PROPERTIES OF SOY PROTEIN ISOLATE-CHITOSAN BIOADHESIVES FOR PRODUCTION OF OIL PALM TRUNK PARTICLEBOARD

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
cm	Centimeter
C_{Ir}	Crystallinity Index
°C	Degree Celsius
°C/min	Degree Celsius per minute
g	Gram
kDa	Kilodalton
M	Molarity
min	Minute (s)
mL	Milliliter
mm	Micrometer
MPa	Megapascal
N/mm^2	Newton per square millimeter
%	Percentage
rpm	Revolution per minute

LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Material
CH	Chitosan
DA	Degree of Acetylation
DD	Degree of Deacetylation
DSC	Differential Scanning Calorimetry
DSF	Defatted Soy Flour
DTG	Derivative Thermogravimetric
EFB	Empty Fruit Bunch
FTIR	Fourier Transform Infrared
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
IB	Internal Bonding
JIS	Japanese Industrial Standard
KBr	Potassium Bromide
MC	Moisture Content
MF	Mesocarp Fibre
MOE	Modulus of Elasticity
MOR	Modulus of Rupture
MPOB	Malaysia Palm Oil Board
MW	Molecular Weight
NaOH	Sodium Hydroxide
OPF	Oil Palm Frond
OPT	Oil Palm Trunk
OD	Oven Dry
PKS	Palm Kernel Shell
POME	Palm Oil Mill Effluent
SBA	Soy Based Adhesive
SEM	Scanning Electron Microscopy
SPC	Soy Protein Concentrate
SPI	Soy Protein Isolate
TGA	Thermogravimetric Analysis

TS	Thickness Swelling
UF	Urea Formaldehyde
WA	Water Absorption
XRD	X-Ray Diffractometry

SIFAT BIOPEREKAT PROTEIN SOYA TERPENCIL – KITOSAN UNTUK PENGHASILAN BOD PARTIKEL BERASASKAN BATANG KELAPA SAWIT

ABSTRAK

Perekat berasaskan formaldehid telah digunakan secara komersial dalam penghasilan komposit berasaskan kayu. Walau bagaimanapun, memandangkan ia mengandungi formaldehid karsinogenik yang berbahaya terutamanya kepada kesihatan manusia, para penyelidik di seluruh dunia telah memberi tumpuan dalam menghasilkan perekat bebas formaldehid daripada sumber semula jadi. Protein soya telah digunakan dalam penghasilan perekat sejak dahulu lagi kerana sifatnya yang baik seperti pertumbuhan yang cepat, biokompatibiliti dan mesra alam. Dalam kajian ini, bioperekat daripada protein soya terpencil dan kitosan dihasilkan dan dicampurkan dengan batang pokok kelapa sawit untuk menghasilkan bod partikel bertujuan untuk memperbaiki sifat-sifat bod partikel tersebut terutamanya kestabilan dimensinya. Bod partikel dengan ketumpatan sasaran 0.80 g/mm^3 dan kandungan perekat sebanyak 10% (berdasarkan berat kering batang kelapa sawit) dihasilkan dengan mencampurkan partikel daripada batang kelapa sawit dan perekat protein soya terpencil dan kitosan pada nisbah berbeza (3:1, 2:1, 1:1, 1:2 dan 1:3) dan ditekan menggunakan mesin penekan panas pada suhu 180°C dan tekanan 5 MPa selama 8 minit. Sifat-sifat perekat yang dihasilkan seperti kelikatan dan kandungan pepejal dinilai dan dibandingkan sesama sendiri. Analisis kimia bagi batang kelapa sawit termasuk kandungan ekstraktif, holoselulosa, selulosa alfa dan kandungan lignin dinilai untuk menentukan kesannya terhadap kekuatan mekanikal bod partikel yang dihasilkan. Bod partikel terikat dengan protein soya terasing dan kitosan yang dihasilkan diuji sifat fizikal,

mekanikal, haba serta imej morfologinya dan dibandingkan dengan bod partikel yang terikat dengan perekat komersial urea formaldehid. Hasil kajian menunjukkan bahawa bod partikel yang dihasilkan daripada perekat protein soya terasing dan kitosan pada nisbah 1:3 menunjukkan peningkatan yang ketara secara keseluruhannya dalam ujian kekuatan ikatan dalaman (IB), kekuatan modulus kepecahan (MOR), kekuatan modulus keanjalan (MOE), pengembangan ketebalan (TS) dan penyerapan air (WA) berbanding formulasi lain. Manakala, komposit yang diikat dengan perekat nisbah 3:1 and 2:1 menunjukkan peningkatan paling sedikit antara formulasi lain. Bod partikel dengan perekat protein soya terasing dan penambahan kitosan menunjukkan peningkatan dalam kekuatan fizikal dan mekanikal berbanding dengan yang dihasilkan dengan perekat protein soya terasing dan kitosan sahaja. Kesemua komposit yang dihasilkan telah memenuhi piawaian dalam Japanese Industrial Standards (JIS A5903:2003) untuk bod partikel jenis 8, jenis 13 dan jenis 18. Berdasarkan keputusan yang diperolehi dalam kajian ini, bio-perekat daripada protein soya terasing dan kitosan serta batang kelapa sawit mempunyai potensi untuk digunakan bagi menggantikan perekat formaldehid dan partikel kayu dalam penghasilan bod partikel.

PROPERTIES OF SOY PROTEIN ISOLATE – CHITOSAN BIOADHESIVES FOR PRODUCTION OF OIL PALM TRUNK PARTICLEBOARD

ABSTRACT

Formaldehyde-based adhesive had been used commercially in production of wood-based composite. However, since it contains carcinogenic formaldehyde that is hazardous mainly to human health, researchers all over the globe are focusing on producing formaldehyde-free adhesive from natural resources. Soy protein had been used to produce adhesive since the early years due to its good properties such as fast growth, biocompatibility, and environmentally friendly. In this study, bioadhesive from soy protein isolate with addition of chitosan were produced then mixed with oil palm trunk to make particleboard with the aim to improve the properties of the particleboard especially its dimensional stability. The particleboard with targeted density of 0.80 g/mm^3 and 10% adhesive content (based on oven dry weight of oil palm trunk) were produced by mixing the oil palm trunk particles with different ratio soy protein isolate and chitosan (3:1, 2:1, 1:1, 1:2 and 1:3) before pressed with hot press machine at 180°C and 5 MPa pressure for 8 minutes. The properties of adhesives made such as its viscosity and solid content were evaluated and compared with each other. The chemical analysis of oil palm trunk including its extractives, holocellulose, alpha cellulose and lignin content were assessed to determine its effect on the mechanical strength of particleboard produced. The particleboards bonded with soy protein isolate and chitosan adhesives produced were tested for its physical, mechanical and thermal properties as well as its morphological images and compared with the particleboards bonded with commercial urea formaldehyde adhesive. The

result showed that particleboard produced with soy protein and chitosan with ratio of 1:3 exhibit the overall substantial improvement for internal bonding strength (IB), modulus of rupture (MOR), and modulus of elasticity (MOE), thickness swelling (TS) and water absorption (WA) compared to other formulations. While, composite bonded with adhesive ratio 3:1 and 2:1 showed the least improvement among all blend formulations. The particleboard with soy protein isolate adhesive with addition of chitosan show improvements in physical and mechanical strength compared to those produced with soy protein adhesive and chitosan only. All the composite produced had met the Japanese Industrial Standard (JIS A5903:2003) for particleboard Type 8, Type 13 and Type 18. Based on the result obtained in this study, the bio adhesive from soy protein isolate and chitosan as well as oil palm trunk have a potential to be utilize to substitute the formaldehyde adhesive and wood particle in production of particleboard.

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm (*Elaeis guineensis*) is the preferable agricultural plant in Malaysia since years ago due to its commercial value and high demand of oil palm product. Malaysia and Indonesia recorded 86.5% of the 57 million of global palm oil production in 2019 (Noirot et al., 2022). In 2021, over 5.74 million hectare of Malaysian land had been used as oil palm planted area (MPOB, 2022) marking this industry as the one of industry that contribute the most on economic growth of Malaysia. Consequently, a large amount of oil palm biomass in form of oil palm trunks (OPT), fronds, empty fruit bunches and leaves were being generated in harvesting site. Normally, a method such as push-fell and shred method by using heavy equipment are used to eliminate OPT in order to clear the land for replanting purpose as well as for nutrient recycling process. (Uke et al., 2021). However, the OPT biomass usually takes more than 2 years to decomposed. Therefore, in order to tackle this improper disposal problem, OPT been used as raw material to produce value added products as stated in several studies (Khalil et al., 2012; Lee et al., 2018; Rattanaporn et al., 2018; Che Ismail et al., 2022).

Adhesive has been used in variety of items from shoes, plasters, packaging, self-adhesive note/envelope to wood composites and so on. In order to produce wood based panel such as particleboard with good physical and mechanical properties, synthetic adhesive had been incorporated in the production of this wood based panel. The formaldehyde based adhesive can offer superior strength and good water resistance to the composite, hence it's become the preference in the board industries (Nordqvist et al., 2010). Until now, formaldehyde-based resin such as urea formaldehyde (UF) was used commercially in panel for interior while phenol

formaldehyde (PF) resins was used in panel for exterior purposed (Frihart and Lorenzo, 2013; Kristak et al., 2023). However, the International Agency for Research on Cancer (IARC) reported that formaldehyde is carcinogenic to human (Beane Freeman et al., 2013). Moreover, due to the growing concern on environment issues, human health as well as the limited reserve of oil, adhesives made from natural and renewable resources has become interesting product for the industry of adhesives (Khosravi et al., 2010; Santoni and Pizzo, 2013). Therefore, increasing number of studies has been conducted to test the compatibility of adhesives from natural resources in composites especially wood composites (Islam et al., 2022; Ma et al., 2023).

There are various types of agricultural biomass product that can be used to produce adhesives for coatings, packaging and furnishing purpose including protein, gelatin, lignin, tannin and polysaccharides (Chen et al., 2017). Protein based adhesive based on animal resource normally originate from blood, hoof, hide, milk and fish scale while, soybean and wheat was reported as a major protein resource from plant (Lambuth, 2003; Frihart, 2010; Wool and Sun, 2011). Among all plant protein, soybeans are the major raw material for preparing vegetable-protein-based adhesives due to their high soy protein content. One of the soy protein product is soy protein isolate (SPI), which is derived from defatted soy flour (DSF) that contains 90% crude soy protein and is easily modified to produce soy-based adhesives (SBA) with high water resistances (Chen et al., 2013, Qin et al., 2022). Moreover, as one of reproducible and renewable resource, SPI has offer good film-forming ability, and biocompatibility (Zink et al., 2016). The utilization of soy-protein as an adhesive had begun in early day when it was used as a binder in plywood manufacturing industry before it was replaced by synthetic resin in 1960s (Nordqvist et al., 2010; Xu et al., 2022). However,

wood composites bonded with soy protein-based adhesive exhibit lower strengths and lower water resistance (Wool and Sun, 2011; Zhang et al., 2013). To overcome this problem, several methods has been utilized to enhance the performance of soy protein-based adhesive including hydrolysis, cross-linking, chemical denaturation, enzyme modification, addition of additives and so on (Chen et al., 2013; Zhang et al., 2013; Lei at al., 2014).

Chitosan is a second most abundant polysaccharide in nature that is obtained from high deacetylation (>50%) of chitin and most attractive polysaccharide used for adhesive development (Paiva et al., 2013; Patel, 2015). It is a polymer of α-(1,4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and easily obtained by N-deacetylation, to a varying extent that is characterized by degree of deacetylation, and consequently a copolymer of N-acetyl glucosamine and 2-amino-2-deoxy-d-glucopyranose (glucosamine) (Dutta et al., 2004). Moreover, chitosan is also a polymer with good biocompatible, biodegradable, non-toxic, non-immunogenic, antibacterial, and antifungal biomaterials (Wang et al., 2016). Chitosan as adhesive find a major application in two distinct fields which are as a biomedical adhesive and natural adhesive (Patel, 2015). Based on previous study, incorporation of chitosan with other natural material also reported successfully improved the mechanical strength mainly thru formation of hydrogen bonding as well as increased the water resistance of the composite (Xing et al., 2018; Cheng et al., 2022, Li et al., 2022).

The limited number of study conducted on the application of soy protein and chitosan as an adhesive as well as the compatibility of using both of the materials in producing bio-adhesive with enhanced properties had led to this study. Therefore, in this study, SPI together with chitosan was used to produce bio-adhesive to improve

the water resistance and strength of SPI adhesive that will be measure through application on particleboard.

1.2 Problem statement

The adhesive synthesize and manufactured from oil-based raw material such for example petroleum and natural gas was still utilized in the production of wood-based adhesives nowadays (Islam et al., 2022). Even though this type of adhesives mainly formaldehyde –based adhesives are well known for their durability and gives superior physical and mechanical properties to the final product, it also gives negative impacts. The main concern arise from the utilization of formaldehyde-based adhesive is the emittance of volatile organic compound and formaldehyde gas which highly harmful to the environment and carcinogenic to human health (Ma et al., 2023). The indoor formaldehyde exposure gives variety of negative impact on human health including skin sensitization, nausea, skin and respiratory tract irritation and so on while the waste water release from this adhesive production contain high level of pollutant that is toxic to environment and animals (Lebkowska et al., 2017; Kristak et al., 2023).

As the decreasing on the petrochemical resources continue and rises of environmental awareness, people are more interested in production of bio-based adhesives in order to lessen environmental pressures and address health concerns as well as to replace formaldehyde based adhesive (Ma et al, 2023). As one of natural resources, soy protein isolate (SPI) with 90% of protein content and derived from defatted soy flour is naturally cheap, renewable and available agricultural byproduct that have potential to be turn into an adhesive and used in wood based panel manufacturing process replacing formaldehyde based adhesive (Chen et al., 2013; González et al., 2019). Conversely, SPI is hydrophilic in nature and form unstable

interaction that subsequently contribute to adhesive with low water resistance and bonding strength (Liu et al., 2017; Zeng et al., 2021). On the other hand, chitosan is a biocompatible, biodegradable, non-toxic, non-immunogenic, antibacterial, and antifungal biomaterial as mention before by Wang et al. (2016) and Zheng et al. (2020) and reported have higher hydrophobicity than SPI (Li et al., 2017). Modification methods including cross-linking, chemical denaturation, hydrolysis, enzyme modification and others were normally used to improve the drawbacks of the soy protein based adhesive as mentioned by Zeng et al. (2021) but there is limited study done on addition of other types of material mainly chitosan in improving the performance of the SPI as in adhesive application even though previous study showed that the interaction between chitosan and protein can form a film with good physical and mechanical properties (Abugoch et al., 2011; Wang et al., 2016; Li et al., 2017; Cheng et al., 2022). Thus, the aim of this study is producing bioadhesive from soy protein with addition of chitosan to improve mainly the physical and mechanical properties of SPI-based that will be measure through application on particleboard.

1.3 Objectives

1. To study the chemical compositions of oil palm trunk and compared to other oil palm biomass its effect on the properties of particleboard.
2. To investigate the effect of different ratios of soy protein isolate and chitosan on the solid content and viscosity of soy protein isolate and chitosan blend adhesive.
3. To determine the effect of different ratio of SPI and CH on physical, mechanical, chemical, thermal and morphology of oil palm trunk (OPT) particleboard.

CHAPTER 2

LITERATURE REVIEW

2.1 Soy Protein

2.1.1 Soy Protein Structure, Characterizations and Properties

Soy protein is one of the most valuable agricultural crops product that consist of about 40 % of protein and about 20% of a high level of edible oil (Qin et al., 2022). Due to it contents significant amounts of essential amino acid, soy protein has been used as an excellent nutritional source mainly in the food industry (Kim and Natravali, 2011). Soy protein itself own good properties such as fast growth, biocompatibility, and biodegradability and due to these properties, it has sparked research interest in development of environmentally friendly adhesives from these raw material (Chhavi et al., 2017; Yue et al., 2019). Currently, there are three common types of soy protein products that available in the market which are defatted soy flour (DSF), soy protein concentrate (SPC), and soy protein isolate (SPI) and these products were classified according to their protein content. The lowest protein content among all soy protein products is DSF with 40-60% protein that combined with carbohydrate and fats (Ciannamea et al., 2014). Then, SPC contains 65-72%, 20-22% and 7.5-10% of protein, carbohydrate and fiber and ash respectively (Schmitz, 2009). The SPI contains the highest amount of protein in its structures with approximately 90% protein (Kim and Natravali, 2011; Bacigalupe et al., 2015; Qin et al., 2022). The different producing method of all the soy protein products mentioned above resulting on different soy protein content in each product. By grinding soybean to a particle size of less than 100 mesh or finer, DSF which is the least refined soy protein product was produced (Singh et al., 2008). Eliminating part of the carbohydrates (oligosaccharide) from dehulled and defatted soybean will result in a more refined form of protein known as SPC (Tian et

al., 2018). According to Singh et al. (2008), there are three different ways that can be used to remove carbohydrates; washing with 60-80% aqueous alcohol, using an acid with a pH of 4.5 and using of moist heat. On the other hand, to produce the most refined form of soy protein named SPI, the non-protein components, fat, and carbohydrates should be removed from DSF. In order to produce SPI, several steps should be done, first the DSF is stirred in warm water at alkali condition (pH 7-8.5). Next, the solution containing the protein and soluble carbohydrates is separated from the insoluble fraction through centrifugation following adjustment of the pH of the solution to 4.2-4.5. In the end, the sugars are washed away to obtain protein precipitate (Kumar et al., 2002; Singh et al., 2008). Ranking all these soy protein products by their cost will put SPI as the most expensive, followed by SPC and lastly SF (Ciannamea et al., 2010; Ciannamea et al., 2012; Chhavi et al., 2017). Soy protein offers significant potential to be enforced in variety products including as in drug delivery products (Abaee et al., 2017; Assadpour and Mahdi Jafari, 2019), fiber products (Liu et al., 2013; Souzandeh et al., 2016), biodegradable film (Chinma et al., 2015; Xu et al., 2015), foams (Zhang et al., 2018), packaging (González and Igarzabal, 2013; de Souza et al., 2020) and adhesives. Soy-based protein compositions consist of a mixture of four main proteins categories. It is distinguished from one another by their Svedberg sedimentation coefficients and is recognized by names 2S (conglycinin), 7S (β-conglycinin), 11S (glycinin) and 15S (globulins) (Nishinari et al., 2014; Sui et al., 2021). Among the four protein categories stated, the two largest proteins reported (7S and 11S) represent more than 80% of the total protein (Saio et al., 1969; Qin et al, 2022)

Proteins are polymers that make up by twenty different types of amino acids and the acidity, basicity and naturalness properties of the protein is determined by the structure of the side chain of amino acid. As one of the most essential components in

living things aside from DNA, fat and polysaccharides, proteins are linear polyamides made up of amino acids, that are bounded together by polypeptide bonds (Alberts et al., 2002; Raydan et al., 2021). The quantity of amino acid residue and its location along the polypeptide chain, as per Gupta and Nayak (2015), are the most important aspects that responsible in determine the chemical and physical characteristics of soy protein. The polypeptide backbone of the protein determines the molecular structure of soy protein. According to Albert et al. (2002), the different types, numbers and sequences of amino acid will formed the polypeptide backbone of protein. Proteins have primary, secondary, tertiary, and quaternary structures. Figure 2.1 demonstrate the complex structure of the protein that will signify the properties of a protein itself. A string of amino acid that coupled by peptide bond will formed a structure known as the primary structure. The secondary structure of α-helices and β-pleated sheets that stabilized by hydrogen bonds are formed from partial assemble of primary structure. When the side-chains interact with each other and stabilized by series of hydrophobic amino acid residue and disulfide bonds formed among two cysteine amino acid indicate that the tertiary structure in form of a 3D-structure are established. In the end, the quaternary structure will be established after the whole protein molecule interconnected with other protein molecule to form an entire unit (Gupta and Nayak, 2015; Norström et al., 2018; Raydan et al., 2021). The hydrophobicity and hydrophilicity of the amino acid was defined by the functional groups in the side-chains of the polypeptide chain. It offers potential interactions with the hydroxyl or carboxyl groups in wood, as well as crosslinking site (Lehninger, 2005).

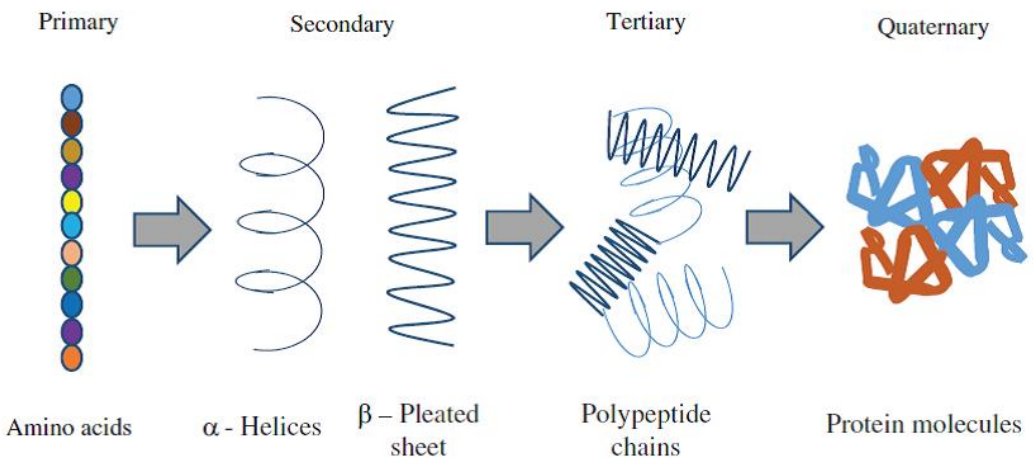

Figure 2.1 Simplify structure of soy protein molecule

2.1.2 Soy Protein as an Adhesive

Adhesive is material that have capacity to bind or hold two surface together. According to Nordqvist et al. (2010), in order for an adhesive to form a proper bond, it needs to fulfil three factors: first, it must be able to wet the surface of the wood substrate, flow over it, and finally penetrate into the wood and all these factors are basically dependent on the viscosity and the dry content of the adhesive. The viscosity of protein-based adhesive is related to the dispersing and unfolding ability of it in the solution that lead to the increases of contact area and adhesion with other substrate (Leiva et al., 2007).

Protein-based adhesives, however, often only meet the requirements for indoor applications because of their poor water resistance, low solid content and high viscosity (Lehninger, 2005; Yue et al., 2019). Thus, to overcome the negative affects come from protein-based adhesive mainly the low water resistance and low adhesive strength, this soy protein-based adhesive has been modified using a variety of techniques including cross-linking, chemical denaturation, hydrolysis, enzyme modification, and others (Lei et al., 2014). As a result of the modification, the secondary, tertiary and quaternary

structure levels of protein molecules will change the soy protein formation from folded structure to loose and disordered structure thus allow for more interaction between the substrate and the protein-based adhesive (Gao et al., 2013; Vnučec et al., 2017). Overall, soy protein is being modified to produce better desirable structure as well as to enhance it physiochemical and functional properties (Djuardi et al., 2020).

2.1.3 Soy Protein Modification

2.1.3(a) Structure Modification (Denaturation Agents)

Soy proteins are compact molecules, folded on themselves at numerous locations (Vojdani and Whitaker, 1994). Any type of modification that change the secondary, tertiary, or quaternary structure of the protein molecules are referred to as denaturation (Huang and Sun, 2000; Schmitz, 2009). On the other hand, Wu and Inglet (1974) defined denaturation as an act of protein unfolding by breaking off the hydrogen and sulphate bonds that exist within the higher orders of protein structure. The accessibility of amino acid side groups that are hidden inside the internal structure of proteins to react with other active groups on denaturation agents increased as a result of denaturation process (Thames, 1994). Multiple factors can cause protein to become denaturized, including exposure to heat, acid/alkali, organic solvents, detergents, and urea (Hettiarachchy et al., 1995).

The modification of soy protein structure involves the use of denaturation agents for example alkali, urea, sodium dodecyl sulphate and guanidine hydrochloride. It is to cause the protein molecules to unfold into a loose and disordered structure (Gao et al., 2013; Zhang et al., 2018). When protein structure is unfolded, more of hydrophobic protein subunit will be exposed but it is important to remember that denaturation will not totally expose all the hydrophobic protein subunit; there will be also hydrophilic subunit that remain in the protein structure to interact with the substrate and form bonds.

Through this interaction formed, the bonding strength and water resistance of the resulting adhesive product will further enhanced.

In order comprehend more on denaturation process of soy protein, the FTIR analysis used. The different secondary structure of soy protein are reflected on the medium IR range present Amide I (1720–1600 cm^{-1}) and Amide III (1400–1200 cm^{-1}) band patterns (Carpenter et al., 1998). Since the modification of soy protein will normally change the secondary structures of protein, it can be detected through changes in FTIR spectra. The changes in the FTIR absorption attributed to hydrogen bonding, dipole-dipole interaction and peptide backbone geometry indicate the changes in the secondary structure of the protein (Bacigalupe et al., 2015). In accordance with Ciannamea et al. (2014), Amide I absorbs between 1720–1600 cm^{-1} range is in response to C=O stretching and C-N stretching, whereas Amide III absorb in between 1400–1200 cm^{-1} due to N-H bending, C-N stretching along with small contribution of C-O bending and C-C stretching (Assadpour et al., 2019).

Urea has been used to modify soy protein to enhance the performances of the soy protein-based adhesive. Urea has an ability to change the structure of soy protein by expending the secondary helix structure of soy protein molecules. Since urea contain amino hydrogen and carbonyl oxygen in its structure, it can reacts actively with the hydroxyl group of the soy protein molecules and break their internal hydrogen bond resulting in unfolding of complex protein structures (Bacigalupe et al., 2020; Hosseini et al., 2020). Modification of soy protein with urea had been done by several of researchers including Pereira et al. (2016), Yue et al. (2019), Wu et al. (2019) and Bacigalupe et al. (2020). Most of them found that addition of urea to the protein-based adhesive did improved the dimensional stability and strength of the soy protein-based adhesive. Moreover, in studied that used soy protein and urea formaldehyde together

reported that the formaldehyde emission from the wood composite was decreased with the addition of soy protein and it also lead to the reduction of formaldehyde in the formulation without affecting the performance of the composite (Pereira et al. 2016). The addition of urea might also affect the viscosity of the adhesive in which the urea modified soy protein adhesive might display low viscosity as reported by Sun and Bian (1999). This could be owing to an increase in number of unfolded protein structures after the addition of urea, which provides a greater surface area for bonding and increases the gluing strength for both adhesives (Lambuth, 2003).

Other than urea, to improve the dimensional stability and adhesive strength of soy protein-based adhesive, guanidine hydrochloride has also been added to the formulation. Huang and Sun (2000) produce plywood from variety of wood known as walnut, cherry and pine and bonded with lab made protein-based adhesive with addition of urea and guanidine hydrochloride in varies concentrations. The addition guanidine hydrochloride, the structure of soy protein molecules was unfolded and changed into more loose and random conformation (Zhong et al., 2002). On the other hand, from the DSC data they obtained show that the peak temperature and total empathy of two main protein subunit (conglycinin (7S) and globulin (11s)) were decreased indicating that urea helps in unfolding and denaturation of protein. This explained the improvement in water resistance and increased in adhesive strength of modified soy protein-based adhesive. In comparison with the unmodified protein-adhesive, they found that the best shear strength and water resistance were displayed by plywood bonded with modified adhesive made with 3M urea and 1M guanidine hydrochloride. This might due to the possibility that there are more amount of secondary structures of protein in the modified protein adhesive and this structures were denatured, exposing more hydrophobic amino acid. The exposed of more hydrophilic amino acid through this modification

contributed to the improvement of water resistance of the resulting product while, the enhancement in the modified adhesive strength was caused by the amount of secondary structure of globular protein.

Adjustment of pH to acid or alkali conditions will also modify the soy protein-based adhesive and this type of modification is known as the acid-base modification technique. Under alkali condition, the carboxyl groups that exist in protein structure are neutralized and form carboxylate anions resulting in repulsive forces between the anions. This is known as alkali denaturation process. Thru this process, the molecular chains of the soy protein will be unfolded and hence exposing its polar functional groups. Alkali denaturation will help in enhancing the dry bonding strength of the adhesives as well as increase the protein molecular chains and their viscosity (Bacigalupe et al., 2015). Hettiarachchy and team (1995) has modified their soy protein with alkali (sodium hydroxide) and trypsin. Based on the results obtained, they discovered that compared to unmodified soy protein-based adhesives, the modified adhesives showed improvement in bond strength and water resistance properties. While, comparing between alkali and trypsin modification, the former displayed higher adhesive strength and water resistance compared to the latter. This is because alkali can promote the unfolding of soy protein molecules by breaking the internal hydrogen bonds of the protein molecules hence exposing more hydrophobic groups for better bonding (Hettiarachchy et al., 1995; Norstrom et al., 2018). Moreover, as stated by Mo et al. (2001), alkali-modified soy protein gives particleboard the best mechanical properties due to the fact that alkali condition can hydrolyze soy protein molecules, creating peptide chains that improve bondability. Besides, Samson et al. (2021) also produced soy protein isolate (SPI) adhesive modified with sodium hydroxide and itaconic acid polyamidoamine-epichlorohydrin (IA-PAE) and applied on *Rhizophora*

spp. particleboard. Among all IA-PAE additional level used, they recorded that particleboard bonded with SPI-based/NaOH/IA-PAE with 15 wt% of IA-PAE showed the best physical and mechanical properties. They also concluded that the formulation stated in medical heath applications as a tissue-equivalentF phantom material.

Sodium dodecyl sulphate (SDS) can be used as a protein denaturation agent since the hydrophobic moieties of SDS can form a strong hydrophobic interaction with the protein hydrophobic side chains (Tanford, 1968). Zhong et al. (2001) studied the effects of sodium dodecyl sulphate (SDS), press temperature, press time and assembly time on the properties of the fibreboard produced. The shear strength of the panel produced was reported to be increased with increasing the SDS concentration to 3wt%. Yet, high concentrations will contribute to poor adhesion due to the increase of viscosity caused by the swelling and unfolding of the protein molecules. The denaturation temperature of the two main protein structures which is 11S and 7S is 90°C and 70°C respectively. So they discovered that the ideal press temperature to produce panels bonded with modified protein adhesive with enhanced adhesion strength and water resistance is above this denaturation temperature. This is because at this temperature the protein molecules were unfolded into loose and disordered structures as well as promote immobilization of protein for better bonding. While, a long press time and assembly time can help in increase the shear strength of the adhesive. In conclusion, the fibreboard bonded with SPI modified with 3 wt% concentration of SDS, pressed at a temperature of 100± °C above for 5 min and more showed the best adhesion and shear strength.

Other types of anion detergent such as sodium dodecylbenzene sulphate (SDBS) can be used as a denaturation agent to modify soy protein as done by Huang and Sun (2000). In their study, they compared two different anion detergents named SDS and

SDBS with different concentrations and their effects on the modified soy protein isolate adhesive. They reported that the increasing of unfolded protein molecules in modified soy protein adhesive with SDS (0.5%) and SDBS (1%) concentration gave good shear strength contrasted to unmodified soy protein adhesives. This claim was supported by the DSC data obtained that showed the decreasing heat capacity upon increasing the SDS concentration in the modified soy protein adhesive. The same result was also reported by Zhong et al. (2001) in a later study indicating that a greater degree of protein unfolding was occur after the addition of SDS. Through anion binding protein modification, the hydrophobicity of the protein adhesive can be increased by exposing of hydrophobic sides chain of protein molecules to interact with hydrophobic moieties of the detergent molecules (SDS and SDBS) and form micelle-like regions hence enhancement in water-resistance properties of modified soy protein adhesive (Tanford, 1968).

On later year, Yue and team (2019) studied the effect of addition denaturation agents name urea, SDS and sodium hydrogen sulfite (SHS) together with a crosslinking agent triglycidylamine (CA) to modify the soy protein isolate adhesives. According to them, unfolding of soy protein structure through addition of denaturation agents have exposing the hydrophobic group of soy protein hence enhance the water resistance and performance of the modified soy protein-based adhesives. The unfolding was done by urea through breaking of hydrogen bonding in protein structure while SDS was prompting protein unfolding by destroying protein structure. On the other hand, the SHS was destroying the disulphide bond in protein structure forcing it to be unfolded. Moreover, the addition of crosslinking agent CA together with the denaturation agents further improved the performance of the modified adhesive. They discover that the

enhancement was due to the development of crosslinking structure between active groups in protein structure and crosslinking agent during curing time.

2.1.3(b) Cross-linking Agents

Nowadays, the modification of soy protein-based adhesive is most frequently accomplished by cross-linking method of modification. Mixing cross-linkers directly into the soy-based adhesive prior to application or adding them while the adhesive is being made are the two ways often used to add cross-linkers to adhesive formulations (Lei et al., 2014). However, the latter method makes managing soy adhesive considerably simpler. The key step in this kind of modification approach is selecting a suitable and efficient cross-linker since it might have an impact on the final modified adhesives. Soy proteins consist of a lot of many reactive groups, including hydroxyl (–OH), thiol (–SH), carboxyl (–COOH), and amino ($–NH_2$), which makes it possible to utilize a lot of different chemicals to cross-linking of soy adhesive (Lei et al., 2014; Lei et al., 2016; Yue et al., 2019). The cross-linked structures that aid in enhancing the adhesive's water-resistance are produced when the cross-linker's active group reacts with the protein's active groups (–NH, –COOH, and $–NH_2$) (Xu et al., 2018).

In the work of Xu et al. (2018), soy protein isolate adhesive (SPIA) is brittle by nature and becomes even more so when a cross-linker is included. This result in low dry bond strength and impact resistance properties of the bonded panel. This is simply because of residual stresses that are present inside the panel during manufacture will be inescapable, and they will intensify when moisture content in the panel changed during application. These residual stresses may often be balanced by the bond forces of cured adhesive. However, if the residua stress increases, this balance can be instantly broken (the cured adhesive is brittle). Increased cross-linking agent dosage can produce a more compact structure of adhesive, but it will olso weaken the bond strength of the resulting

panel as dictated by previous research done by Gao and team (2013). Therefore, increasing the toughness of the SPIAs will benefit from balancing the internal forces, thus enhance the bond strength and water resistance of the adhesive.

In order to synthesize a high-performance SPI-based adhesive with improved in toughness, soy protein isolate (SPI)/ triglycidylamine (TGA) formulation can be combined with thermoplastic polyurethane elastomer (TPU) and -(2,3-epoxypropoxy) propyltrimethoxysilane (KH-560) (Xu et al., 2018). They found that the reactive groups of TGA and KH-560 will react with the active of –NH2 and -OH in soy protein, enhancing the water-resistance of the resulting adhesive when they were added to the formulation. The FTIR result obtained showed that there are reducing of –COOH spectra and blue shift of amide I/II detected strongly prove their claimed. KH-560 has capability to serve as a bridge to link with protein molecules and TPU to form joined crosslinking structure. In addition to improving the interfacial force between the adhesives and substrate (polplar veneer plywood), which improves board performances, the thermostability and toughness, this joined crosslinking network also allows for the development of an adhesive with a uniform ductile fracture section.

Usually, a larger amount of cross-linking agent is used in adhesive formulation to enhance its properties. Zhang et al. (2018) found that the amount of cross-linking agent added to the formulation could be decreases without jeopardizing the water resistance properties by addition of hyperbranched aminated SSPS soybean soluble polysaccharide (A-SSPS) to the blend of soy protein isolated and bio-based triglycidylamine (TGA). A-SSPS will increase the adhesive's reactivity and create an adhesive with hyperbranched cross-linked structure. The introduction of A-SSPS to the system, it aid to formation of a highly cross-linked compact three-dimensional network in the adhesive system and as well as reduced the dose of cross-linking agent added into

the system, the adhesive's water resistance has improved. Additionally, the toughness and thermal stability of the cured adhesive are improved upon addition of A-SSPS and TGA to SPI adhesive since they can form a compact and homogeneous fracture section. Furthermore, it also assisted in lowering the moisture absorption of the adhesive, which improved the dry/wet bond strength of the finished plywood. However, the researchers found that the adding A-SSPS in exceeding 3 wt% simply increased the amount of hydrophilic amino group exposure which in turn will increased moisture absorption and hence reduce water-resistance.

Zhang and team (2020) study the possibility to initially use high-pressure homogerization (HPH) on soy protein adhesives prior to the addition of TGA. They reported that the enhancement in water resistance of the sample is due to reaction between TGA and soy protein that consumed the reactive hydrophilic groups in soy protein adhesive surface and a dense cross-linked network is formed. Their finding is similar with previous study done by Luo et al. (2016). Moreover, treated soy protein with HPS before addition with TGA shown a great improvement on adhesives properties. This is because HPS can gives homogenous (Chen et al., 2017) and bring out the active group within protein molecules (Yuan et al., 2012) that can promote effective cross-linked with TGA to increase adhesive's cross-linking density (improve water-resistance).

Other than that, protein-based adhesive with enhanced bonding strength, water resistance and antibacterial properties can be produced by incorporating of Tannic acid/hyperbranched silicone (HBSi) into soybean meal adhesive (Jin et al., 2020). The resulting soybean meal adhesive consisted of hyperbranched crosslinked structures that contributed to the enhancement of the properties of the adhesive. Moreover, the addition of copper ion to the adhesive also helps in the formation of variety of interface

coordination bonds hence improves the antibacterial properties of the adhesive. The enhanced cross-linking density and cohesion of the adhesive as a result of the metal coordination bonds and covalently cross-linked double network in the adhesive increased the bonding strength and water resistance of the final adhesive product. Their modified adhesive reported had maximum wet shear strength of 1.27 MPa, a swelling ratio of 52.4% and showed resistance against *Staphylococcus aureus* and *Escherichia coli* bacteria.

On the other hand, Xu and team (2020) modified the SPI adhesive with multifunctional crosslink agents (DDE) that were synthesized by reacting soybean daidzein with epichlorohydrin in order to improve its water and mildew resistance. They found that the reaction between the polymers can form a double cross-linked network and increased adhesive toughness hence improving adhesive properties. This is proven by the dry and wet shear strength of plywood bonded with SPI modified with 6% DDE reported increased by 52.3% and 164.4% respectively. Besides, the addition of DDE boosted the adhesive's mildew resistance properties by extending its shelf life and the plywood's durability.

2.1.3(c) Enzymatic Modification

The usage of enzyme commonly proteolytic enzymes as one of modification agents was also another types of modification method that had been used to improve the performance of soy protein-based adhesives by modifying the protein structures and this modification method known as enzymatic modification. Kumar and team (2004b) in their study had modified their SPIs adhesives with variety of proteolytic enzymes like chymotrypsin, trypsin, urease, papain, and pepsin in order to improve the adhesive properties. They also test the modified SPIs adhesives on several types of wood

substrate such as rubberwood, Bhutan pine, teakwood and plywood to study their compatibility. According to them, unmodified SPI adhesive displayed the lowest hydrophobicity while chymotrypsin modified SPI (CSPI) reported having the highest value. Thru extent of hydrolysis, more of hydrophobic group in native soy protein structures will be exposed that contributed to the increasing of hydrophobicity properties of resulting SPI adhesive. The past research by Kumar and team (2004b) discovered that the number of the hydrophobic groups exposed is more in CSPI compared to papain modified SPI (PSPI) and trypsin modified SPI (TSPI). As mention before this is related to the extension of hydrolysis where the extension of enzymatic hydrolysis is less for PSPI and TSPI compared to CSPI. However, extensive hydrolysis will led to decreasing of viscosity and poor adhesion as showed by the CSPI in contrast with urease modified SPI (USPI) that displayed improvement in adhesion. Moreover, their final finding also reported that among all wood substrates that used, rubberwood substrate was found to be the best adherent while teakwood is the least adherent.

On the later years, Xu and team (2018) study the possibility of using protein endonuclease-bromelain enzyme to modified SPI in order to break down the protein molecules into polypeptide chains before addition with TGA to produce bio-adhesive and tested on a three layers plywood. The plywood bonded with SPI/bromelain adhesive showed the lowest wet shear strength among all formulations tested. Upon addition of TGA to the SPI/bromelain adhesive formulation, the wet shear strength displayed positive improvement. This enhancement was due to the breakage of protein molecules into polypeptide chain by bromelain that help in expose more active hydrophilic groups of soy protein hence more reaction can take placed between SPI and TGA that led to the production of a denser cross-linked network in adhesive. The denser cross-linked network formed helps in improvement of water resistance and thermal stability of the

final product. Moreover, enzymatic modification of soy protein by bromelain also aim to alleviate the solid content and reduced the viscosity of the SPI adhesive produced.

On the other hand, Zheng et al. (2019) experimented on production of self-crosslinked soy protein adhesive through modification of DSF with enzyme complex from fermented broth of *Aspergillus niger*. The hydrolysis of polysaccharides in DSF by this enzyme complex used in their study was able to produced reducing sugar that felicitate crosslinked with soy protein. Reported that the wet shear strength of the adhesive along with the water resistance and mechanical strength of the adhesive upon application improved after modified with *A. niger* enzyme complex in comparison with soy protein adhesive without enzyme modification. The is because, posterior of enzyme modification on the DSF adhesive, the degree of crosslinking of cured modified adhesive seem to be significantly improves hence enhancement in strength properties as mention before. They also stated that, the enzyme modification with *A. niger* enzyme appeared to be more effective and cost friendly compared to the usage of other enzyme such as Viscozyme L as done previously by Chen et al. (2015).

Despite the fact that there have been few research done on the impact of enzyme modification on soy protein adhesive and its application on composite material, several studies have documented employing enzyme modification to enhances soy protein properties in food applications. As noted by Kim et al. (1990), the use of the proteolytic enzyme (trypsin, alcalase and α-chymotrypsin) can significantly enhance SPIs functionality. By monitoring the time frame of proteolytic treatment, the types of proteases utilized, and the functional properties of the final SPI adhesive, it is possible to increase the emulsifying capacity, solubility and thermal aggregation of SPIs to a particular extent. Additionally, they found that as compared to 11S globulins, protein 7S sub-units exhibit a more through enzymatic breakdown. This might due to the fact

that 11S globulins may have a more compact structure (Wolf, 1972), whereas 7S sub-units possibly contain more hydrophilic surface (Lewis and Chen, 1979. Soy protein hydrolysates must be hydrolyzed to a certain degree of hydrolysis under tightly monitored conditions in order to get desired functional properties. A limited degree of hydrolysis frequently improves solubility, emulsifying and foaming capacities, whereas excessive hydrolysis typically causes the loss of some of these functionalities (Barać et al., 2004).

2.1.3(d) Additives

Few studies had been carried out to test the compatibility of adding different types of additives into soy protein-based adhesive to enhance the water-resistance and adhesive strength for wood composite application.

In order to enhance the mechanical properties of soy protein-based adhesive, Qi and colleagues (2016) used nanoscale fillers such as sodium montmorillonite clay (Na MMT). According to Pojanavaranaphan et al. (2010), Na MMT is a type of silicate clay that have an interesting nanoscale layered structure, and a high expect ratio polymer. As a result of their research, they found that, SP/Na MMT modified adhesive have increased in dry and wet adhesion strength, with the most remarkable water resistance recorded at 8% Na MMT loading. The hydrogen bond and electrostatic bond were responsible in formation of intercalation structure between Na MMT and protein resulting in a compact cross-linking protein structures. The water resistance of the final product is improved due to the existence of this compact structures that make the interfacial layer between wood and adhesives are impenetrable by water.

2.2 Chitosan

Chitin (Figure 2.2) is the most common biopolymer found in nature after cellulose and it also one of the most abundant polysaccharide in nature (Umemura et al., 2009; Basturk, 2012). Chitin or also known as poly (β-(1→4)-N-acetyl-D-glucosamine) is a polysaccharide that can be extracted from eukaryotic species such as crustacean, insects and fungi (Kean & Thanou, 2011; Younes & Rinaudo, 2015; Ahmed & Ikrem, 2017). Chitosan is a random copolymer with a molar fraction DA (degree of acetylation) of β-(1→4)-N-acetyl-D-glucosamine and a fraction (1-DA) of β-(1→4)-D-glucosamine can obtained by partial deacetylation of chitin under alkaline condition (Younes & Rinaudo, 2015; Homez-Jara et al., 2018).

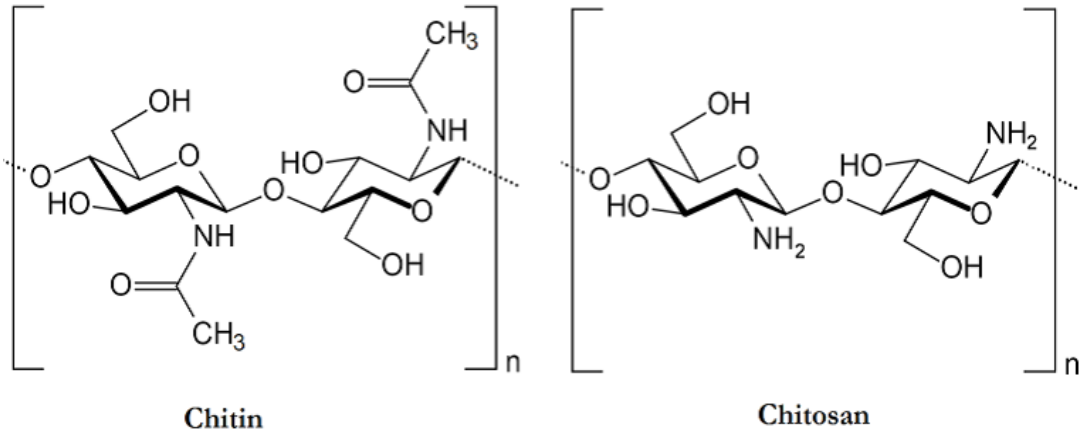

Figure 2.2 Chemical structure of chitin and chitosan (Younes and Rinaudo, 2015)

The degree of acetylation (DA) is defined as the mole fraction of N-acetylated repeating units, whereas the percentage of repeating units of β-1,4-D-glucosamine in polysaccharides is defined as the degree of deacetylation (DD) (Khan et al., 2002; Younes and Rinaudo, 2015). In other words, deacetylation process is the process removing acetyl group from the molecular chain of chitin and leaving behind a complete amino groups ($-NH_2$) and the adaptability of chitosan is depending on the degree of reactive amino groups present in it (Hussain et al., 2013). Chitosan can be classified

according to its degree of deacetylation (DD), molecular weight and distribution, protein residues, and so on. As reported by Hussain et al. (2013) and Jana et al. (2013) , the degree of deacetylation is normally the most important parameter for chitosan since it can affect the physical, chemical and biological properties of chitosan (acid base and electrostatic characteristics, biodegradability, self-aggregation, sorption properties, and the ability to chelate metal ions). Furthermore, Chitin can be distinguished from chitosan by the degree of deacetylation, which affects the amount of free amino groups in the polysaccharide (Li et al., 1992). The higher the DD value, the higher concentration of amino groups in the molecules (Nilsen-Nygaard et al., 2015; Kou et al., 2021). Since the properties and application of chitosan is based on their degree of deacetylation, it can be increase or decreases through several ways including enhancing the removal of acetyl groups by increasing the temperature or strength of sodium hydroxide solution (Baxter et al., 1992; Hussain et al., 2013). The DD of commercial chitosan sample reported was between 70–90 % (Sanford, 1988; Basturk et al., 2012; Hussain et al., 2013).

Other than DD, molecular weight (MW) of chitosan is also important factor that affect the bioactivity of chitosan where compared to high MW chitosan, a lower MW chitosan showed more significant bioactivities (Huang et al., 2004; Benhabiles et al. 2012; Younes and Rinaudo, 2015). On the other hand, chitosan with higher DD will have higher antimicrobial activities (Benhabiles et al., 2012). High MW of chitosan can give a greater problem such as poor solubility at neutral pH and high solution viscosity that limits its application in food, cosmetic, agriculture and health industry (Xia et al., 2011; Benhabiles et al., 2012). Chitosan can be classified into three categories based on their MW cut-off value; high, medium and low MW chitosan (Shuai et al., 2013; Pavinatto et al., 2013; Patil et al., 2015). The relationship between MW of chitosan and