.15	20.14	20.26	20.24
3380	20.27	20.48	20.35	20.85	20.47	20.24	20.29	20.32	20.16	20.20
3360	20.91	21.49	21.23	22.33	21.56	20.80	20.98	21.12	20.54	20.74
3340	21.61	22.54	22.32	23.99	22.91	21.62	21.92	22.21	21.25	21.62
3320	22.07	23.35	23.35	25.48	24.23	22.51	22.95	23.40	22.17	22.73
3300	22.57	24.13	24.40	26.82	25.49	23.44	23.97	24.57	23.17	23.90
3280	23.67	25.40	25.77	28.28	26.85	24.59	25.02	25.75	24.26	25.12
3260	25.89	27.58	27.67	30.16	28.49	26.13	26.16	27.00	25.41	26.39
3240	28.44	30.05	29.81	32.26	30.32	27.88	27.42	28.40	26.69	27.78
3220	30.60	32.19	31.80	34.26	32.17	29.64	28.76	29.91	28.14	29.33
3200	32.63	34.18	33.73	36.20	34.01	31.44	30.17	31.47	29.73	30.99
3180	34.92	36.42	35.87	38.27	36.02	33.45	31.69	33.14	31.45	32.76
3160	37.38	38.79	38.13	40.39	38.13	35.62	33.30	34.88	33.29	34.62
3140	39.63	40.95	40.26	42.35	40.17	37.73	34.92	36.62	35.19	36.51

Table D.9 FT-IR spectrum of plasticized fish protein/ Kraft lignin biomaterial (Continues)

Wave number (1/cm)	% Transmittance									
	0% KL		20% KL		40% KL		60% KL		70% KL	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
3120	41.30	42.54	42.04	43.96	41.99	39.64	36.49	38.29	37.07	38.37
3100	42.12	43.33	43.27	45.10	43.49	41.24	38.03	39.94	39.08	40.34
3080	42.45	43.64	44.02	45.82	44.57	42.42	39.37	41.40	40.96	42.17
3060	43.27	44.41	44.93	46.68	45.63	43.62	40.59	42.71	42.59	43.77
3040	45.25	46.24	46.57	48.14	47.13	45.26	41.92	44.10	44.10	45.22
3020	46.82	47.76	47.94	49.30	48.34	46.57	42.89	45.08	45.07	46.14
3000	47.84	48.75	48.80	50.06	49.09	47.39	43.54	45.72	45.69	46.74
2980	44.73	45.89	46.54	48.13	47.55	45.67	42.74	45.00	45.10	46.16
2960	38.96	40.66	41.48	43.57	42.62	40.49	38.62	40.67	40.34	41.59
2940	37.24	39.14	39.58	41.81	40.24	38.08	36.20	38.01	37.17	38.52
2920	37.15	39.15	40.49	42.54	41.43	39.43	37.54	39.42	39.06	40.40
2900	42.38	43.99	43.86	45.76	44.17	42.32	39.44	41.46	41.21	42.50
2880	42.27	43.93	43.48	45.49	43.69	41.86	38.90	40.90	40.55	41.87
2860	44.24	45.86	45.88	47.57	46.14	44.53	41.22	43.29	43.34	44.59
2840	48.98	50.22	49.23	50.61	48.62	47.27	42.99	45.06	45.08	46.28
2820	51.82	52.85	52.04	53.12	51.72	50.60	46.13	48.20	48.65	49.69
2800	53.79	54.68	53.96	54.80	53.73	52.74	48.29	50.33	50.97	51.89
2780	55.28	56.09	55.39	56.05	55.17	54.29	49.90	51.88	52.60	53.45
2760	56.56	57.28	56.53	57.04	56.25	55.44	51.08	53.02	53.77	54.55
2740	57.67	58.33	57.49	57.89	57.12	56.38	52.01	53.90	54.65	55.40
2720	58.80	59.41	58.52	58.79	58.06	57.40	53.05	54.88	55.61	56.31
2700	59.73	60.30	59.45	59.60	58.98	58.39	54.13	55.88	56.62	57.26
2680	60.27	60.83	60.03	60.11	59.61	59.05	54.91	56.62	57.36	57.97
2660	60.77	61.34	60.51	60.54	60.09	59.57	55.52	57.19	57.92	58.50
2640	61.39	61.95	61.06	61.02	60.57	60.08	56.09	57.72	58.39	58.95
2620	62.13	62.65	61.74	61.62	61.21	60.77	56.88	58.44	59.07	59.60
2600	62.90	63.39	62.49	62.27	61.89	61.51	57.71	59.19	59.76	60.26
2580	63.51	63.99	63.18	62.87	62.57	62.24	58.59	59.97	60.50	60.97
2560	63.95	64.42	63.72	63.36	63.17	62.87	59.40	60.71	61.21	61.65
2540	64.33	64.86	64.22	63.80	63.73	63.45	60.17	61.42	61.90	62.30
2520	64.68	65.32	64.65	64.18	64.22	63.97	60.89	62.06	62.53	62.89
2500	65.13	65.85	65.14	64.63	64.74	64.52	61.62	62.72	63.15	63.49
2480	65.78	66.43	65.69	65.22	65.30	65.12	62.41	63.41	63.80	64.11
2460	66.45	67.02	66.28	65.83	65.90	65.75	63.25	64.15	64.48	64.76
2440	67.08	67.58	66.86	66.44	66.51	66.40	64.14	64.92	65.20	65.45
2420	67.69	68.11	67.43	67.03	67.11	67.03	65.01	65.67	65.89	66.11
2400	68.20	68.56	67.93	67.57	67.66	67.61	65.85	66.40	66.57	66.75
2380	68.60	68.88	68.34	68.01	68.12	68.07	66.59	67.05	67.20	67.32
2360	68.81	69.10	68.65	68.33	68.43	68.40	67.17	67.55	67.66	67.70
2340	69.00	69.28	68.90	68.60	68.69	68.69	67.64	67.95	68.04	68.05
2320	69.20	69.43	69.12	68.85	68.89	68.96	68.03	68.22	68.34	68.36
2300	69.48	69.62	69.37	69.14	69.19	69.23	68.41	68.60	68.69	68.73
2280	69.74	69.81	69.65	69.44	69.50	69.52	68.90	69.06	69.09	69.12
2260	69.91	69.95	69.85	69.68	69.73	69.76	69.34	69.45	69.46	69.46

Table D.9 FT-IR spectrum of plasticized fish protein/ Kraft lignin biomaterial (Continues)

Wave number (1/cm)	% Transmittance									
	0% KL		20% KL		40% KL		60% KL		70% KL	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
2240	69.98	70.00	69.96	69.84	69.88	69.91	69.64	69.71	69.73	69.71
2220	70.00	70.00	70.00	69.94	69.96	69.98	69.83	69.88	69.91	69.87
2200	69.99	69.98	69.99	69.98	69.96	69.98	69.90	69.95	70.00	69.95
2180	69.94	69.93	69.94	69.93	69.90	69.91	69.83	69.90	69.98	69.92
2160	69.84	69.84	69.83	69.83	69.80	69.80	69.69	69.79	69.88	69.82
2140	69.75	69.75	69.73	69.74	69.73	69.71	69.59	69.71	69.80	69.75
2120	69.67	69.67	69.64	69.66	69.64	69.63	69.48	69.62	69.71	69.67
2100	69.63	69.62	69.56	69.58	69.56	69.55	69.35	69.51	69.60	69.57
2080	69.61	69.59	69.47	69.49	69.42	69.41	69.10	69.30	69.39	69.38
2060	69.67	69.65	69.48	69.48	69.37	69.38	68.90	69.13	69.19	69.21
2040	69.79	69.77	69.66	69.63	69.57	69.59	69.22	69.37	69.42	69.43
2020	69.91	69.89	69.85	69.80	69.79	69.81	69.60	69.69	69.72	69.71
2000	69.98	69.97	69.96	69.93	69.93	69.96	69.88	69.92	69.96	69.93
1980	69.95	69.96	69.94	69.94	69.92	69.95	69.87	69.90	69.96	69.93
1960	69.93	69.96	69.94	69.95	69.89	69.94	69.81	69.85	69.91	69.88
1940	69.89	69.97	69.98	69.99	69.92	69.98	69.86	69.89	69.95	69.91
1920	69.70	69.89	69.92	69.95	69.93	69.92	69.85	69.97	69.96	69.92
1900	69.31	69.50	69.66	69.82	69.85	69.78	69.82	69.92	69.95	69.89
1880	68.82	69.04	69.35	69.64	69.70	69.60	69.72	69.87	69.92	69.85
1860	68.18	68.43	68.93	69.37	69.50	69.38	69.61	69.79	69.89	69.83
1840	67.24	67.58	68.32	68.93	69.17	69.11	69.52	69.70	69.93	69.91
1820	66.08	66.46	67.44	68.25	68.60	68.46	69.10	69.40	69.81	69.83
1800	64.47	65.02	66.15	67.18	67.46	67.37	67.83	68.28	69.07	69.13
1780	62.31	62.95	64.16	65.49	65.51	65.28	65.02	65.95	67.03	67.18
1760	58.75	59.69	61.11	62.77	62.71	62.44	61.85	63.15	64.51	64.74
1740	53.01	54.41	56.27	58.32	58.18	57.73	57.22	59.17	61.24	61.59
1720	48.54	49.63	51.72	54.35	53.82	52.79	52.78	55.30	57.68	58.25
1700	37.95	39.13	43.47	47.24	47.77	46.16	48.76	51.66	54.84	55.62
1680	27.00	28.88	33.61	38.26	39.18	36.72	43.59	47.19	53.22	54.03
1660	21.57	23.75	28.31	33.25	33.97	31.17	40.02	43.98	51.85	52.62
1640	22.81	24.70	29.71	33.77	35.30	32.62	40.43	43.77	51.06	51.75
1620	32.84	34.50	38.78	41.50	42.27	39.93	41.98	44.23	47.63	48.58
1600	42.19	43.01	43.32	46.66	43.32	41.21	39.18	41.35	41.09	42.54
1580	39.18	40.37	42.88	46.94	45.59	43.22	43.56	46.17	47.52	48.77
1560	33.82	35.80	39.54	44.26	44.58	41.88	46.73	50.25	55.37	56.21
1540	30.43	32.82	36.97	42.01	42.33	39.51	45.08	48.75	55.05	55.94
1520	34.49	36.23	37.72	42.43	38.32	35.65	34.91	37.04	35.97	37.62
1500	43.65	44.32	43.95	48.13	43.38	41.12	38.79	41.11	40.27	41.87
1480	50.33	50.79	48.31	51.99	47.06	45.11	41.01	43.47	42.77	44.21
1460	44.67	45.73	42.48	46.54	40.71	38.44	34.94	37.06	35.13	36.70
1440	44.60	45.57	43.03	47.12	42.20	40.02	36.85	39.22	37.90	39.37
1420	44.50	45.58	42.30	46.54	41.16	38.81	35.42	37.54	35.76	37.28
1400	41.22	42.68	41.57	46.00	41.78	39.25	36.93	39.01	37.67	39.20
1380	45.75	46.79	44.41	48.61	43.48	41.26	38.15	40.30	39.28	40.88

Table D.9 FT-IR spectrum of plasticized fish protein/ Kraft lignin biomaterial (Continues)

Wave number (1/cm)	% Transmittance									
	0% KL		20% KL		40% KL		60% KL		70% KL	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
1360	50.13	51.04	47.07	51.03	45.29	43.33	39.27	41.42	40.35	41.97
1340	50.26	51.11	47.38	51.40	46.10	43.88	39.96	42.14	41.33	42.98
1320	49.81	50.60	46.85	51.02	45.55	43.38	39.51	41.71	40.61	42.29
1300	50.55	51.22	46.53	50.74	44.29	42.16	37.81	39.88	38.08	39.84
1280	50.96	51.49	44.42	48.72	40.04	37.82	33.14	34.69	31.55	33.38
1260	50.22	50.48	44.22	48.51	39.92	37.70	33.06	34.57	31.59	33.46
1240	47.75	47.73	44.08	48.38	41.14	38.90	34.77	36.58	34.27	36.13
1220	49.39	48.76	44.21	48.34	40.09	38.06	33.34	34.94	32.30	34.15
1200	52.51	51.00	47.07	51.05	42.84	41.03	35.42	37.26	35.19	37.06
1180	55.03	52.44	50.52	54.43	47.02	45.22	39.53	41.75	40.43	42.24
1160	55.24	51.94	50.26	54.26	46.61	44.76	38.91	41.04	39.11	40.94
1140	55.86	51.72	49.67	53.83	45.60	43.66	37.33	39.33	36.76	38.60
1120	44.24	41.35	41.42	46.04	39.04	36.69	32.44	34.29	31.76	33.54
1100	43.43	39.93	41.34	45.96	39.49	37.30	32.78	34.76	32.85	34.68
1080	45.22	41.92	43.07	47.63	41.30	39.24	34.38	36.39	34.59	36.42
1060	38.60	37.28	37.58	42.52	36.55	34.29	30.94	32.78	30.99	32.78
1040	34.88	34.49	32.92	38.15	30.81	28.50	25.58	26.89	24.50	26.00
1020	47.02	45.30	42.30	47.43	38.32	36.67	30.12	32.16	30.55	32.36
1000	52.56	50.41	48.46	53.57	45.90	44.39	36.95	39.51	38.99	40.73
980	55.90	53.74	51.24	56.42	48.67	47.33	39.30	42.04	41.80	43.49
960	62.66	59.90	57.31	62.39	54.17	53.14	44.15	46.95	46.91	48.43
940	64.10	61.68	59.23	64.21	56.98	55.83	47.99	50.67	50.75	52.12
920	59.89	58.61	54.83	60.15	52.82	51.41	44.02	46.77	46.78	48.33
900	64.86	63.13	59.57	64.76	57.37	56.39	48.79	51.37	51.73	52.98
880	65.98	64.36	60.34	65.64	58.26	57.14	50.16	52.64	52.70	53.87
860	61.31	60.38	54.89	60.58	52.37	50.81	43.61	46.15	45.80	47.23
840	63.01	61.90	57.03	62.56	54.66	53.21	45.72	48.34	48.48	49.73
820	63.67	62.41	57.54	62.81	54.84	53.34	45.84	48.37	48.23	49.55
800	62.68	61.24	57.37	62.63	55.36	53.81	46.77	49.29	49.49	50.69
780	60.21	59.27	55.85	61.23	54.63	52.96	46.64	49.18	49.61	50.82
760	58.25	57.61	54.53	60.21	53.99	52.19	46.54	49.11	49.78	50.97
740	55.54	55.23	52.54	58.38	52.49	50.54	45.47	48.03	48.90	50.09
720	53.07	53.07	50.90	56.86	51.32	49.33	44.74	47.28	48.42	49.60
700	50.09	50.36	48.59	54.75	49.45	47.35	43.37	45.98	47.34	48.55
680	48.32	48.58	46.46	52.78	47.19	45.04	41.06	43.72	44.92	46.16
660	48.11	48.24	46.00	52.34	46.55	44.48	40.32	43.02	44.30	45.50
640	48.18	48.24	45.85	52.03	46.03	44.19	39.62	42.25	43.49	44.70
620	47.57	47.48	44.66	50.74	44.06	42.31	37.48	40.11	41.16	42.44
600	47.14	46.91	44.74	50.57	44.80	43.32	38.53	41.12	42.86	44.01
580	47.29	46.87	44.49	50.23	44.42	42.99	38.08	40.74	42.63	43.72
560	47.00	46.45	43.82	49.62	43.47	42.09	36.87	39.64	41.35	42.56
540	47.80	47.17	44.50	50.25	44.03	42.87	37.28	40.06	42.08	43.23
520	48.53	47.88	45.38	50.81	44.84	43.86	37.83	40.65	42.90	44.04
500	48.89	48.30	45.90	51.15	45.07	44.36	38.24	40.89	43.19	44.18

Table D.9 FT-IR spectrum of plasticized fish protein/ Kraft lignin biomaterial (Continues)

Wave number (1/cm)	% Transmittance									
	0% KL		20% KL		40% KL		60% KL		70% KL	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
480	49.22	48.53	45.96	51.46	45.46	44.97	38.59	40.81	43.47	44.33
460	50.64	49.68	47.35	52.52	46.60	46.20	39.16	41.64	44.34	45.21
440	52.25	51.85	48.77	53.83	47.67	47.60	39.94	42.53	45.50	46.22
420	53.37	53.56	49.64	54.71	48.54	48.61	40.54	43.31	46.24	47.11
400	53.68	55.82	48.98	55.51	49.02	48.83	40.92	44.47	46.66	47.95

Table D.10 Mechanical properties of plasticized fish protein/ Kraft lignin biomaterial at 100 ºC

Sample	Young (MPa)	Tensile strength (MPa)	Elongation at break (%)
0% KL	35.872	2.055	10.270
	19.890	0.993	7.095
	22.961	1.456	20.995
	23.124	1.342	24.234
	24.332	1.762	25.012
	21.439	1.181	30.325
	42.303	2.601	31.432
	22.438	1.042	30.542
10% KL	29.879	3.508	15.695
	20.351	2.439	15.795
	24.797	3.089	21.195
	32.493	3.248	14.695
	29.953	2.989	24.873
	31.181	3.233	23.986
	32.410	2.490	22.974
	33.639	3.017	30.865
20% KL	36.969	3.227	20.395
	39.842	4.480	14.820
	32.460	4.875	18.195
	26.908	4.326	23.595
	50.324	4.840	18.620
	45.203	4.754	23.987
	41.874	4.103	26.972
30% KL	45.732	5.448	15.120
	37.229	5.505	23.170
	43.584	3.758	20.070
40% KL	40.667	3.821	10.020
	26.008	3.568	15.320
	27.807	3.643	11.270
	27.005	3.347	11.345
	29.267	3.642	12.895
50% KL	18.674	1.248	7.070
	18.007	1.158	5.170

Table D.11 Mechanical properties of plasticized fish protein/ Kraft lignin biomaterial at 130 ºC

Sample	Young (MPa)	Tensile strength (MPa)	Elongation at break (%)
0% KL	25.639	1.505	8.545
	28.875	2.318	14.770
	25.989	1.337	8.245
	31.101	2.153	11.995
10% KL	40.190	3.713	10.945
	36.797	3.191	11.295
	43.093	3.138	11.045
	40.634	3.101	10.095
20% KL	58.861	4.727	10.370
	48.782	3.574	9.020
	61.172	3.680	6.995
	86.396	4.447	6.170
	75.163	5.471	10.520
30% KL	60.583	5.123	10.020
	63.717	4.906	6.295
	71.588	5.508	10.370
	72.400	5.565	7.245
40% KL	66.206	4.487	8.645
	60.826	3.412	5.195
	68.934	3.902	6.895
	62.414	2.911	5.145
50% KL	8.631	0.336	1.870
	16.283	0.225	0.370
	1.156	0.060	1.370
	-2.851	0.114	0.170

Table D.12 Water absorption of plasticized fish protein/ Kraft lignin biomaterial at 100 ºC

Fiber content	**Replication**	**Water absorption (%)**
0 % KL	1	59.14
	2	58.10
	3	54.17
	4	55.05
10 % KL	1	56.88
	2	54.68
	3	53.80
	4	55.49
20 % KL	1	43.53
	2	41.55
	3	41.25
	4	43.26
30 % KL	1	42.21
	2	42.81
	3	41.37
	4	42.25
40 % KL	1	33.28
	2	33.55
	3	33.56
	4	32.95
50 % KL	1	30.64
	2	33.77
	3	32.91
	4	32.81
60 % KL	1	31.07
	2	32.25
	3	31.40
	4	33.26
70 % KL	1	30.44
	2	31.51
	3	30.15
	4	31.16

Table D.13 Water absorption of plasticized fish protein/ Kraft lignin biomaterial at 130 ºC

Fiber content	Replication	Water absorption (%)
0 % KL	1	55.91
	2	53.84
	3	54.25
	4	54.96
10 % KL	1	50.53
	2	51.66
	3	51.24
	4	49.68
20 % KL	1	41.26
	2	38.55
	3	40.68
	4	42.56
30 % KL	1	36.35
	2	35.47
	3	35.48
	4	35.46
40 % KL	1	32.09
	2	32.39
	3	33.36
	4	33.20
50 % KL	1	32.53
	2	32.18
	3	30.95
	4	32.03
60 % KL	1	30.99
	2	30.53
	3	31.53
	4	30.24
70 % KL	1	30.43
	2	29.42
	3	29.42
	4	29.92

APPENDIX E

Statistic analysis

Table E.1 ANOVA table of water absorption of biomaterial at 100ºC

Water absorption

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3053.274	7	436.182	283.669	.000
Within Groups	36.903	24	1.538		
Total	3090.177	31			

	KL	N	Subset for alpha = .05			
			1	2	3	4
Duncan(a)	70.00	4	30.8150			
	60.00	4	31.9950	31.9950		
	50.00	4	32.5325	32.5325		
	40.00	4		33.3350		
	30.00	4			42.1600	
	20.00	4			42.3975	
	10.00	4				55.2125
	.00	4				56.6150
	Sig.		.075	.161	.789	.123

Means for groups in homogeneous subsets are displayed.

Table E.2 ANOVA table of water absorption of biomaterial at 130ºC

Water absorption

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2553.588	7	364.798	489.910	.000
Within Groups	17.871	24	.745		
Total	2571.459	31			

	KL	N	Subset for alpha = .05						
			1	2	3	4	5	6	7
Duncan(a)	70.00	4	29.7998						
	60.00	4	30.8199	30.8199					
	50.00	4		31.9208	31.9208				
	40.00	4			32.7608				
	30.00	4				35.6930			
	20.00	4					40.7629		
	10.00	4						50.7805	
	.00	4							54.7403
	Sig.		.108	.084	.181	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table E.3 ANOVA table of Young's modulus of biomaterial at 100 ºC

Young's modulus

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1333.342	5	266.668	6.276	.001
Within Groups	1147.316	27	42.493		
Total	2480.658	32			

	KL	N	Subset for alpha = .05			
			1	2	3	4
Duncan(a,b)	50.00	2	18.3405			
	.00	8	26.5449	26.5449		
	10.00	8		29.3379		
	40.00	5		30.1508	30.1508	
	20.00	7			39.0829	39.0829
	30.00	3				42.1817
	Sig.		.079	.457	.057	.496

Means for groups in homogeneous subsets are displayed.

Table E.4 ANOVA table of Tensile strength of biomaterial at 100 ºC

Tensile strength

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	48.754	5	9.751	36.439	.000
Within Groups	7.225	27	.268		
Total	55.979	32			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a,b)	50.00	2	1.2030		
	.00	8	1.5540		
	10.00	8		3.0016	
	40.00	5		3.6042	
	20.00	7			4.3721
	30.00	3			4.9037
	Sig.		.334	.103	.148

Means for groups in homogeneous subsets are displayed.

Table E.5 ANOVA table of elongation at break of biomaterial 100 ºC

Elongation at break

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	730.167	5	146.033	4.030	.007
Within Groups	978.495	27	36.241		
Total	1708.662	32			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a,b)	50.00	2	6.1200		
	40.00	5	12.1700	12.1700	
	30.00	3		19.4533	19.4533
	20.00	7		20.9406	20.9406
	10.00	8		21.2598	21.2598
	.00	8			22.4881
	Sig.		.156	.053	.512

Means for groups in homogeneous subsets are displayed.

Table E.6 ANOVA table of Young's modulus of biomaterial at 130 ºC

Young's modulus

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	13082.422	5	2616.484	39.218	.000
Within Groups	1267.605	19	66.716		
Total	14350.027	24			

	KL	N	Subset for alpha = .05			
			1	2	3	4
Duncan(a,b)	50.00	4	5.8048			
	.00	4		27.9010		
	10.00	4			40.1785	
	40.00	4				64.5950
	20.00	5				66.0748
	30.00	4				67.0720
	Sig.		1.000	1.000	1.000	.685

Means for groups in homogeneous subsets are displayed.

Table E.7 ANOVA table of Tensile strength of biomaterial at 130 ºC

Tensile strength

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	69.004	5	13.801	51.416	.000
Within Groups	5.100	19	.268		
Total	74.103	24			

	KL	N	Subset for alpha = .05				
			1	2	3	4	5
Duncan(a,b)	50.00	4	.1838				
	.00	4		1.8283			
	10.00	4			3.2858		
	40.00	4			3.6780	3.6780	
	20.00	5				4.3798	
	30.00	4					5.2755
	Sig.		1.000	1.000	.290	.066	1.000

Means for groups in homogeneous subsets are displayed.

Table E.8 ANOVA table of elongation at break of biomaterial 130 ºC

Elongation at break

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	275.395	5	55.079	15.489	.000
Within Groups	67.564	19	3.556		
Total	342.959	24			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a,b)	50.00	4	.9450		
	40.00	4		6.4700	
	30.00	4		8.4825	8.4825
	20.00	5		8.6150	8.6150
	10.00	4			10.8450
	.00	4			10.8887
	Sig.		1.000	.137	.107

Means for groups in homogeneous subsets are displayed.

Table E.9 ANOVA table of storage modulus (E') of biomaterial

Storage modulus

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1000.656	7	142.951	25.622	.000
Within Groups	44.634	8	5.579		
Total	1045.290	15			

	KL	N	Subset for alpha = .05				
			1	2	3	4	5
Duncan(a)	70.00	2	3.4275				
	60.00	2	5.9269	5.9269			
	50.00	2	7.1025	7.1025			
	40.00	2	8.0647	8.0647			
	30.00	2		10.6793	10.6793		
	20.00	2			15.2568	15.2568	
	10.00	2				16.9420	
	.00	2					29.7163
	Sig.		.102	.095	.089	.496	1.000

Means for groups in homogeneous subsets are displayed.

Table E.10 ANOVA table of Tan δ peak high of biomaterial

Tan δ peak high

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.344	7	.049	16.318	.000
Within Groups	.027	9	.003		
Total	.371	16			

	KL	N	Subset for alpha = .05				
			1	2	3	4	5
Duncan(a,b)	.00	2	.3199				
	10.00	2	.3441				
	20.00	2	.4349	.4349			
	30.00	2		.4880	.4880		
	40.00	2			.5857	.5857	
	60.00	2			.5922	.5922	
	50.00	2				.6385	.6385
	70.00	3					.7358
	Sig.		.070	.349	.096	.372	.103

Means for groups in homogeneous subsets are displayed.

Table E.11 ANOVA table of Glass transition temperature of biomaterial

Glass transition temperature

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	166.175	7	23.739	2.543	.097
Within Groups	84.007	9	9.334		
Total	250.182	16			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a,b)	.00	2	105.5004		
	60.00	2	106.6045	106.6045	
	10.00	2	107.7780	107.7780	107.7780
	70.00	3	109.6424	109.6424	109.6424
	50.00	2	111.3008	111.3008	111.3008
	40.00	2		113.0432	113.0432
	20.00	2		113.0673	113.0673
	30.00	2			115.1222
	Sig.		.107	.079	.052

Means for groups in homogeneous subsets are displayed.

Table E.12 ANOVA table of consistency (K) of biomaterial

Consistency (K)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	21530582652.600	4	5382645663.150	53.763	.000
Within Groups	500587311.000	5	100117462.200		
Total	22031169963.600	9			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a)	60.00	2	28458.5000		
	70.00	2	30235.0000		
	40.00	2	47726.5000		
	20.00	2		105266.0000	
	.00	2			145693.0000
	Sig.		.120	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table E.13 ANOVA table of power law index (n) of biomaterial

Power law index

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.047	4	.012	42.107	.000
Within Groups	.001	5	.000		
Total	.049	9			

	KL	N	Subset for alpha = .05	
			1	2
Duncan(a)	70.00	2	.0000	
	20.00	2	.0250	
	60.00	2	.0250	
	40.00	2		.1450
	.00	2		.1650
	Sig.		.206	.286

Means for groups in homogeneous subsets are displayed.

Table E.14 ANOVA table of soluble protein of biomaterial

Soluble protein

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4347.213	4	1086.803	50.216	.000
Within Groups	216.426	10	21.643		
Total	4563.639	14			

	KL	N	Subset for alpha = .05		
			1	2	3
Duncan(a)	0.00	3	18.1607		
	20.00	3	21.1877	21.1877	
	40.00	3	22.7010	22.7010	
	60.00	3		27.9977	
	FP	3			64.3187
	Sig.		.280	.117	1.000

Means for groups in homogeneous subsets are displayed.

Table E.15 ANOVA table of insoluble protein of biomaterial

Insoluble protein

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	469.312	4	117.328	12.117	.001
Within Groups	96.829	10	9.683		
Total	566.141	14			

	KL	N	Subset for alpha = .05	
			1	2
Duncan(a)	0.00	3	8.3227	
	60.00	3	9.0810	
	20.00	3	10.9717	
	40.00	3	11.5387	
	FP	3		23.6473
	Sig.		.265	1.000

Means for groups in homogeneous subsets are displayed.

CURRICULUM VITAE

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The 12th ASEAN Food Conference 2011, pp 235-243.