Development and Characterisation of Waste Kibisu Silk Reinforced Biodegradable Polymer Composite

Doctoral Thesis

by

“Papiya Bhowmik”

(2017MEZ0021)

DEPARTMENT OF MECHANICAL ENGINEERING

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Name: *Development and Characterisation of Waste Kibisu Silk Reinforced Biodegradable Polymer Composite*

DEDICATED

TO

FAMILY & FRIENDS

DECLARATION

I hereby declare that the work which is being presented in the thesis entitled **Development and characterisation of waste Kibisu silk reinforced biodegradable polymer composite** has been solely authored by me. It presents the result of my independent investigation/research conducted from January 2018 to December 2022 under the supervision of **Dr. Ravi Kant**, Assistant Professor, Department of mechanical engineering, IIT Ropar and **Dr. Harpreet Singh**, Professor, Department of mechanical engineering, IIT Ropar. To the best of my knowledge, it is an original work, both in terms of research content and narrative, and has not been submitted or accepted elsewhere, in part or in full, for the award of any degree, diploma, fellowship, associateship, or similar title of any university or institution. Further, due credit has been attributed to the relevant state-of-the-art and collaborations (if any) with appropriate citations and acknowledgements in line with established ethical norms and practices. I also declare that any idea/data/fact/source stated in my thesis has not been fabricated/ falsified/ misrepresented. All the principles of academic honesty and integrity have been followed. I fully understand that if the thesis is found to be unoriginal, fabricated, or plagiarised, the Institute reserves the right to withdraw the thesis from its archive and revoke the associated degree conferred. Additionally, the Institute reserves the right to appraise all concerned sections of society for their information and necessary action (if any). If accepted, I hereby consent for my thesis to be available online in the Institute's Open Access repository, inter-library loan, and the title & abstract to be made available to outside organisations.

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CERTIFICATE

This is to certify that the thesis entitled **Development and characterisation of waste Kibisu silk reinforced biodegradable polymer composite,** submitted by **Papiya bhowmik (2017MEZ0021)** for the award of the degree of **Doctor of Philosophy** of Indian Institute of Technology Ropar, is a record of bonafide research work carried out under our guidance and supervision. To the best of my knowledge and belief, the work presented in this thesis is original and has not been submitted, either in part or full, for the award of any other degree, diploma, fellowship, associateship or similar title of any university or institution.

In our opinion, the thesis has reached the standard of fulfilling the requirements of the regulations relating to the degree.

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LAY SUMMARY

The contamination of plastic pollutants in the soil, water, and air is increasing globally, raising severe concerns among environmentalists. The outbreak of SARS-COV-2 has elevated the use of one-time plastics due to the sudden merge of improved hygiene practices. Scientists are continuously working towards developing a biodegradable alternative to plastic that should be non-toxic, renewable and affordable. Over the past decade, green composite has taken over many synthetic composites and plastics in various applications. Developing natural fibre-based composites is well practised worldwide because of their easy availability, renewability, outstanding mechanical properties, non-toxicity and excellent biodegradability. Natural fibres have the potential to replace conventional petroleum-based synthetic fibres. The large availability in the market has also triggered its wide application. However, the low degradation temperature of the fibres doesn't allow them to be used in high-temperature applications or curing processes. Research showed hydrophobic surfaces bond with proteins more definitely than hydrophilic surfaces. Hydrophobic surfaces attach to another hydrophobic surface more viciously because of a good adsorption process. As hydrophilic surfaces bind water to them, it is difficult for the hydrophobic surfaces to set a homogeneous contact with them. Interfacial water displaces more quickly when two hydrophobic surfaces are in contact. The hydrophilic property of the natural fibres leads to poor adhesion between the fibre and the composite matrix. To tackle this problem, the researchers have taken different approaches to modify the surface texture of the fibres and matrix material. The modified fibres and matrix have better adhesion because the changed surface texture allows better bonding. There are other approaches to enhance the physicochemical properties of the developed composites by adding crosslinkers and plasticisers along with them. The plasticiser influences the protein to form a clay-like structure, allowing the protein to be thermally extruded or moulded at a temperature range of 100 to 120 °C. Various plasticisers are being used for different natural polymers, considering their plasticising capacity, hydrophilic compatibility with the polymer, and the capacity to produce many hydrogen bonds. Plasticiser enhances the elasticity of the protein by enabling more fluid melt, flexibility and elongation. Besides plasticisers, crosslinkers are also used to modify natural polymers' microstructural rheological features. Crosslinking increases the mechanical properties of the polymer by making longer polymeric chains connected with intermolecular bonds or links.

In this current study, efforts have been made to develop a hundred percent biodegradable composite sheets with all-natural ingredients to reduce the carbon footprint. The fibre used here is waste Kibisu silk (bombyx Mori), an industrial waste available in abundance in silk-textile industries of India. To bind the fibres, organic wheat gluten was used as a biopolymer, a byproduct of wheat flour. After optimising among different parameters through different objectives of the work conducted, castor oil has been opted as an effective natural plasticiser for plasticising the wheat gluten. The natural lemon extract was used instead of synthetic citric acid for crosslinking. To better wheat gluten dispersion into the water, sodium hydroxide (NaOH) was used. The prepared composites showed outstanding physio-chemical properties, which can be used to prepare mouldable sheets to produce biodegradable seedling pots, hospital trays, egg trays, nursery sampling pots, table tops and ceiling tiles etc.

Along with the composite, two different all-natural biodegradable coating materials were developed to coat the composite products to diversify their applications. The coatings were developed with Agar and gelatine, respectively. The Agaragar coating was developed with the help of distilled water, castor oil and calcium chloride, which helped in increasing the flexibility and stability of the coating. For the second coating, gelatine solution was developed in tea extract and aloe vera media which was used to reduce the oxidisation of the product. In Agaragar coating, water absorption was found to be higher, whereas water absorption in the gelatine-based coating was comparatively lower. The coatings showed outstanding biodegradability at the biocabinet in bacteria culture just after one week. The coating can be used to develop all-natural coated composites, which can be used as biodegradable disposables.

ABSTRACT

In India's current plastic management policy, single-use plastics are the most significant contributor to littered waste. Millions of plastic waste end up in the environment vis-a-vis soil, water bodies, watercourses, etc. It takes an average of one thousand years to decompose plastics completely. In India, the Plastic Waste (Management and Handling) Rules, 2011 included plastic waste management as its priority to address the issue of scientific plastic waste management. The Government has announced the Plastic Waste Management Rules, 2016, in suppression of the earlier Plastic Waste (Management and Handling) Rules, 2011, focusing on developing alternative biodegradable plastic with a faster degradation rate. An eco-friendly product, a complete substitute for plastic in all uses, has not been found to date. In the absence of a suitable alternative, it is impractical and undesirable to impose a blanket ban on the use of plastic all over the country. The real challenge is to improve plastic waste management systems or develop a feasible alternative to plastic disposables. So, the dire need of the hour is to furbish a viable low-cost alternative to plastic with zero or minimised carbon footprint. The current work aims to develop a hundred percent biodegradable composite by reinforcing waste Kibisu silk into wheat gluten as a possible replacement for plastic disposables. The fibre used here is waste Kibisu silk (bombyx Mori), an industrial waste available in abundance in silk-textile industries of India. To bind the fibres, organic wheat gluten was used as a biopolymer, a byproduct of wheat flour. After optimising among different parameters, castor oil is used as an effective natural plasticiser for plasticising wheat gluten. The natural lemon extract was used instead of synthetic citric acid for crosslinking. For better dispersion of wheat gluten into the water, sodium hydroxide (NaOH) was used. The prepared composites showed outstanding physio-chemical properties, which can be used to prepare mouldable sheets to produce biodegradable seedling pots, hospital trays, egg trays, nursery sampling pots, table tops and ceiling tiles etc. Based on the findings of this study, it can be concluded that waste Kibisu silk and wheat gluten with natural plasticisers and crosslinkers can make easily mouldable composite sheets which can be used for different purposes to reduce the usage of disposable plastics. The final result shows composite prepared with 50:50 six-hour treated waste Kibisu silk and wheat gluten with 10% castor oil, 2.5% NaOH, and 2.5% lemon extract gives the most promising properties in terms of mechanical strength and thermal stability. The bio-coating of aloe vera-gelatine is proven to be best suited for effectively coating the developed composites to prevent moisture absorption and water resistance.

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Notations and Abbreviations

AKD	Alkyl letene dimer
CMC	Ceramic matrix composite
E. coli	Escherichia coli
FTIR	Fourier-transform infrared spectroscopy
IP	Isoelectric point
MMC	Metal matrix composite
NF	Natural fibres
NFP	Natural fibre-reinforced polymer
OHP	Overhead projector sheet
PBS	Polybutylene succinate
PBSA	Polybutylene succinate adipate
PLA	Polylactic acid
PMC	Polymer matrix composite
SEM	Scanning electron microscopy
TF	Treated fibre
T_g	Glass transition
TGA	Thermogravimetry analysis
UF	Untreated fibre
W_0	Weight before water absorption
W_1	Weight after water absorption
WA	Water absorption
WFS	Waste filature silk
WG	Wheat gluten
WKS	Waste Kibisu silk
W_t	Weight reduction after heating
W_x	Weight before degradation
W_y	Weight after degradation
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

Composite material combines two or more constituents completely insoluble in one another and has different physical properties. The primary purpose of composite materials is to develop low weight, high stiffness, high specific strength and low-density materials, which is impossible to acquire with a single material [1,2]. The main constituent of composite material is matrix and reinforcement materials. The reinforcements can be continuous, discontinuous, unidirectional, bidirectional or randomly oriented and the matrix materials are always in a continuous phase [3]. In composite material, the fibre and matrix should be more than five percent to impart distinctive properties better than the individual properties of the constituents. The purpose of the reinforcements is to provide strength to the developed composite, whereas the matrix holds the reinforcement together like glue and provides structure and shape to the composite [4]. Over the last decade, researchers have developed various composite materials combining different synthetic and natural fibres with the matrix to enhance the performance for various end applications. Depending on the matrix materials, composites are classified into three different categories, metal matrix composite (MMC), ceramic matrix composite (CMC), and polymer matrix composite (PMC). In MMC metal, ceramic or polymer is reinforced into metal; in CMC, alumina, carbides, and nitrides are reinforced into ceramic material; in PMC, different synthetic or natural fibres are reinforced into thermoplastic or thermosetting polymer [1,5,6]. PMCs are among the most popular composites of the different types of composites because of their impressive mechanical, thermal and chemical properties [3,5,7].

1.1 Introduction to polymer composite

Polymer is a combination of two Greek words, *poly* and *mer*, representing many and parts. By definition, the polymer is a big molecule of consecutive subunits of monomers [8]. From the beginning, both organic and synthetic polymers have taken a great place in manufacturing composites [9]. The entire composite industry depends on polymers due to their low cost, high specific strength and stiffness compared to conventional metal alloys or ceramics [1,10–12]. In addition, polymer matrix composites (PMC) offer excellent corrosion resistance and provide better flexibility [1,2,13,14]. PMCs have gained popularity in the aerospace and automobile industries in the last two decades. In recent developments, PMCs are also considered to be a very suitable option for marine, infrastructure, energy and biomedical applications [15–18]. The prime goal of polymer is to hold together the fibres and shield them from the surrounding chemical changes, uneven mechanical loading and shielding them from ambience moisture [19,20]. Upon loading, it distributes the load uniformly throughout the structure, thus reducing the chances of early failure [21]. Apart from the polymer, the strength of the composite also depends on the orientation of the fibres[22,23]. There are two types of polymers: synthetic and natural. Synthetic polymers are derived from petroleum oil and made by scientists and engineers [24]. Examples of synthetic polymers include nylon, polyethylene, polyester, Teflon, and epoxy [5,19,25–27]. Natural polymers occur in nature and can be extracted. They are often water-based . Naturally occurring polymers are cellulose and proteins. Natural polymers are of two types: thermosets and thermoplastics. Thermoplastics have poor wettability and high viscosity, while thermosetting polymers possess low viscosity and better wettability with the fibre surface [28]. Adding coupling agents/compatibilisers to thermoplastic polymers improves interfacial adhesion. Thermoplastic polymers like polyvinyl chloride, polyethylene, and polypropylene become softer at higher temperatures and regain their original properties when cooled down [23,25,29].

Despite having better properties, synthetic polymers are not considered a viable option nowadays because of the growing environmental pollution. The disposal of such composites is a severe environmental problem as they are non-biodegradable. Burning non-biodegradable polymer composites is raising the global warming issue by emitting more carbon dioxide into nature [30–32]. Biodegradable polymers are viable alternatives to synthetic polymers for a variety of applications. Synthetic polymer-based composites require a long and expensive process of collection, recycling and reusing, which can be easily eliminated with green polymers as they are environment friendly [21,23,33,34]. Natural polymers are developed in an organism-rich environment during their growth cycle. They are often called as biopolymers also [35–37]. The most distinctive family of these natural polymers belongs to the polysaccharide class, which includes starch and cellulose [38]. Two main sources of biodegradable polymers, proteins and other natural polymers, can be combined to create biodegradable products [39,40]. Due to easy mouldability and easy manufacturing processes, Bio-PMCs are more widely manufactured for various applications [41–43]. Despite acquiring lightweight, high strength, and high stiffness, Bio-PMCs have their own disadvantages. They have high sensitivity to moisture and radiation [44]. Degradable polymers are hydrophilic and thus decompose rapidly. Because of its hydrophilic nature, it absorbs moisture and abolishes the desirable mechanical properties of the composite [45–48].

These properties can be tailored vigilantly with some chemical alteration, blending with other constituents and adding fibre and/or particle reinforcements. However, the growing popularity of PMCs has evolved a great interest among researchers to work on sustainable PMCs. The Bio-PMCs consist of biodegradable polymer matrix and fibres that are economical, readily available, renewable, and have competitive properties, making them a sustainable option in the global market [21,23,33]. Researchers around the world are trying to apply natural polymers like wheat gluten (WG), soya protein, starch, gelatine, polylactic acid (PLA), Polybutylene adipate terephthalate, Polyhydroxy butyrate etc. for developing products with competitive properties like synthetic polymer-based composites [114933445034]. These biopolymers have competitive mechanical properties, are chemically inert, non-toxic, rapidly biodegradable and easily renewable.

WG is gaining popularity as a viable natural polymer for a wide range of applications due to its advantageous properties. WG holds complex proteins with long polymeric chains, which can be used for different food or non-food applications. The increasing pollution due to plastic waste has significantly drawn the attention of researchers to work towards renewable biopolymers like WG. WG-based bioplastics and composites can replace conventional petroleum-based plastics because of their large-scale production, easy availability, eco-friendly approach and non-toxicity. Despite that, the use of WG is restricted due to its extreme brittleness [30,42,51]. Various plasticisers, fibre reinforcements and fillers are extensively experimented upon to enhance the flexibility of WG. To enhance the mechanical properties and reduce the brittle fracture of WG under various loading conditions, natural fibres (NF) are reinforced into it [30,42,51–53].

1.2 Development of natural fibre-reinforced polymer composites

Natural fibres are organic, non-toxic, biodegradable and environment friendly in contrast to synthetic fibres made of chemical synthesis. Synthetic fibres are vigorously used in the polymer composite industry to make aerospace and automobile parts because of their anti-corrosive properties and high specific strength [54,55]. Despite adhering to high mechanical, chemical and thermal properties, synthetic fibre-reinforced composites pose a high threat to the environment. Manufacturing synthetic fibres like Kevlar, nylon, acrylic, glass, and carbon produces harmful gases which can cause severe health issues for employees. Additionally, synthetic polymer-based composites are non-biodegradable and toxic to soil and water [56]. Natural fibres are derived from plants, insects and animals, which makes them renewable, non-toxic and readily available [9,22]. Similarly, natural fibres can be categorised depending on their source of extraction, as shown in Table 1.1.

Table 1.1: *Sources of different types of natural fibres.*

Source	Variety
Wood fibres	Soft and hardwood
Straw fibres	Rice and wheat straw
Seed fibres	Cotton, coconut
Leaf fibres	Abaca, cantala, hemp, pineapple, sisal
Stem fibres	Bamboo, jute, banana, hyacinth, sugarcane
Insect fibres	Silkworm silk, Spider silk
Animal fibres	Hairs of cow, pig, horse, human
Avian	Bird feathers

Natural fibres have almost similar mechanical properties but lesser density than synthetic fibres, thus making them lightweight substitutes to manufacture composites. It makes them produce lightweight structures for automobile, building and aerospace applications. Because of their faster biodegradability and non-toxicity, it is catching the attention of researchers worldwide [23,42,57]. Developing natural fibre-reinforced polymer (NFP) composites requires reinforcing natural fibres to thermoplastic or thermoset polymers matrix. Depending on the application and requirement, continuous, discontinuous, unidirectional, bidirectional, chopped, and mat-type fibres can be reinforced in the matrix. Matrix materials provide structural integrity to the composite and provide safety to the fibre materials from external attack [3]. The polymer matrix can either be synthetic or naturally derived, as shown in Table 1.2. The synthetic polymer matrix offers better, more controlled and faster structural formation but lacks in biodegradability factor. On the other hand, naturally derived polymers degrade faster but are naturally very brittle and fragile. Therefore, it requires customised tailoring or pre-processing with other components for better fibre-matrix adhesion and enhanced flexibility. Natural fibre-reinforced synthetic polymer-based composites are partially biodegradable, whereas natural fibre-reinforced green polymers are entirely biodegradable [20,23,58]. The natural polymer reinforced with natural fibre-based composites is drawing the interest of environmentalists to be used for different applications.

Table 1.2: *Types of different polymer materials according to the source.*

Status	**Source**	**Variety**
Nondegradable	Synthetic	Epoxies, phenolics, polycarbonate, polyvinylchloride, nylon, acrylics, polyethylene, polypropylene.
Synthetic biodegradable polymers	Processed Natural	Polylactic acid (PLA), Polyglycolic acid (PGA), poly (lactictide-co-glycolide) (PLGA), Polycaprolactone (PLC), Polybutylene succinate, Polybutylene succinate adipate.
Natural degradable polymers	Natural	Wheat gluten, soya protein, tapioca starch, potato starch, corn starch, gelatine, agar, arrowroot starch, chitosan, gums, and whey proteins.

The properties of the natural fibre, like flexural modulus, tensile modulus, density and impact strength, majorly influence the properties of the composite. Apart from that, fibre fraction (% weight) also impacts the strength of the composite. As the polymer works as a binder to structure the composite, a satisfactory fibre-matrix adhesion is a must while developing a composite. Fibres are grafted by different processes like alkali, silane, acetylation, benzoylation, peroxide, and enzymatic treatment for better fibre-matrix adhesion [11,59]. Matrix material also holds great importance in the wettability of the fibre-matrix to improve the interfacial adhesion [60]. Different crosslinkers and plasticisers are used for this purpose to enhance the crosslinking and flexibility of the developed composites. As natural fibre-reinforced polymer composites are light in weight, they are used in various applications like automotive panels and frames, construction industries, door and ceiling panels, bicycle frames, electronic boards etc [3,25,61,62].

Natural silk (Bombyx Mori) is one of the most popular fibre reinforcements to produce natural protein-based green composites. The waste natural silk, also known as waste filature silk (WFS) or waste Kibisu silk (WKS), produced in textile industries during silk processing, possesses excellent mechanical and thermal properties [63–66]. India is the second-highest silk manufacturer after China on the global map. Along with the production of high-end clothing, the Indian silk industry generates huge silk waste yearly, making it an economical option to use as fibre reinforcement [67]. The well-aligned internal structure of the WKS makes them susceptible to more tensile load, high elongation, and good resilience. Due to long chains of amino acids, these silks possess higher heat resistance capacity. The WFS consists of two parts, fibroins (75%) in the inner section and sericin (25%) on the outer layer. The silk fibroins have a semicrystalline structure that provides inner strength to the silk fibres, and sericin acts like amorphous glue, holding the fibroins and structure together[65,68]. Because of this hydrophilicity of the sericin layer, it is often challenging to have a proper fibre-matrix adhesion with natural polymers like wheat gluten. Although removing the sericin layer reduces the toughness of the silk fibre, but the process benefits the fibre-matrix adhesion, thus improving the overall composite properties [69].

1.3 Role of crosslinkers and plasticisers in polymer composites

1.3.1. Plasticisers

The role of plasticisers is to modify the thermal and mechanical properties of polymers. Plasticisers are generally low molecular weight substances, which enhances the flexibility, extensibility and processibility of polymers that are generally very brittle. Adding plasticisers makes the polymer and polymer composites more suitable for different manufacturing processes [42,70,71]. Plasticisers should have chemical affinity and similarity towards the polymer. The molecules of the plasticisers embedded between the polymeric chains increase the gap between them and interrupt the polymer-polymer interaction as shown in Figure 1.1. The chain mobility of any polymer is represented by its glass transition (T_g) temperature. At $T_{g,}$ the polymer transforms from a hard, brittle state to a soft and rubbery state. Adding plasticiser reduces the T_g without altering the basic chemical characteristics of any polymer [39,72]. Increasing the percentage of the plasticiser increases flexibility but reduces the tensile strength and hardness. Plasticisers are widely used in plastic and composite industries as an additive.

The classification of plasticisers depends on their chemical composition and their influence on the structural elements of the base polymer. Plasticisers belong to different chemical families that affect the physiochemical properties of the polymer. The most commonly used polymers are classified in

Table 1.3 [73]. Plasticisation can be done either internally or externally. A polymer can be internally tailored by chemically modifying the monomers or polymeric chains to enhance flexibility and extensibility [74]. Internal plasticisation can be done by copolymerisation of low T_g plasticisers into high T_g polymeric chains. Internally plasticised polymers show structural instability at higher temperatures [75].

Table 1.3: *Classification of different types of plasticisers.*

Classification	Name
Polyos	Glycerol, ethylene glycol, diethylene glycol, triethylene glycol.
Organic esters	Phthalate esters, triacetin, diethyl phthalate, dibutyl phthalate, tributyl citrate.
Oils/glycerides	Canola oil, corn oil, linseed oil, rapeseed oil, sunflower oil, epoxidised soybean oil, castor oil, coconut oil, acetylated monoglycerides.

External plasticisation is more commonly used because of its low cost and high compatibility with polymers. They enhance the elongation and flexibility, and fire resistance of the polymers. External plasticisers may or may not contain functional groups to solvate the polymers at different temperatures [76]. Considering biocompatibility and easy processability, natural oil-based plasticisers are gaining popularity. Being renewable and biodegradable makes them an eco-friendly option for composite manufacturers. Several independent studies have revealed castor oil, epoxidised soyabean oil, sesame oil and linseed oils to be feasible bio alternatives as a plasticiser [77–81]. Study says the increase in plasticiser decreases Young's modulus, the ultimate tensile strength of the composites but increases the breakpoint elongation [75]. To keep the mechanical properties integral, crosslinkers are blended with the biopolymers to maintain the structural integrity of the composite under heavy loading [82,83].

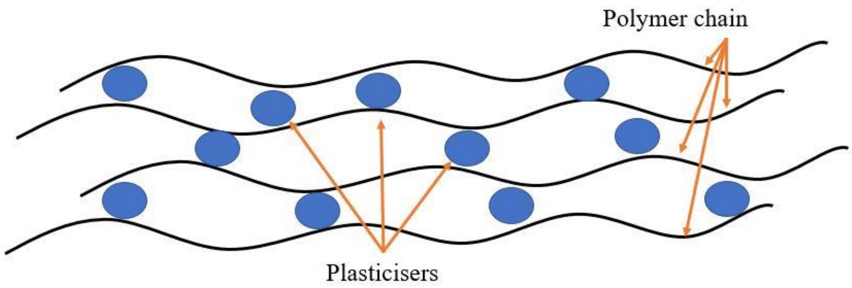


Figure 1.1. Plasticised polymer chains.

1.3.2 Crosslinkers

Bioconjugation or crosslinking refers to the joining of two or more molecules of a polymer by chemically forming a covalent bond. Crosslinkers are used to control the structure of the polymer matrix by crosslinking the monomer imprinted polymers. It forms a highly packed network of polymers with crosslinking, thus improving the mechanical properties of the polymer matrix [35,46,78].

The crosslinking agents connect the monomers with chains and keep the structure firm and strong by polymerisation as shown in Figure 1.2. Molecules with two or more reactive ends capable of chemically connecting to particular functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules are known as crosslinking reagents (or crosslinkers) [84]. Selecting the appropriate crosslinker and its percentage highly influences the properties of the polymer. In short, crosslinkers can tailor the morphology, ensure the structural stabilisation of the monomer-monomer bonds, and enhance the polymer porosity and mechanical strength. Polymers with a high crosslinker ratio make the monomer-monomer bonding very close to each other, making them harder and non-mouldable [85]. That's why optimising the amount of crosslinker added to the polymers is very important. There are various kinds of synthetic crosslinkers like ethylene glycol dimethacrylate (EGDMA), glutaraldehyde (GA), dialdehyde polysaccharides, citric acid, polyurethane, glutaraldehyde, carbodiimide, boric acid, sodium trimetaphosphate, N,N′-methylene bisacrylamide, and polycarboxylic acid preferred for polymer crosslinking [35,82,83,86]. Among the natural crosslinkers, transglutaminase, tyrosinase, horseradish peroxidase, laccase, sortase A, genipin, vanillin, tannic acid, and phytic acid are primarily used [86].

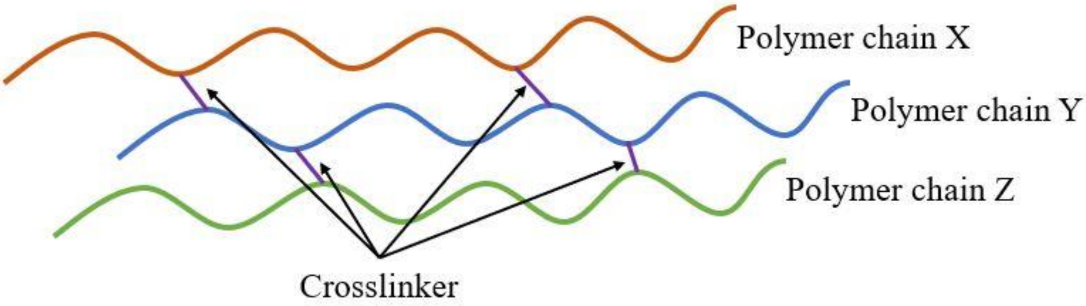


Figure 1.2. Crosslinked polymer chains.

Natural polymers are very brittle in nature, that's why they undergo brittle failure very often under heavy loading. Being a natural hydrophilic protein, WG absorbs too much ambient moisture and reduces the mechanical properties of the polymer composite. Crosslinking plays a crucial role in improving organic polymers' overall properties. Crosslinking enhances the mechanical properties as well as water resistance of any biopolymer [46,83]. Among the compound class of carboxylic acid, citric acid is the most commonly used crosslinker, improving the mechanical properties of natural polymers and specially WG-based composites [46,87]. Natural lemon extract holds 45 g of citric acid per litre, making it a viable resource of naturally extracted citric acid [88]. Citric acid is highly reactive and forms ester bonds with WG. The polar group of most of the food proteins ranges from 30 to 45% and has a negative charge. Wheat gluten is a water-insoluble complex protein separated from the wheat. The longer polymeric chains form due to protein–protein interaction in an aqueous medium which intensifies due to hydrophobic interaction among the non-polar groups. Wheat gluten has a polar charge of 10% but with a net positive charge. This makes it repulse excess water and resist complete dispersion in water. The poor solubility of WG in water at its natural pH level leads to chemical modification of WG to enhance its solubility. Research has shown citric acid, and sodium hydroxide enhances the deamidation and crosslinking of WG at a higher temperature. Therefore, the problem of the insolubility of WG has been addressed by adding sodium hydroxide (NaOH) as a dispersion agent to the wheat gluten and water mixture [78,89].

1.4 Natural coatings

Coatings are essential to prevent moisture, water or toxic environmental contaminations from entering the composite body. It allows minimum friction and dust build-up on the substrate, thus enhancing various composites' lifespan [90–92]. With the growing popularity of natural fibre-based polymer composites, the need to develop biodegradable natural coatings is increasing too. Natural fibres have strong non-polar characteristics, leading to poor matrix materials adhesion. It leads to the nonhomogenous dispersion of fibre in the polymer matrix. On the other hand, they are prone to moisture absorption when exposed to a humid atmosphere. It leads to early failure of the material during mechanical loading or thermal stress [30,93–95]. Many studies reported improving the fibre-matrix adhesion and different processes to coat the composite naturally. These coatings make the composite less susceptible to moisture or a toxic atmosphere. Hygroscopic ageing is one of the most popular methods to form a biobased coating on the fibres and the biocomposite [96].

Natural edible coatings are gaining popularity in the food and packaging industries [80,97]. Edible coatings are coated on the surface of the food products, which are thin and easily soluble. They mostly give better protection to the substrates against the oxygen, moisture and other toxic elements from the food surface. Composites of edible coatings can also reduce bacterial proliferation by containing antimicrobial compounds [98,99]. Adding vegetable oil to edible food coating also increases the food's moisture barrier, which helps them stay juicy [100]. Though the concept of biodegradable coating is gaining interest globally, these coatings aren't practical considering the current solid waste disposal process. As we know, in landfills or dump yards where solid waste is disposed of, they don't get the compostable environment to degrade themselves naturally. So it is also a global concern to find an appropriate method for managing solid degradable waste. However, biocoatings are still required to create moisture barriers for end products to increase their shelf life [91].

Agar-agar is one of the most commercially used coating materials. Being a porous and inexpensive polymer, Agar-Agar is used as a promising absorbent material. Being hydrophilic, it is often used as the skeleton adsorbent. Agar-Agar is used to prepare edible polymeric composite sheets in various applications with these many properties [99,101]. As a part of the natural and sustainable coating, gelatine plays a significant role. Gelatine is a biopolymer with multifaced nature. Gelatine is a filmogenic polymer with a sound barrier against moisture, oxygen and other atmospheric elements. It makes transparent films with antimicrobial properties, which can be used as a highly functional biodegradable coating. The adhesive property of gelatine helps it to stick firmly to any surface. Researchers have combined gelatine with other substances to make an effective coating for various applications [92,102–104]. Different biopolymer-based coatings act differently with added oil, fats, and acids, improving the shelf life, quality, physical properties, and chemical barrier, delaying discolouration of the substrate to it has been applied. These edible green coatings keep the food-grade products last longer, thus keeping the system sustainable [98].

1.5 Applications

Natural fibre-reinforced composites can be used in different structural or nonstructural applications of different areas of engineering. Because of their low cost, non-toxicity, high degradability rate and better weight-to-strength ratio, they are becoming a popular choice in the manufacturing industry. With the diversified properties of polymer composites, new sustainable products are being developed worldwide, starting from door panels, ceiling tiles, table tops, electronic boards, car panels, shoe soles, aerospace body parts, packaging, disposables, interior designing etc [17,25,36,105,106].

Few automobile manufacturers plan to develop a car’s entire body part using sustainable polymer composites. All-natural composites are increasingly used to solve many problems in our society. The field of application mainly varies according to the end-user. In such an approach, all-natural composites made of either industrial waste fibres or natural fibres are replacing disposable plastics. Disposable plastics are used worldwide for takeaway-based restaurants, eateries and hotels. Streetside food vendors, grocery stores, flea markets, vegetable and fish markets require disposable plastic packets or containers. It generates massive non-recyclable waste worldwide, becoming a prime concern for all environmentalists [20,107].

Microplastic pollution has reached the level where it has been found in the body fluid of unborn babies to the unexplored deep trenches in the ocean where even human interference has not been made yet. The only solution to avoid this problem is to reduce plastic usage and go sustainable wherever possible [108,109]. Many researchers and upcoming startup giants promote and produce sustainable and rapidly biodegradable cutleries and packaging units globally. The products are mainly developed using naturally available biodegradable fibre and polymer protein. Several researchers have developed biodegradable counterparts of plastic disposables using bagasse and starch-based composites. Besides, bamboo fibre, textile waste, banana fibre, rice or wheat husk and straws have been used widely to develop natural composites [21,110,111].

1.6 Need for development and characterisation of all-natural polymer composite

The previous sections have emphasised the sustainable approaches of the polymer-based composite and how they are the need of the hour. The petroleum-based synthetic fibres and epoxies are highly toxic to nature, so sustainable natural alternatives are in high demand worldwide. As the natural polymer-based composite industry is snowballing, the necessity to understand the properties of the developed products has become evident. Unlike conventional material, composite consists of two different materials, which are entirely distinct, and their integrated properties depend on how good the fibre-matrix adhesion is. Hence, this is required to characterise the developed composites to study their behaviour under different conditions.

Experimental characterisations are essential to understand the mechanical, morphological and thermal behaviour of the natural composites. The intrinsic characterisation is essential for natural polymer-based composites to understand their behaviour for structural applications. Different non-destructive characterisations like Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscopy (SEM) provide physical information on the bonding and adhesion of the constituents in the composite. The tensile and impact tests are used to study the mechanical behaviour of the composites. Thermogravimetric analysis (TGA) provides the thermal behaviour of the composite at elevated temperatures. Soil burial tests and time-dependent biodegradability by bacterial culture provide the degradation rate of the composites.

1.7 Organisation of thesis

The thesis consists of five chapters and is organised as follows.

Chapter 1: Introduction

This chapter includes the contextual research of the thesis with an extended overview of the introduction to natural fibre-reinforced green polymer composites. This chapter briefs the essential components used to develop all-natural composites with bio-coatings and their possible applications and characterisations.

Chapter 2: Literature Review

This chapter covers the extensive literature review of the work in the broad field of processing and characterising natural fibre-reinforced polymer composites and natural coating. The research gap and objectives of the study are outlined at the end of the chapter.

Chapter 3: Materials and Methods

This chapter covers the materials and methods of the present research work. Here the steps of developing different composites with different fibre fractions, crosslinkers, plasticisers, and treated fibres are discussed. The methodology to develop natural coatings for the composite is also briefly demonstrated.

Chapter 4: Results and discussion

This chapter focused on the mechanical properties (Tensile and impact properties), chemical characterisation (FTIR Spectroscopy, XRD), thermal analysis (Thermogravimetric analysis), morphological analysis (SEM), biodegradability (Soil burial test and Time-dependent biodegradability by bacterial culture) of developed Kibisu fibre-reinforced wheat gluten composite and the developed natural coatings for different compositions.

Chapter 5: Conclusion and future scope

This chapter summarises the research outcomes of the study. It outlines a specific conclusion from all the objectives of the study and suggests ideas for the future scope of this study.

CHAPTER 2

LITERATURE REVIEW

Natural composites exhibit outstanding biodegradability, non-toxicity, and good density-to-strength ratio with an economically viable and renewable market. Natural polymers are easy to process and require low heating conditions for moulding. With the introduction of green fibre reinforcement, they exhibit remarkable physical, chemical and thermal properties. Because of its renewable nature, the market for green polymer composites is increasing worldwide. The literature review explores all the information currently available on the topics that will be addressed in this thesis and emphasises the importance of the current study. This thesis concentrates on the green composite developed with waste Kibisu silk (Bombyx Mori), wheat gluten, castor oil and lemon extract as fibre reinforcement, matrix, plasticiser and crosslinker. Hence this chapter summarises current research in this direction that has been broadly discussed in this chapter. In this first section, the recent developments of biodegradable polymer-based composites have been explored, along with their drawbacks and advantages. The second section demonstrates the different options of natural fibres available in the market and how they enhance the properties of polymer composites as a reinforcement. In the next section, a closer look is imparted at the various works of literature dealing with wheat gluten-based composites and their advantages and drawbacks. The next section covers the literature on waste Kibisu silk-reinforced wheat gluten composites and their properties with varying constituents. The following section presents different literature on the role of crosslinkers and plasticisers used to develop wheat gluten-based composites. Different degradable coating development of bio-based coatings for different food-grade products and other applications is discussed after that. As biodegradability is one of the crucial factors for green composites and polymers, the next section describes different degradation methods for polymers and polymer-based composites. This chapter also includes the research trends, gaps, objectives of the proposed thesis, and plan of work in the direction of the proposed thesis.

2.1 Types of biodegradable polymers

The demand for bio-composites or green composites has increased drastically in the past few decades to reduce the usage of nonrenewable petroleum-based products. The usage of bio-composites is increasing both in the household and commercial sectors. Researchers are coming up with different manufacturing processes for the economical and easy production of bio-based composites. The hand-layup method is one of the fairly simple methods to manufacture biocomposites. Various researchers have reported hand lay-up methods for biocomposites followed by curing them in a hot air furnace and autoclave. Curing the composite sheets helps the entrapped air to come out, enhancing their mechanical properties and crack resistance. In addition, compression moulding is frequently employed further to shape the composite sheets in a desired structure [112]. Compression moulding is the most common and fast way to reshape thermoset and thermoplastic polymers. In a study, Serpil Edebali et al. showed that hemp-reinforced wheat gluten plastic showed better integrity among the constituents upon compression moulding. Compression moulding produces large-volume products that enable short cycle time, better part uniformity, and fast moulding of inserts, grooves, and holes in component [113]. Because of the advantages compression moulding imparts, it is widely used to manufacture biocomposites of different sizes and shapes in a controlled pressure and heating environment [114–116].

Biodegradable polymers extracted from natural and renewable sources are gaining popularity because of their non-toxicity and biodegradability in the past decade. The faster fossil depletion and growing awareness of a greener environment are grabbing the attention of scientists to develop more products using natural polymers. Isabelle Vroman et al. have classified natural polymers into two broad categories: natural polymers or agro-polymers and bacterial polymers. Natural polymers or agro polymers are generated in the lap of the natural growth cycle among different organisms. They are often called biopolymers because of their faster rate of biodegradability. Bacterial polymers are attained from the polymerisation of monomers through a series of fermentation processes. They are semi-synthetic polymers which require different culture media, environments and microorganisms to tailor their properties to become a microbial polymer. The detailed classification of natural biodegradable polymers is shown in Figure 2.1 [117].

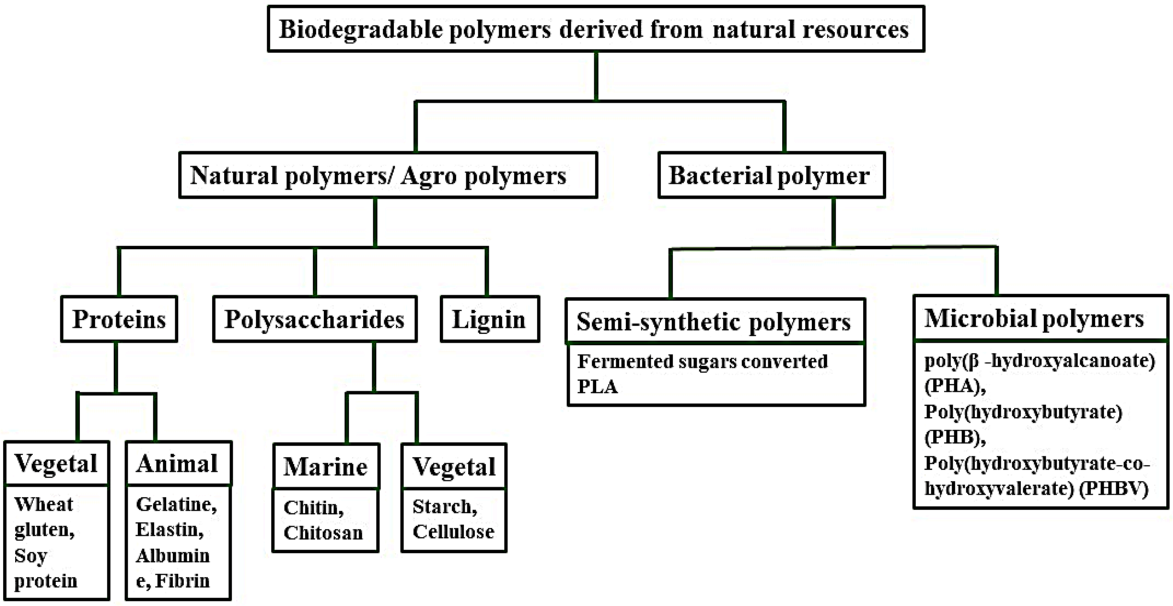


Figure 2.1. Classification of natural biodegradable polymers.

Clement M. Chan et al. manufactured wood flour-based PHA biocomposite and compared them with Polylactic acid (PLA) and polyethylene (PE) based wood flour composite. The authors concluded that the PHA composite performed better indoors in the ageing study. Whereas during the outdoor degradability study, PHA composites start to grow microorganisms faster, resulting in crack formation in the structure leading to early mechanical failure compared to others. The low degradability rate for PLA and PE-based composites in the outdoor environment shows better composite water resistance properties, resulting in similar mechanical strength after year [118].

Many different types of bacteria manufacture polyhydroxybutyrate (PHB), a natural biodegradable polymer that serves as an intracellular energy storage substance [119]. In a study, Kelen C. Reis et al. studied the effect of coffee waste as reinforcement in the PHB biocomposites. The inclusion of the reinforcement enhanced the impact strength significantly. Still, there was not much change in the thermal properties, and the thermal degradation was higher in one of the variations. Adding different coffee waste increased the water absorption also [120]. Polyhydroxy-co-3-butyrate-co-3-valerate (PHBV) is a bacterial aliphatic copolyester produced as a byproduct in the food industry that is entirely biodegradable upon composting [121]. Sarah Lammi et al. studied that reinforcing valorised olive pomace filler in PHBV accelerated the biodegradation kinetics of the composites, which exceeded 100% after 4 months [122].

Poly(lactic acid) or polylactide (PLA) is the most popular biodegradable polyester used for different biomedical, packaging and structural items. PLA inherits several advantageous properties like high tensile strength and, Young’s modulus, good flexural strength. However, PLA is very brittle to process and has less than ten percent elongation, which makes it very difficult to manufacture complicated parts. Fibre reinforcements are widely used to improve the overall properties of PLA-based products [34,123,124]. A study by Kyoung Ja Sim et al. reinforced bleached red algae fibre in PLA and PP matrix. The results showed greater storage modulus and better fibre-matrix adhesion were achieved in PLA-based biocomposites than in PP-based composites [125].

Cellulose is the most utilised renewable polysaccharide polymer on earth, and they are widely used for manufacturing biocomposites for bone and tissue engineering. Despite having excellent biocompatibility, cellulose is slowly degradable because of its tightly packed intermolecular bond [126]. Starch is an extensively used natural polymer matrix for producing biocomposites. It is economical and rapidly renewable from agricultural products like potatoes, corn, rice, and wheat [33,127]. C.R.di Franco et al. studied the effect of sisal-reinforced starch blended biocomposite, which showed enhanced hydrolytic stability with increasing fibre percentage [128]. Chitin and chitosan are the second most popular polysaccharide biopolymers. They possess long chain structures, which gives them unique physiochemical and optical properties like low density, high porosity, renewability, and high biodegradability. However, its widespread applications have been constrained by its subpar mechanical and thermal qualities compared to the conventional alternatives [129].

Gelatine is a protein-based polymer matrix extracted from animal-source collagen. Due to its great functional qualities, gelatine is widely employed in the food sector. Gelatine films can be used to package food, but their use is constrained because they are moisture-sensitive and easily dissolve, swell, or break when in contact with water. To enhance its barrier properties, different chemical agents are added to it [45,91]. Among different vegetal proteins, soy protein and wheat gluten are the most widely used natural proteins. Zein extracted from corn and soy proteins are the main source of plant proteins and collagen [130]. C. M. Vaz et al. studied the effect of casein reinforcements in soybean protein-based thermoplastic composite, which confirmed that soybean protein-based thermoplastic composite has a suitable range of mechanical and degradation properties. Soya protein-based composites can be used as a viable replacement for biomaterials [131].

Wheat gluten is one of the most inexpensive, biodegradable, renewable natural protein source extracted from agricultural resources. Wheat gluten (WG) is made of polar and non-polar groups of amino acids, which can be used for different thermomechanical interactions. It has excellent oxygen barrier properties making it a suitable bioplastic which can also completely break down in farming soil without generating harmful byproducts. Despite having many positive features, WG lacks flexibility and water resistance. There are several methods to enhance the flexibility, moisture barrier and water resistance of WG, like fibre reinforcement, filler addition and incorporating crosslinker and plasticiser. In a study by Pakanita Muensri et al., coconut-reinforced wheat gluten was compared with plasticised WG. The results showed that fibres within the range of fifty percent to twenty-one percent lignin content showed good thermo-mechanical properties. Reducing the lignin content slightly reduced the water absorption of the composite [132]. In several studies, wheat gluten is reinforced with banana fibres, crosslinked banana fibres, shrimp shell, wool, wood, waste silk and coconut fibres, increasing the overall mechanical properties, thermal properties and water and fire resistance [11,33,39,46,62,89,133].

2.2 Types of natural fibres

The main constituents of natural fibre-reinforced composites are plants, minerals and animals. In-plant fibres, mainly five types of fibres are used: wood fibres, straw fibres, seed fibres, leaf fibre, stem fibres. In animal fibres, mostly animal hairs and silk fibres have been used, and in mineral fibres, amosite, crocidolite, tremolite, actinolite, and anthophyllite fibres are popularly being employed. The performance of green composites or natural fibre composites mainly depends on their length, shape, and interfacial adhesion with the matrix. These fibres have good thermal and acoustic insulation, specific mechanical properties, and the most desirable property, i.e. biodegradability. As per renewability concerns, most researchers prefer plant-based fibres to develop biocomposites. The popularly used plant fibres and their relevant mechanical properties are shown in Table 2.1.

On the other hand, Animal fibres are mostly found as waste material from meat factories, textile factories or fish markets. Table 2.2 depicts the popularly used animal fibres and their mechanical properties reported by different researchers. Natural silk (Bombyx Mori) is one of the most popular and strong fibre reinforcements to produce natural protein-based green composites [44,134]. The waste natural silk has several popular names like chindi silk, filature silk or Kibisu silk [10,89,135,136]. India has a vast silk industry with a variety of silk products [67]. Because of the enormous silk industry in India, it generates an ample amount of silk fibre waste known as waste Kibisu silk every year. It is either discarded or sold by a third-party seller at a very cheap price [137,138].

Silk fibre has two different parts, fibroins and sericin. The silk fibroins provide inner strength, and they have a semi-crystalline structure. Whilst sericin is like an amorphous adhesive, holding the fibroins together [139]. Among the 18 amino acids that sericin possesses, serine aspartic acid and glycine are the three most essential amino acids responsible for its extreme hydrophilicity [40,140]. Research showed hydrophobic surfaces bond with proteins more definitely than hydrophilic surfaces. Hydrophobic surfaces attach to another hydrophobic surface more viciously because of a good adsorption process [141]. The sericin layer's hydrophilicity makes it often challenging to have a proper fibre-matrix adhesion with natural polymers like wheat gluten [140].

Different types of fibre-degumming or fibre-grafting methods are taken to reduce hydrophilicity. When used in developing composites, these degummed and detangled fibres help enhance adhesion and distribute the stress evenly in the structure, thus improving the overall properties of the composite [69].

Table 2.1: *Properties of different commercially used plant-based fibres to manufacture composites.*

Name	**Scientific name**	**Tensile strength**	**Modulus**	**Significant property**	**Ref**
Hemp	Cannabis sativa	1200 MPa	60 MPa	Industrial hemp is a source of high cellulose fibres and possesses superior properties such as high specific strength, stiffness and low density.	142
Ramie	Bohesmeria nivea	615 MPa	20.5 GPa	Ramie fibres are widely known for long, silky and strong plant fibres, making outstanding textile fibres with impressive mechanical properties.	143
Kenaf	Hibiscus cannabinus	692 MPa	10.94 GPa	Kenaf is considered an economically viable natural fibre with impressive properties to replace glass fibres in polymer composites as a reinforcement.	144
Jute	Corchorus	399 MPa	24.70 GPa	Because jute has high tensile strength, low thermal expansion, and high strength-to-weight ratio, it is being widely used for bio composite manufacturing.	
White coir	Cocos nucifera. L.	192 MPa	3.44 GPa	White Coir fibres possess low strength, low modulus of elasticity, and a high strain to failure due to their low cellulose content and large micro fibrillar angle.	145
Brown coir	Cocos nucifera. L.	343 MPa	4.94 GPa	On the contrary brown coir has an advantage over white coir. It improves the hybrid composite by improving the impregnation of small-diameter fibres.	
Bamboo	Bambusa vulgaris	206.2 MPa	20.1 GPa	Bamboo fibres are famous for their easy availability, low cost, fast renewability, high strength-to-weight ratio, high tensile strength, non-toxicity.	146
Bagasse	Saccharum officinarum L.	70.9 MPa		Bagasse fibres have the advantage over other natural fibres due to their excellent thermal property, energy recovery, good specific strength and low density.	146,147
Rice husk	Oryza sativa	135 MPa	1.8 GPa	The ultimate tensile strength of rice husk is compared to nylon fibres. The micro-arrangements of cellulose and hemicellulose help rice husk to attain high strength.	148
Napier grass fibre	Pennisetum purpureum	12.4 MPa	2.2 GPa	Napier grass fibres hold the lowest densities compared to other natural fibres. The tensile strength of the Napier fibre is greater than bamboo and coir but lower than flax, hemp, jute, kenaf, and banana.	149
Sisal	Agave sisalana	68 MPa	3.8 GPa	Sisal fibres has outstanding wear resistance, indicated by lower mass loss, specific wear rate and coefficient of friction.	150
Abaca	Musa textilis	308.7 MPa	6.19 GPa	The high cellulose and low water content of Abaca fibres acquire a high value of mechanical properties with impressive tensile strength.	151
Banana	Musa acuminata	550 MPa	3.5 GPa	With high strength and high flame resistance, banana fibres are preferred in making fire retardant panels, strong garments and yarns.	14,152
Betelnut	Areca catechu	128.79 MPa	2.56 GPa	Dried Betelnut fibres exhibit good Young's modulus compared to ripe Betelnut fibres. Betelnut fibres are preferred for applications with high dimensional stability because of their low moisture content and water uptake.	94

Table 2.2: *Properties of different animal/insect-based fibres.*

Name	Scientific name	Tensile strength	Modulus	Significant property	Ref
Human Hair	Homo sapiens (Trichology)	280 MPa	4.1 GPa	Hair executes high tensile strength, but it varies depending on the strain rate and humidity. The strain rate of hair is comparable to Keratinous materials and other common synthetic polymers.	153
Human nails	Corpus unguis	120 MPa	4.5 GPa	At low humidity, nails are more brittle and flexible at high moisture. The reason behind this is a reduction in torsional stiffness with increasing relative humidity.	154
Chicken feather	Gallus gallus zsdomesticus	203 MPa	3.59 GPa	The study showed chicken feather fibres have high resistivity and low dielectric constant, which helps them to be used as composite reinforcements to develop electric insulator panels.	155
Silk	Bombyx Mori	623 MPa	14.96 GPa	Natural silkworm silk fibres consist of two core of fibroin filaments bonded together by a layer of sericin. Silk fibres are abundantly studied because of their several unique properties such as good processability, desirable biocompatibility and biodegradability.	66
Pig hair	Sus scrofa	135 MPa	6.39 GPa	Being coarser and thicker, pig fibres can only be used in non-woven applications. As the tensile properties of pig fibres are comparable with other natural fibres, they can be used as low-cost environment-friendly biocomposites.	61,156
Alpaca	Vicugna pacos	190 MPa	2.7 GPa	Alpaca fibre is a soft, durable, luxurious and silky natural fibre. Alpaca is also valued because it is lustrous, extremely strong, very warm	157
Camel hair	Camelus bactrianus	212.15 MPa	3.87 GPa	Camel fibre adheres to tremendous breaking stress, higher breaking strain and significant rupture energy. The outstanding texture and mechanical properties encourage it to be a viable natural fibre to produce composites.	158
Spider silk	Araneae	1 GPa	10 GPa	spider frame silk can be considered the best fibre in terms of stiffness and strength, which makes it stronger per unit weight than high tensile steel.	54,159

2.3 Wheat gluten-based composites

Due to growing environmental concerns and depleting petrochemical resources, developing renewable polymer materials from agricultural feedstocks for developing sustainable products has become a significant concern for material scientists. Being a viscoelastic material with excellent tensile strength, good gas barrier properties, consistent quality, excessive availability and low cost, WG has become one of the most vital natural polymers. Natural polymers can be easily processed through thermal processing. Still, it’s an extra challenging job to process WG as it has complex intermolecular interaction and crosslinking, making it very brittle. A significant amount of external plasticisers were added to increase the mobility of the polymeric chains by reducing intermolecular bonds. Plasticisers reduce the strength of the products developed with WG. To enhance the mechanical properties of WG, different chemical crosslinking is also performed to extend its application [160].

In a study by N. Godard et al., wheat gluten was reinforced with beeswax and diacetyl tartaric ester of monoglycerides, increasing its strength and reducing water-vapour permeability without compromising its transparency [161]. Wantani Chaiwong et al. evaluated the effect of treated oil palm empty fruit bunch fibre reinforcement in WG composites. The tensile strength of the composites with surface-modified fibres (in NaOH solution of 1% and 5%) and unmodified fibres were compared. The results showed composite made of 15% fibre weightage and 5% NaOH treatment gave the best tensile strength and lowest water absorption [161]. SupakornThammahiwes et al. studied the effect of fish scale reinforcement on the tensile and degradation properties of the composite. The results showed that the WG/fish scale-based composites showed increased tensile strength (6.5–7.5 MPa) than pure wheat gluten samples (3.40 MPa). The addition of the fish scale also increased the weight loss of the WG composite after 120 hours of accelerated weathering compared to pure WG samples [162]. B. Ferrero et al. developed a WG-based composite with high cellulose-based Posidonia oceanica waste with the help of compression moulding. The composites made of 40% WG showed improved samples’ mechanical properties and water resistance [9]. T.Kunanopparat et al. developed composites of WG plasticised with glycerol and reinforced with hemp and wood fibres. The addition of fibres increased the tensile strength and Young’s modulus but reduced the elongation at break. The glass transition temperature of the composite was also observed, which attributes towards the plasticiser migration from matrix to fibres. This phenomenon is known as matrix deplasticisation, which increases the overall mechanical performance of the composite [53].

Divya Nataraj et al. developed composites of WG with reinforced banana stem fibre by solution casting. The composite films were crosslinked with citric acid and glutaraldehyde, and the results were compared. The crosslinking enhanced the strength of the composite from 3.5 MPa to 13 MPa, enhanced resistance to thermal degradation and reduced water sorption by 200%. M. Chandrasekhar et al. studied the effect of waste silk (Bombyx Mori) fibres reinforced in wheat protein isolates with different fibre loading. To develop the composites, WG was plasticised with glycerol and crosslinked with glutaraldehyde. The tensile properties of the composite increased with increased fibre loading, and the elongation decreased significantly. Though the fibre-matrix interaction was found to be very narrow, and the increased tensile strength of the composite was the outcome of surface modification of fibre and fibre weightage [135].

2.4 Waste Kibisu silk-based composites

The application of natural silk-reinforced composites to replace different petroleum-based products is gaining popularity nowadays. From small-scale disposable industries to big automotive industries are leaning towards natural fibre-based composites due to the recent shift of paradigm towards green manufacturing. Due to climate change and increased pollution, customers are also leaning towards more eco-friendly products [163]. Natural fibres have two essential factors; they are renewable and biodegradable. Among all the natural fibres, natural silks are a decent option for developing biocomposites. Adhering to their impressive properties, natural silks are widely used for sustainable applications and high-performance biocomposites. Two different sources of natural silks are available: silkworm silk (Bombyx Mori) and spider silk (mostly Nephila Clavipes). Spider silk possesses a specific modulus more than Kevlar, that’s why they are used in high-strength applications. However, the lack of mass production, limited availability and high-cost spider silks aren’t used to produce biocomposites for low-strength applications.

Silkworm silk has recently captured the interest of researchers worldwide for its remarkable properties over cellulose-based fibres. In India, the silk industry produces a considerable amount of silk waste annually. The waste silk fibres are also called Chindi silk, Filature silk or Kibisu silk. Kibisu silk is made of fibroin protein and a sericin layer. Fibroins offer core strength and stiffness, whereas the sericin layer acts like glue to bind the fibroins together. As a result of millions of years of evolution, natural silkworm silk possesses excellent mechanical strength, elongation and energy absorption. It holds better competitive properties than glass fibre and plant-based natural fibres [164]. For all these reasons, silkworm silks are being used as reinforcements in biocomposites. As a waste silk, Kibisu silk doesn’t obtain proportional diameter or mechanical strength throughout its length. That’s why waste silkworm silk or waste Kibisu silk is often used for low-strength applications [138]. Lalit Ranakoti et al. used Tasar silk waste (4 to 14 % fibre loading) and jute fibres (constant loading) to reinforce epoxy with the help of compression moulding. The results showed that composites with 12 % fibre loading showed optimized mechanical strength and specific wear rate [165]. Munir Taşdemır et al., the waste silk and cotton fibre of various lengths were reinforced in polycarbonate polymer, and the properties were characterised and compared. The results showed that increasing the silk length reduces the composite’s tensile strength, elongation and impact strength but the hardness and elasticity modulus increase. In cotton-based composite with increasing length, the tensile strength, elasticity modulus, and hardness decrease while elongation and impact strength decrease. The morphological study observed that cotton adheres to better fibre-matrix adhesion, where the sericin layer stopped the waste silk from having proper fibre-matrix adhesion [166].

In another approach, A. Nadhan et al. developed waste silk fibre and cellulose green composite, where they varied the fibre fraction in the composite. With increasing fibre content, the tensile strength increased, whereas the optical clarity and thermal stability decreased. The opaqueness of the silk and different refractive indices of the silk and matrix might be responsible for that. The fibre-matrix bonding is found to be moderate under morphological study [167]. Shaoyong Chen et al. developed waste silk fibre-reinforced PBS biocomposite using a hot press. The results concluded composites with 60 % fibre content delivered superior properties [15]. Moving towards all-natural green composite, M. Sekhar et al. developed short waste fibre-reinforced wheat protein isolate composites in the presence of synthetic plasticisers and crosslinkers. The results showed that with the increase of fibre loading, the tensile strength and modulus increase, but the elongation decreases [135]. Various studies implied that reinforcing different matrix polymers with waste silk positively changes the composite's properties. All these studies imply the possibility of developing biocomposites using waste silk fibre or Kibisu silk as a fibre reinforcement.

2.5 Types of crosslinkers and plasticisers for polymer composites

2.5.1 Plasticisers for polymer composites

Despite all these advantages, the major drawback of using natural polymer is its intrinsic brittleness. Various modifications to the molecular chain of the natural polymers have been made to address this problem with plasticisers, crosslinkers, and reinforcing fibres or introducing fillers into WG. The most widely used method to reduce the brittle fracture of natural polymers is reinforcing natural fibres. The rest of them requires toxic chemicals, which is not desirable to reduce the carbon footprint of the final product. To enhance the efficacy of the plasticiser, reactive crosslinkers are also employed to enhance the interfacial bonding among the polymer and fibres. Many researchers have proposed different synthetic plasticisers to improve the gelatinisation of the polymer for composite manufacturing, as shown in Table 2.3. In an eco-friendly approach, vegetable oils are used to plasticise the natural polymers. Vegetable oils are renewable and inexpensive. The long polymeric chains of fatty acid in vegetable oil help enhance the natural polymer’s flexibility through plasticising. In such an approach, Siti Kamarudin et al. discussed the possibility of using epoxidized jatropha oil as a plasticiser for kenaf fibre-reinforced PLA biocomposite. The results showed that plasticised PLA with 40% treated kenaf fibre-reinforced composites showed improved mechanical properties and better adhesion [168].

Table 2.3: *Different types of organic and synthetic plasticisers reported by the researchers.*

Major components	Name of the plasticiser used	Advantage/ Disadvantage	Ref
Wheat straw reinforced Linseed oil-based resin.	Wheat straw, resin made of n-butyl methacrylate and divinylbenzene comonomers, conjugated linseed oil as a plasticiser.	Apart from the natural resources, many chemicals have been used, which leaves the carbon footprint behind.	169
Bamboo fibres reinforced poly(lactic acid) biocomposite	A biobased monomer, i.e., epoxidised soybean oil (ESO), was used to in-situ polymerise at the interface between Bamboo fibres and PLA matrix for forming a polymerised ESO interfacial layer.	Epoxidation of the soyabean oil requires lots of time and the manufacturing of the composite using melt compounding and compression technique requires lots of chemicals.	111
Alginate cast films	Alginate cast films were plasticised by two plasticisers, glycerol and sorbitol, in different concentrations.	The plasticisers showed a positive influence on the mechanical properties of the prepared samples. But the plasticisers are synthetic, which cannot offer a sustainable all-natural composite.	70
chitosan/alginate bio-nanocomposites	chitosan/alginate bio nanocomposites were plasticised in different plasticiser environments (glycerol or 1-ethyl-3-methylimidazolium acetate).	Again synthetic plasticisers and ionic liquid ions are used to improve the interaction between the biopolymers.	170
Gelatin–olive oil composite films	Gelatin–olive oil composite films were prepared through emulsification to improve the water barrier ability of gelatine-based films.	It requires a microfluidiser to improve the particle size distribution in film-forming dispersions. It offers excellent water barrier ability but poor mechanical properties.	171
Polylactic acid/ halloysite nanocomposites	This mainly focuses on the thermal, rheological and thermomechanical properties of PLA with an impact modifier, plasticiser (triethyl citrate, TEC) and reinforcement (halloysite nanotubes, HNTs).	Synthetic plasticisers improved tensile properties but reduced extensibility and had a negligible effect on the thermal properties.	72

2.5.2 Crosslinker for polymer composites

Natural polymers have certain disadvantages as discussed in the previous sections. They need necessary modifications to obtain certain features like thermal stability, enhanced impact response, improved elongation, stiffness, and multiphase physical response. Sometimes through physical change, insoluble polymers develop solubility and vice versa. Polymer modification makes it easier to process them to achieve different shapes and sizes. Different modification processes, such as grafting, blending, and crosslinking, are popularly used by researchers. The physical properties of the polymers can be altered with the degree of crosslinking, uniformity of the network formation and the presence and absence of crystallinity in the polymer. With a low degree of crosslinking, the polymers with high crystallinity reduce their crystallinity. Reduced crystallinity hinders chain orientation, making the polymer softer and more flexible with a lower melting point. Crosslinking reduces free volume and modifies the local molecular packing; increased glass transition temperature of the polymer can verify that. The creep behaviour of the polymer also improves as crosslinking restricts the viscous flow. That’s why crosslinking is necessary for property synergism in the current polymer industry [172].

Yiqi Yang et al. studied the effect of fibre formation condition and tensile properties before and after crosslinking the casein in alkali solution with the help of citric acid. The results showed that crosslinked casein fibres had high tensile strength (110 MPa) and elongation at break (13.6%) when compared to uncrosslinked casein [130]. In another approach Yuanyuan Liu et al. studied the Structural property of crosslinked chitosan/silica composite films modified by genipin and glutaraldehyde under alkaline conditions. The results showed that genipin forms more hydrogen bonds with chitosan than glutaraldehyde resulting in better mechanical properties, crystallinity and a lower swealling ratio [173]. In a study Zeshan Tian et al. to improve the shelf life of green asparagus, a hemicellulose-based coating is developed with Alkyl ketene dimer (AKD) as a crosslinker. The results showed that adding AKD improves the respiratory barrier properties, slowed down the secretion of lignification-involved enzymes, and regulated the conversion of phenolic compounds of the hemicellulose-based coatings in highly humid conditions [174]. Ting Wu et al. tried to improve the flexibility of gelatine after gelation at low temperatures by adding PVA and sodium acetate trihydrate/urea deep eutectic solvent (SAT/U-DES) as a solvent, crosslinker and plasticiser. The results showed that with the addition of crosslinker SAT/U-DES, new hydrogen bonds formed while gelation, which improved the flexibility and ductility of the film [175]. To improve the properties of organic polymers, crosslinking plays a key role. Crosslinking enhances the mechanical properties as well as water resistance of any biopolymer [176,177].

Among the compound class of carboxylic acid, citric acid is the most commonly used crosslinker, improving the mechanical properties of WG based composites [46,178]. Research reveals that synthetic citric acid plays a crucial role in crosslinking the WG protein chains. Citric acid is highly reactive and forms ester bonds with WG [46]. To enhance the crosslinking among the WG molecules, organic lemon extract can be a viable source of citric acid. Natural lemon extract holds 45 g of citric acid per litre, making it a viable resource of nature extracted citric acid [78,88].

2.6 Bio-coatings for the composite

Coatings are essential to prevent moisture water or toxic environmental contaminations from entering the composite body. It allows minimum friction and dust build-up on the substrate, thus enhancing various composites' lifespan [179]. With the growing popularity of natural fibre-based polymer composites, the need to develop biodegradable natural coatings is increasing too. Natural fibres have strong non-polar characteristics, which leads to poor adhesion with the matrix materials. It leads to nonhomogenous dispersion of fibre in the polymer matrix [180]. On the other hand, they are prone to moisture absorption when exposed to a humid atmosphere. It leads to early failure of the material during mechanical loading or thermal stress [179]. Many studies reported improving the fibre-matrix adhesion and different processes to coat the composite naturally. These coatings make the composite less susceptible to moisture or a toxic atmosphere [98].

Hygroscopic ageing is one of the most popular methods to form a biobased coating on the fibres and the biocomposite. In a recent study, Mokhothu TH et al. have shown that the moisture absorption of a composite reduces to a great extent when coated with biobased polyurethane and polyfurfuryl alcohol [96]. The study shows that the flex/phenolic laminates coated with PU and PFA showed lower water absorption when compared to uncoated laminates. Among PU and PFA, PFA displayed better hydrophobic properties as a coating material. In a recent study by Tian, Zeshan et al., It was shown that little moisture absorption has no significant effect on the mechanical and thermal stability of the composite with coatings on it [174]. Recent studies have shown that biocoatings can also be applied to metallic substrates [179,181]. Biobased materials like hemicellulose are extracted from plants and are also a byproduct of pulping industry. It is water-soluble polysaccharides with lower molecular weight and hydrodynamic radius. However, hemicellulose is sensitive to moisture under high humidity conditions.

The water absorption reduces the binding capacity and weakens the hemicellulose-based film's structural stability and respiratory barrier. Alkyl Letene Dimer (AKD) is used as a crosslinker and a hydrophobic modifier to prepare a novel hemicellulose-based nanocomposite coating to improve the moisture barrier [174]. Pérez Gago et al. studied about polysaccharide bio-coating generated from brown seaweed that is sodium alginate. It is constituted of α-D-mannuronic and α-L-glucuronic Acid [182]. Sodium alginate is a thickening agent in the food industry as it is non-toxic, biodegradable, and has a high consumption acceptance rate. If applied as a coating, it improves the shelf life of food during storage [183]. Another promising candidate in the field of biobased coating is propolis. Propolis is a plant-derived resin product collected by bees to develop and protect the hive. In a study Gabriela Pires et al. showed that green propolis produced by Apis mellifera bees from plant-extracted nectar can be used as a coating for its excellent antioxidant and antimicrobial properties [184]. In a recent approach, commercial epoxidised cardanol from cashew nutshell liquid (CNSL) has proven to be an outstanding bio coating when blended with sucrose epoxy derivative, sorbitol and isosorbide [185]. Bisphenol A diglycidyl ether (BADGE) is a popular coating because of its unique molecular structure. Finding a substitute for BADGE is very difficult, but various biobased epoxy reactants can tailor the required properties of the biobased coatings for different applications [185]. Preserving fish or fish byproducts using green coatings is of tremendous interest in food industries. Alexsandra Cruz et al. studied that Sodium alginate bilayer coating combined with green propolis can increase the shelf life by up to eleven days [183].

Natural coatings are also used to protect the indoor microbial colonisation of higher places that require a high level of environmental hygiene. M. V. Revuelta et al. studied that Melamine formaldehyde as a capsule shell and Lavandin-Teatree essential oils as core material can be reinforced into painting as a biocide agent [186]. A novel UV-curable waterborne polyurethane acrylate was developed in a greener approach by P. Tsupphayakorn-aek, et al., comprising polymerisation of hydroxyl telechelic natural rubber (HTNR). It provides enhanced wettability with the surface, thus increasing the mechanical and thermal properties of the substrate [187]. Coatings are also used to enhance the anti-crease properties of cotton fabric. Cong Huang et al. studied that, the α-lipoic Acid (ALA) has improved cotton fabric’s crease-resistant and hydrophilic properties without loss of strength. The ALA attaches itself to cotton fibre through esterification between the carboxyl group and disulfide bond of cotton and ALA[188]. N, Prakashkumar et al. found that Neem gum is used as an effective biodegradable glue. It has hydrophilic polysaccharides obtained from exudates of neem tree which can be used to coat nanoparticles of PbNPs using the sonochemical method [189]. Natural edible coatings are gaining popularity in the food and packaging industries. Agar-agar is a plant-derived polymer with promising results when used as a coating. It has worked wonderfully to develop effective coatings for different fresh fruits, vegetables, fish and meat [80].

Edible coatings are coated on the surface of the food products, which are thin and easily soluble. They mostly give better protection to the substrates against the oxygen, moisture and other toxic elements from the food surface. Composites of edible coatings can also reduce bacterial proliferation by containing antimicrobial compounds. Adding vegetable oil to edible food coating also increases the food’s moisture barrier, which helps them stay juicy [189]. Thuong Nguyen et al. have deloped an active nanocomposite in an approach to enhancing bananas’ shelf life from physical and biological deterioration during transport and storage. The nanocomposite consists of polyvinyl alcohol (PVOH), Agar-agar and maltodextrin with silver nanoparticles. This coating helped the fruits to prevent bacterial inhibition against Escherichia coli and Staphylococcus aureus.

The addition of silver nanoparticles enhances the flexibility of the composite films, allowing them to coat different surfaces of complicated shapes [190].

Agar-agar is one of the most commercially used coating materials. Being a porous and inexpensive polymer, Agar-Agar is used as a promising absorbent material. Being hydrophilic, it is often used as the skeleton adsorbent. Agar-Agar is used to prepare edible polymeric composite sheets in various applications with these many properties [99]. Ashok Shrestha et al. studied that Agar-Agar usually produces a thick homogeneous gelled structure, and it takes a long time to dry off completely. But when mixed with xanthan gum or locust bean gum, it produces a translucent flexible film that is easy to dry and prevents water for a long time [191]. In an approach by Robson Geraldine et al., Agar-agar (1%) based coatings with 0.2% chitosan and 0.2% acetic acid showed promising properties when used as an edible coating. The study showed when applied to raw garlic cloves, the coating delayed the decolouration, fungal and bacterial growth, moisture loss and respiration rate, thus increasing the shelf life [101]. The cohesive structural layer of agar and ethanol reduces the water vapour permeability of polymeric composites from 75% to 95%, depending on the layer thickness. The gelation of agar creates a dense network which increases the water vapour resistance and mechanical resistance [99].

As a part of the natural and sustainable coating, gelatine plays a significant role. Gelatine is a biopolymer with multifaced nature. Gelatine is a filmogenic polymer with a sound barrier against moisture, oxygen and other atmospheric elements. It makes transparent films with antimicrobial properties, which can be used as a high functional biodegradable coating. The adhesive property of gelatine helps it to stick firmly to any surface [102,104,192,193]. Researchers have combined gelatine with other substances to make an effective coating for various applications. In one of the approaches, Gelatine-based coating is used to increase the shelf life of strawberries. It showed the coating only consists of gelatine, and starch doesn’t offer good antioxidant properties. To overcome this problem, plant-derived essential oils are used to improve the coating’s mechanical, antimicrobial and barrier properties. A study by M. Aitboulahsen et al. showed that to further enhance the barrier properties of Gelatine-based biopolymer coatings, Mentha pulegium can be used. It further enhances the barrier and antioxidant properties of the coating, thus providing better resistance to the strawberry surface [104,192].

The gullibility of gelatine is different for different surfaces. So, other substrates need a different approach to be coated with gelatine-based coatings. In a different approach Huiyun Zhang et al., gelatine and chitosan are used to develop an edible coating to delay meat discolouration in retail displays. The chitosan-gelatine edible coatings are incorporated with tarragon essential oil (TEO). Nanoparticles of chitosan gelatine were prepared with the gelation method. The nano-encapsulated TEO helps in the controlled release of TEO in the system, thus improving the meat’s antioxidant and anti-micro-bacterial properties. It also showed that glycerol instead of TEO can be used with different Chitosan and gelatine blends, reducing weight loss due to water shrinkage and lipid oxidation of the meat in display storage [103]. Gelatine and native cassava starch (NCS), sorbitol, and a different fraction of tetradenic riparia extract were used by J. Friedrich et al. to develop an advanced edible coating for strawberries. The films produced gives better protection from bacterial growth and antioxidant activity losses [192]. But with the increased thickness, the solubility reduces, directly influencing the water vapour transmission between surfaces. Porosity refers to small cavities or pores within the coating material, which can affect its physical and chemical properties. The degree of porosity depends on various factors, such as the composition of the coating, its thickness, and the presence of surface features like hairs or scales. In general, porosity can enhance the water and gas permeability of natural coatings, allowing them to regulate moisture and gas exchange with the environment. However, excessive porosity may also compromise the protective function of the coating, by allowing harmful agents to penetrate the surface or altering its mechanical properties. Thus, using gelatine-based antimicrobial edible coating is gaining popularity in the food industry. Different biopolymer-based coatings act differently with added oil, fats, and acids, improving the shelf life, quality, physical properties, and chemical barrier, delaying discolouration of the substrate it has been applied on. These edible green coatings keep the food-grade products last longer, thus keeping the system sustainable.

2.7 Biodegradability and environmental aspect of polymers

Biodegradability tests can be performed using ASTM D6003-96. In popular practice, the natural soil burial test is considered one of the most effective methods to check the rate of biodegradability [124,194–196]. Apart from that, the simulated solid waste aerobic compost test is also used for the same [194]. The specimens are buried six to seven cm deep inside the soil surface in the soil burial test. The soil is kept moist by watering it in a regulated manner. The compost soil is kept in a controlled environment in the compost test. The temperature is generally controlled at 30 °C with 50-60% water content. The pH level of the compost soil is maintained at 7. As per the design of the experiment, the samples are taken out from the soil and washed with distilled water until clean. Then the samples are air-dried in a hot air furnace at a temperature of around 65 to75 °C for 24 hours. The weight loss of the sample during the soil burial test and composite tests signifies the rate of biodegradability of sample [196].

Polymers are divided into two categories, biodegradable and nonbiodegradable. Though nonbiodegradable polymers have better properties but they face challenges during disposal. Synthetic, nonbiodegradable polymers are a matter of concern globally as they do not degrade fast. So, the popularity of degradable and recyclable polymers is increasing. Poly(lactic acid) (PLA) is one of the most commercialised degradable polymers. The PLA is a starch-derived natural polymer that reserves the exclusive properties of commercially used plastics [197]. Chitosan is another feasible option to be used as a biodegradable polymer. It is derived from shrimp shells and is completely non-toxic. Being biodegradable and having all the necessary qualities, shrimp shells are used mostly for biomedical studies [197,198]. Starch is another naturally found biopolymer that is widely used to manufacture substitute sustainable counterparts of plastic bags. Starch-based resins are biodegradable and have considerable thermomechanical and rheological properties. The main advantage of starch is its easy processing properties. It has been used for many years in plastic manufacturing as fillers, but adding starch reduces the mechanical properties of starch-based composites. This is one of the main reasons to keep starch content below 40% in any composite [197,199].

Apart from natural biodegradable polymers, other commercially used synthetic resins like polybutylene succinate (PBS), polybutylene succinate adipate (PBSA), Poly (butyleneadipate-co-terephthalate) are popularly used as biodegradable polymers [28]. Biodegradable polymers don't show a competitive advantage over synthetic polymers due to their low mechanical properties. Keeping the biodegradability factor in the count, natural cellulose is blended with natural polymer instead of integrating these natural polymers and synthetic polymers, making it more mechanically advanced yet 100% natural. Microorganisms play a significant role in the faster degradation of composites. The degradation study includes bacterias and fungi with the highest hydrolytic activity. Studies showed soil bacterias degrades the material faster than the isolated bacterias away from soil or compost [198]. Different hydrolase enzymes like lysozyme glycosides are responsible for faster degradation of materials upon soil burial or composting. The degradation of the composite materials depends on which bacteria or fungal growth can penetrate onto the surface faster [200].

In a case study of Chitosan and tannic Acid-based composite, it has been observed that it is very difficult for most of the microorganisms to grow in it due to antimicrobial and antifungal properties. The SEM analysis showed bacterial growth occurs on the composites' surface as a thin film. Further study shows that composite has 99% antibacterial shielding against Staphylococcus aureus but only 28.42% shielding against Escherichia coli. Oxidation is the key parameter for all microorganisms and fungi to access the material surface quickly. It bio-assimilates the molecular structure and breaks the hydrophilic oxidants, thus facilitating the biodegradation process [190,201]. Plenty of microorganisms are found in soil responsible for faster material surface degradation. For example, Yeast, Streptomyces strains, Fungi Mucor rouxii NRRL 1835, Aspergillus flavus, Penicillium simplicissimum YK etc. are to name a few [101,104,186,202].

2.8 Research trends, gaps and scope

Most of the studies found in the literature survey have developed green composite with chemical constituents with complex processing techniques. No all-natural simplified approach was found to develop low-strength biocomposites to reduce plastic waste. The biodegradability study of most of the biocomposites has not been discussed. The current study uses all-natural ingredients to develop a hundred percent green composites, including natural crosslinkers and plasticiser. As the final product will replace one-time plastic disposables, natural bio-coating has also been developed to protect the composite body from water vapour penetration. The biodegradability study of the developed composites and coatings has also been studied effectively as well. To the authors best knowledge in the light of the literature, such combination of green composites with all natural constituents (Matrix, fibre, plasticiser, crosslinkers and coating) has never been studied for their mechanical, morphological and molecular studies.

The main focus of the work is to study the physiochemical properties of waste Kibisu fibre-reinforced (treated and untreated) wheat gluten biocomposite. As natural plasticiser, castor oil is used, and crosslinking natural lemon extract is used. To develop the composite hand lay-up method is used, followed by hot compression moulding. According to the literature, green composites comprising this combination of constituents have never been developed so far. The effect of different natural vegetable oil-based plasticisers on the properties of the composite has not been studied earlier as well. The developed coatings are based on Agar Agar, and gelatine which is again made from all-natural constituents only. The biodegradability study of the samples in soil burial and bio-cabinet bacterial degradation has also been performed.

2.9 Objectives of the proposed thesis

The overall objective of the proposed work is to develop and characterise a novel green composite as a viable replacement for single-use plastic disposables using biopolymer wheat gluten and waste Kibisu silk as fibre reinforcement.

Based on the requirement to fully develop an effective natural alternative to plastic disposables, the following specific objectives have been achieved,

1. To investigate the effect of fibre weightage on Kibisu-reinforced wheat gluten biocomposites.
2. To investigate the possibility of replacing synthetic crosslinkers with natural lemon extract in the composite.
3. To investigate the possibility of replacing synthetic plasticisers with organic oil in the composite.
4. To investigate the role of surface modification of the Kibisu silk in their effect on the tensile properties of the developed composite.
5. To develop an effective biodegradable coating for the prepared composite to prevent moisture and water absorption.

2.10 Plan of work

The detailed plan of work has been illustrated in Figure 2.2. The experimental part consists of manufacturing waste Kibisu fibre-reinforced wheat gluten biocomposite using the hand lay-up method and compression moulding method. Different constituents have been altered to obtain the best possible combination for developing the final composite. Tensile testing, SEM, TGA, XRD, FTIR, and biodegradability tests are performed to characterise different composites based on different objectives.

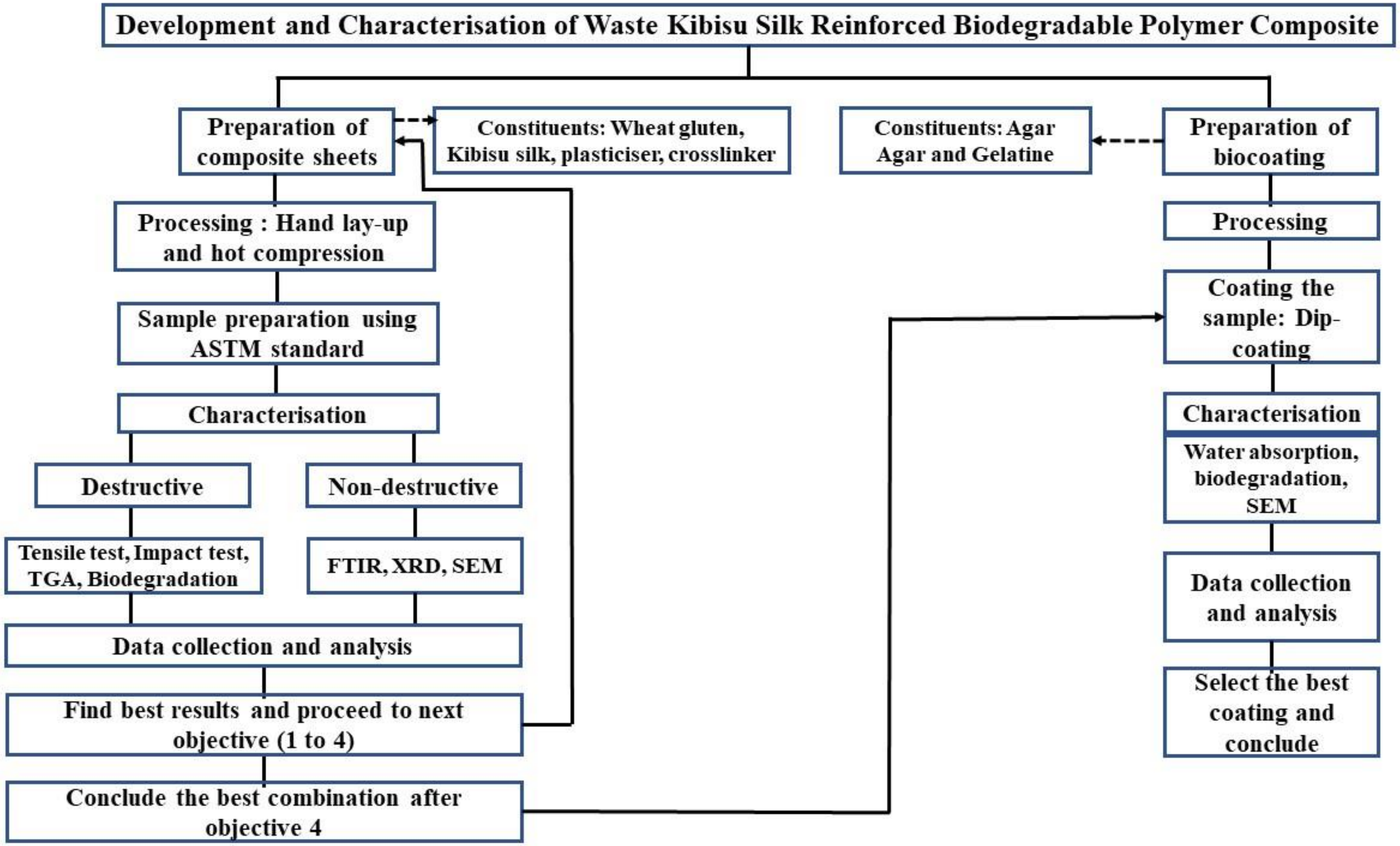


Figure 2.2. Plan of work.

CHAPTER 3

METHODOLOGY

This chapter presents in detail the material and methodology to develop waste Kibisu fibre-reinforced wheat gluten (WKF/WG) biocomposite as per different objectives of the thesis. This chapter will also cover the development of biocoatings for the composite. The detail of the processing and different characterisation techniques adapted for testing the developed composite samples have been reported in this chapter. This chapter will be divided into five different sections. The first section will discuss the fabrication method of different WKF/WG composites with varying fibre fractions. The required materials and their quantifying data to prepare the composite sheets are discussed in detail. This section also describes different destructive and nondestructive characterisation techniques used to characterise the developed samples effectively. The gathered data will be analysed and reported in the next chapter. The second section will study the effect of adding a natural crosslinker to the WKF/WG composites. The samples will be tested with and without a crosslinker to check the significance of adding a crosslinker to the composite properties. This section will also discuss the characterisation techniques used to study the physiochemical changes in the composite due to the addition of crosslinker. The third section will discuss the methodology to develop different WKS/WG composites with different organic plasticisers. The necessary characterisation techniques will also be discussed here to gather data to find the best-suited plasticiser for the developed composites. In the fourth section, composites will be prepared using treated and untreated fibres to check the effect of fibres' degumming on the composite's properties. This section also contains characterisation techniques employed for testing the treated/untreated fibres and the developed WKS/WG composites from treated/untreated fibres. The fourth section will cover the methodology to develop two different biocoatings for the WKS/WG composites. The characterisation techniques employed to check the efficacy of the developed coatings have also been discussed here.

3.1 Composite preparation with different fibre weightage

3.1.1 Materials and methodology

3.1.1.1 Materials

In this section, a novel composite has been developed which is environment-friendly and hundred percent biodegradable by reinforcing waste Kibisu silk into WG. The low cost and enormous availability of wheat gluten making it a good choice as a matrix material. Glycerol was used as a plasticiser to improve the extensibility of the matrix material to shape it into different products for commercial usage. Waste Kibisu silk has been used as fibre reinforcement for its outstanding mechanical property, low cost and abundant availability in Indian silk industries as a waste product. Moreover, organic lemon juice was used as a crosslinker to improve the mechanical properties by making longer polymeric chains. Commercially available wheat gluten (WG) powder was obtained from Urban Platter, Mumbai, India. The procured product consisted of 80% protein, 15% carbohydrate, 4% fat, 0.6% ash and 0.4% foreign substituents, as per the manufacturer's catalogue. 98% purified glycerol was obtained from Avantor Performance Materials India Limited, Thane, India. For maintaining the pH level of the wheat gluten solution, 98% pure analytical grade sodium hydroxide (NaOH) pellets were procured from Avra Synthesis Private Limited, Hyderabad, India. Waste Kibisu silk was used to reinforce the composite procured from Aliya Silk Traders, Sadahali, India. The fibres were golden brown in colour, having varying diameters throughout their length, with an average diameter of 1.3 (±0.5) mm and a length varying from 750 to 1000 mm, as shown in Figure 3.1.

Figure 3.1. Waste Kibisu silk with varying diameter.

3.1.1.2 Methodology

Three different kinds of samples were prepared with various mass fractions of the fibre. All the samples were prepared following the same process, where the WG and chopped WKF fibres of 20 ± 2 mm length were mixed in the ratio of 60:40 (Type 1), 50:50 (Type 2) and 40:60 (Type 3) to make the composite sheets. The constituents and weight percentage of the composites are described in Table 3.1. To make it a gluey paste, water has been added with the wheat gluten in the weight ratio of 1:5. The gluey mixture spreads homogeneously while mixing with the chopped fibre. The process requires hot water in which the wheat gluten was poured slowly and mixed gently by stirring the water at a fixed temperature of 80 °C with 10% glycerol as a plasticiser and 2.5 % NaOH plus 2.5 % freshly extracted lemon juice as the crosslinker. The pH level of the fruit juice extracted from citric fruits depends on the citric acid concentration. For consistency, the pH levels of freshly extracted lemon juices were tested every time before sample preparation. The lemon extracts with similar acidic levels showed similar colour, indicating consistency of the quality of lemon extract. The pH strip showed a dark pink colour representing the pH range of 1-2, strongly acidic in nature, as shown in Figure 3.2. The fibres were dipped into hot water for 15 minutes till they were soft enough to be added to the mixture. After 15 minutes, the water was drained out gently from the fibres, the WG mixture was poured onto it, and proper mixing was done with the hand. To keep the process consistent, hand mixing was done for 15 minutes until the mixture looked homogenous.

When the mixture became homogeneous, a thin sheet was formed out of it using the hand-lay-up method on a transparent overhead projector sheet (OHP) and kept inside a hot-air oven at 70 °C until it was dried entirely, as shown in Figure 3.3. The hand lay-up method helps remove extra air bubbles from the surface by compression, making the prepared composite sheets very compact. The dried sample sheets had a thickness of approximately 1.5 mm each and were stacked in layers inside a stainless-steel mould to achieve a sample dimension of 160 mm length, 105 mm width and 2.5 mm depth. The mould was then placed in the hot compression mould and compressed under a pressure of 120 bar for 5 minutes at 120 °C, followed by 15 minutes of the second compression cycle without applying any heat. The prepared samples were then cooled inside the mould and finally taken out for in-depth analysis. The graphical representation of the detailed methodology is shown in Figure 3.4. The samples prepared with the hand lay-up method may look slightly different from each other, but the tensile test results showed good repeatability in the mechanical properties [89].

Table 3.1: *Constituents and their weight percentage to prepare three different composites Type 1, Type 2 and Type 3 of wheat gluten and waste Kibisu silk.*

Name	Constituents	Weight %
Type 1	Wheat Gluten, Waste Kibisu silk, Glycerol, Sodium hydroxide (NaOH)	WG:WKS = 60:40 1 0% glycerol, 2.5% NaOH, 2.5% lemon extract of wheat gluten dry matter
Type 2	Wheat Gluten, Waste Kibisu silk, Glycerol, Sodium hydroxide (NaOH)	WG:WKS = 50:50 10% glycerol, 2.5% NaOH, 2.5% lemon extract of wheat gluten dry matter
Type 3	Wheat Gluten, Waste Kibisu silk, Glycerol, Sodium hydroxide (NaOH)	WG:WKS = 40:60 10% Castor oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter

Figure 3.2. pH test for the lemon extract used to prepare Composite of wheat gluten and Kibisu silk, (a) lemon extract used in Kibisu-Wheat gluten Type 1 composite (b) lemon extract used in Kibisu-Wheat gluten Type 2 composite (c) lemon extract used in Kibisu-Wheat.

Figure 3.3. Synthesised composite sheets of wheat gluten (WG) and waste Kibisu silk (WKS) with glycerol as plasticiser.

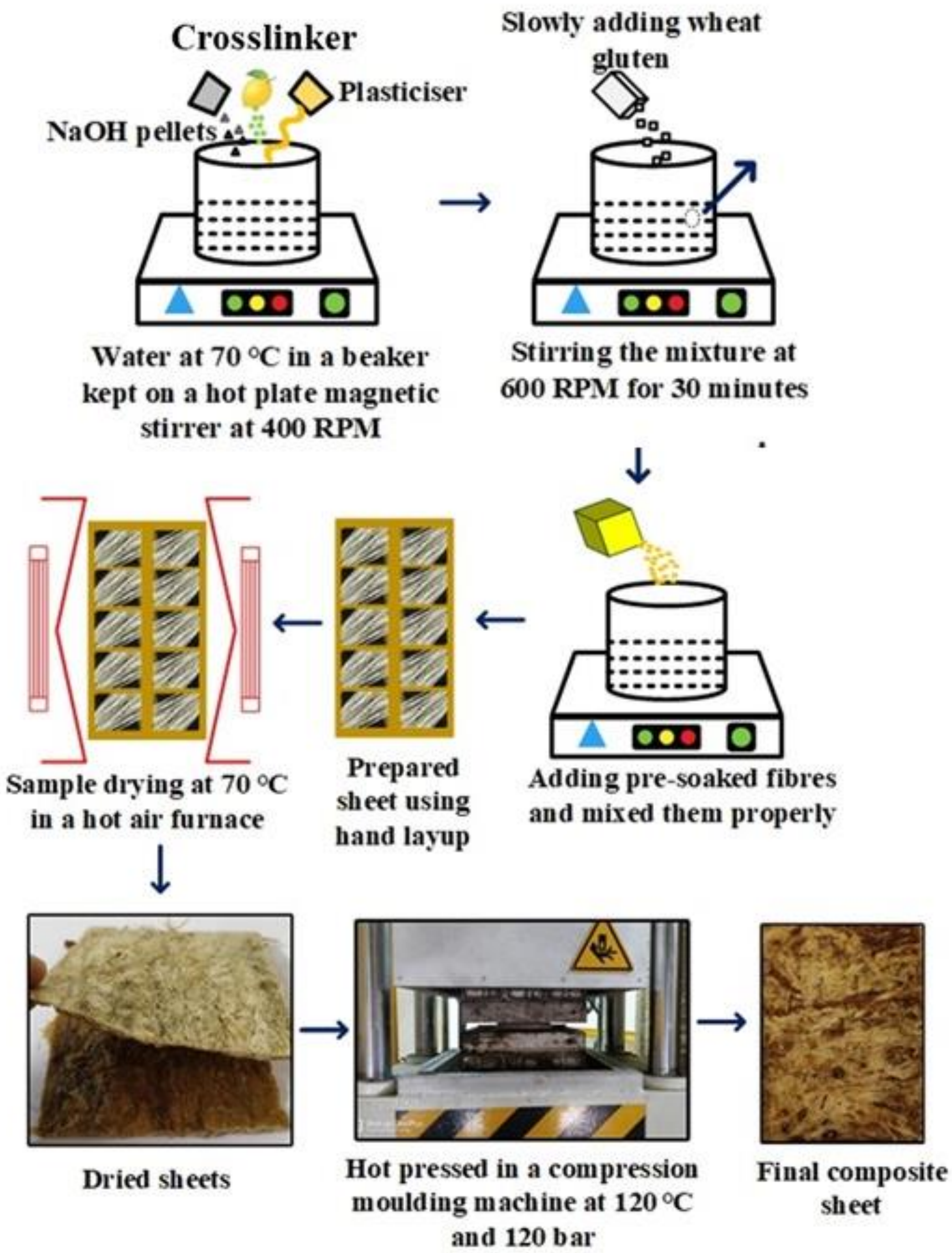


Figure 3.4. Graphical representation of the methodology to develop the WG/WKF composite.

3.1.2 Characterisation techniques

3.1.2.1 Mechanical properties

The tensile testing specimens were prepared as per ASTM D412 standard and shown at Figure 3.5. The Young's modulus, ultimate tensile strength and maximum elongation were measured using a universal tensile testing machine (Tinius Olsen H50KS, capacity 50 kN, Aimil india pvt ltd). The prepared specimens were subjected to tensile loading with a crosshead speed of 2 mm/min using a load cell of 5 kN. Eight specimens of each type i.e. Type 1, Type 2 and Type 3 were tested and mean values of Young's modulus, ultimate stress, and percentage elongation were reported.

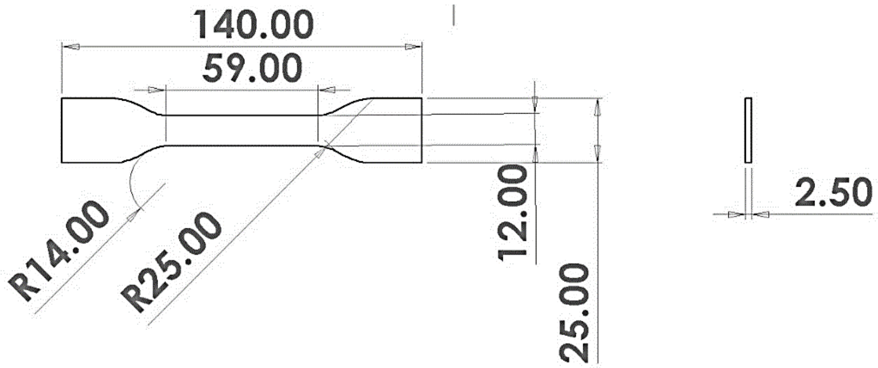


Figure 3.5. Dimension of the tensile specimen prepared by ASTM D412 standard.

3.1.2.2 Thermogravimetric analysis (TGA)

As the prepared composites can be used in different atmospheric conditions, finding the thermal stability for each type is extremely important. Therefore, Type 1, Type 2 and Type 3 composites were subjected to thermogravimetric analysis (TGA) to find out the percentage of weight loss (W_t) under changing temperatures. This thermal analysis of the samples was conducted on a Thermogravimetric Analyser instrument SDT-650, country of origin USA, model number SDT-650. The sample weight was kept at 5 mg, and the heating rate was 10 °C/min. The temperature range was from room temperature to 650 °C. The air supply rate for the test was 50 ml/min.

3.1.2.3 Fractographs by scanning electron microscopy (SEM)

The surface and cross-sectional morphology were studied using JSM-7500F Field Emission Scanning Electron Microscope (country of origin Japan) to check the fibre matrix interface and layer-to-layer adhesion after hot compression moulding. As all the samples were biological and non-conducting, they were platinum coated using a Fine Coater Machine [JEOL, JFC-1600 Auto, Japan] for 20 seconds.

3.1.2.4 Biodegradability test using soil burial test

The biodegradability test was carried out as per the soil burial test method of ASTM G21-70 standards. The samples were cut into 40mm × 20mm × 2mm sheets and washed adequately with ethanol to remove the surface impurities. Then, the samples were kept inside the hot air furnace at 80 °C for 2 hours and weighed (W_x) before placing them in the soil. The samples were buried inside a plastic tub with a wall thickness of 4 mm and 75 mm beneath the soil for consecutive seven, fourteen and twenty-one days, respectively, under natural atmospheric conditions. After every seven days, the samples were taken out of the soil and rinsed twice with distilled water. The cleaning of samples was followed by rinsing with ethanol to remove soil and oil/water particles. The clean samples were further kept in the furnace at 100 °C until a constant weight was achieved. The weight

(W_y) of dried samples was again taken to find out the mass degradation rate. Mass degradation was calculated by using Equation 3.1 as mentioned below,

$$\text{Mass degradation (\%)} = \frac{W_x - W_y}{W_y} \times 100 \quad \text{.........} \quad (3.1)$$

3.1.2.5 Soil test

The soil quality was tested using a soil test kit before and after degradation to compare the overall soil health. Four major parameters of the soil, i.e. Nitrogen (N), Phosphorus (P), potassium (K) and pH have been considered here. The soil sample was mixed with distilled water in a ratio of 1:2 and mixed thoroughly with the help of a magnetic stirrer for 15 minutes at 1200 RPM. After mixing, the solution was kept for 30 minutes to settle down until a clear separation between water and soil was found. After 30 minutes, the clean water from the surface is collected for different tests. To identify the percentage of each nutrient in the soil, synthetic reagents were utilized to colour-code the samples. The concentration of the nutrients is directly proportional to the intensity of the colour. The following reagents in Table 3.2 were used for colour-coding the soil's nutrients.

Table 3.2: *Reagents used for measuring different nutrients of the soil by colour coding.*

Soil Nutrients	**Reagents used**
Nitrogen (N)	cadmium metal, sulfanilic acid, gentisic acid
Phosphorus (P)	sodium molydate, potassium pyrosulfate, ascorbic acid
Potassium (K)	sodium tetraphenylborate
***p*H**	halochromic chemical compound

3.2 Composite preparation with the crosslinker

3.2.1 Materials and methodology

3.2.1.1 Materials

In this section, the efficacy of the addition of the crosslinker was verified. Preparation of the proposed composite sheets requires two main components, wheat gluten and Waste Kibisu silk. The primary component of the composite, WG powder, was procured locally. As received, the procured item comprises 75 % protein, 2% Fat, 1% Sodium, 14 % Carbohydrate, 6.5 % moisture, 1% ash and rest is foreign particles as per the composition received from the manufacturing company. As a fibre reinforcement, WKS was used, and it was obtained from the same vendor as discussed in section 3.1.1.1, with almost similar properties. The 100% pure and natural cold-pressed castor oil was procured from Rey Naturals, Gujrat, India. Pellets of sodium hydroxide (NaOH) of 98% purity were procured from Alpha chem, Haryana, India. As a natural source of citric acid, organic fresh lemon was procured from the Indian vegetable market at a very nominal price.

3.2.1.2 Methodology

Three different samples (C1, C2 and C3) were prepared with and without crosslinker. The constituents of each type of sample are mentioned in Table 3.3. The preparation of all the samples follows an approximately similar approach, as shown in Figure 3.6. An equal proportion of WG and chopped WKS (length 20 ± 2 mm) were taken to prepare all the samples as this combination showed optimised properties in the previous study.

Table 3.3: *Constituents and their weight percentage to prepare three different composites C1, C2 and C3 of wheat Gluten and Waste Kibisu silk.*

Name	Constituents	Weight %
C1	Wheat Gluten, Waste Kibisu silk, Castor oil	WG:WKS = 50:50 10% Castor oil of wheat gluten dry matter
C2	Wheat Gluten, Waste Kibisu silk, Castor oil, Sodium hydroxide (NaOH)	WG:WKS = 50:50 10% Castor oil and 2.5% NaOH of wheat gluten dry matter
C3	Wheat Gluten, Waste Kibisu silk, Castor oil, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 10% Castor oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter

For preparing the gluey paste of the matrix, WG was mixed with the water in the weight ratio of 1:10. For preparing the first sample C1, water was preheated to 70 ℃ with the addition of 10% castor oil and continuously stirred at 200 RPM for ten minutes. Then WG was slowly added to the water at 400 RPM for 30 minutes until castor oil plasticised the WG and made a smooth paste. In the next step, the WG mixture was poured slowly into the WKS and mixed properly by hand. The final composite sheets were obtained by hand lay-up. Then the prepared sheets were dried in the hot air oven at 70 ℃ until dry. To prepare C2, in addition to castor oil, 2.5% NaOH was added as a dispersion agent to prepare the gluey mixture of WG. To prepare C3, 2.5% NaOH and 2.5% lemon extract were added to the gluey mix for better crosslinking. The prepared sheets are shown in Figure 3.7, which visibly illustrates the WG cluster formation on the prepared sheets of C1 and C2, whereas C3 showed uniform distribution of WG. The rest of the process remains similar to preparing the composite sheets for C2 and C3. For uniformity, the pH level of the lemon extracts was checked every time with pH strips, and the lemon extracts with similar acidic levels (pH range 1 to 2) were considered. The dried sheets were hot-pressed at 120 ℃ and 120 Bar for 15 minutes and cold-pressed for another 15 minutes in a stainless steel mould. After cooling, the final sheets were taken out of the mould for different characterisations [78].

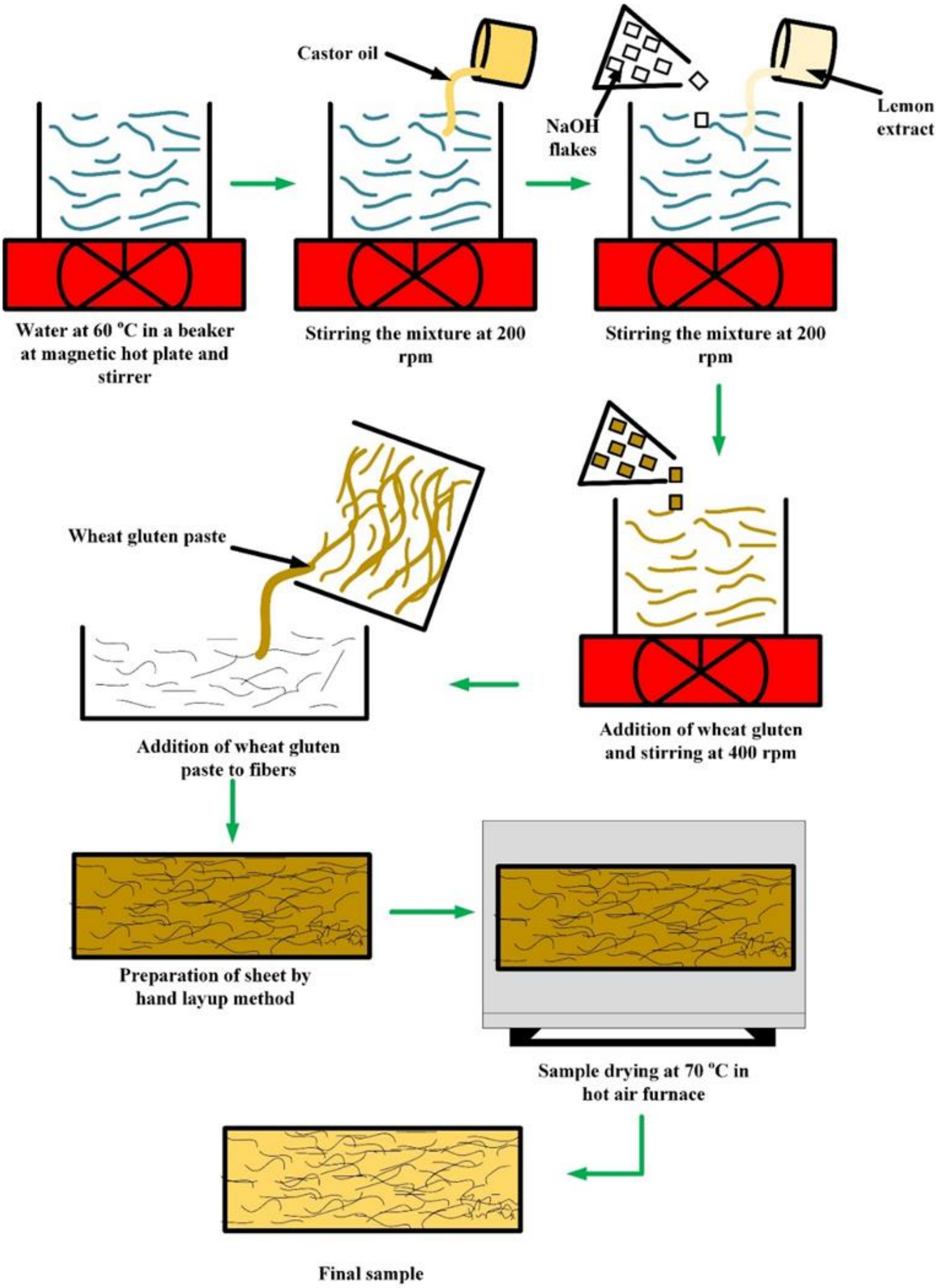


Figure 3.6. The stepwise fabrication method of the waste Kibisu silk-reinforced wheat gluten composite sheets.

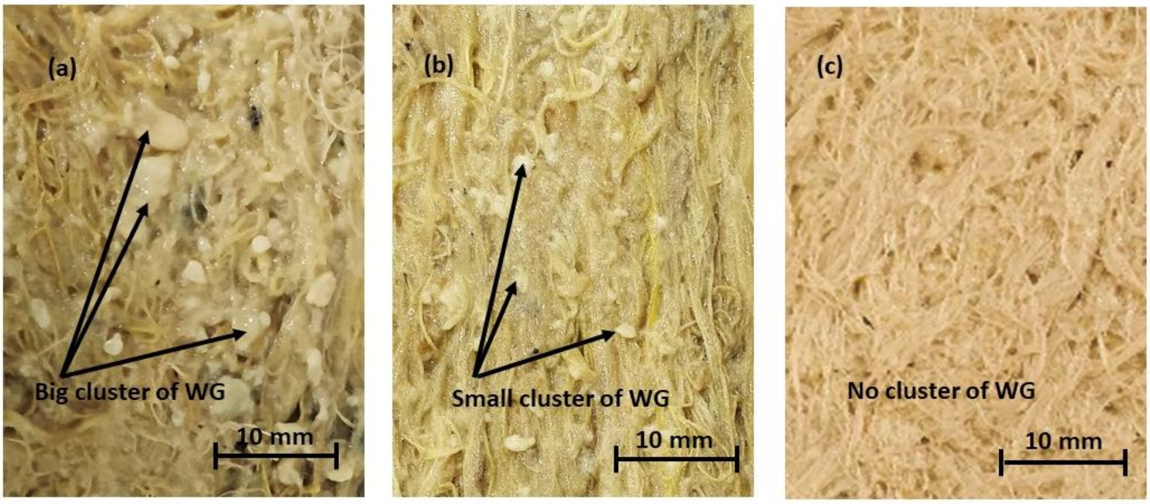


Figure 3.7. Prepared composite sheets of wheat gluten and waste Kibisu silk (a) C1 composite sheet with clustered WG formation (b) C2 composite sheet with small size clusters of WG (c) C3 composite sheet with no visible cluster formation of WG.

3.2.2 Characterisation

3.2.2.1 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy (FTIR) can analyse the molecular structure and level of interaction among the constituents and identify the types of functional groups of material. FTIR spectrometer was used to determine the effect of the lemon extract on the interaction among the different constituents of the developed samples mentioned in Table 3.3. The functional group and degree of interaction between the constituents (with and without the crosslinker) were determined using Nicolet iS50 II FTIR spectrometer in the wavenumber range of 400–4000 cm^{-1}. Number of scans are 32 per minute, resolution 16, assemble ATR with a smart iTX diamond crystal.

3.2.2.2 Tensile properties of the composite

Tinius Olsen H50KS of capacity 50 kN, a universal testing machine, has been used to study the mechanical properties of WG-WKS composites. The samples were prepared according to ASTM D412 standards. The extension rate of the machine was kept at 2mm/min using a load cell of 5 kN. A comparison of Young’s modulus (elastic modulus), ultimate tensile strength and elongation at break (% elongation) for each composite were studied.

3.2.2.3 Thermogravimetric analysis (TGA)

To find out the effect of crosslinking on the thermal stability of the samples, thermogravimetric analysis was performed in Analyser instrument SDT-650, country of origin USA, model number SDT-650. The weight and heating rate for the samples were 5 mg and 10 °C/min, respectively. The behaviour of samples was studied from room temperature to 600 °C.

3.2.2.4 Micrographs by scanning electron microscopy (SEM)

Before characterisation, the samples were cleaned with ethanol and dried at 50 °C for one hour. The cross-sections of the samples were studied using JSM – 7500F Field emission scanning electron microscope. The samples were platinum-coated using a fine coater machine JEOL JFC – 1600 Auto, Japan, for 15 seconds to make them conductive.

3.3 Composite preparation with different natural plasticisers

3.3.1 Materials and methodology

3.3.1.1 Materials

Commercially available wheat gluten (WG) powder was obtained from Urban Platter, India, with similar properties as described in previous studies. A 100% purified cold pressed Castor oil was obtained from Rey naturals India Limited, Gujrat, India. A 99% pure Linseed oil was procured from Camel, Mumbai, India. Cold and naturally pressed mustard oil was procured from Fortune group, Gujrat, India. 100% pure cold-pressed olive oil was procured from Figaro olive oil, Mumbai, India. Cold-pressed pure coconut oil was procured from Patanjali, Uttarakhand, India. As a dispersion agent of wheat gluten, 98% pure analytical grade sodium hydroxide (NaOH) pellets were procured from Avra Synthesis Private Limited, Hyderabad, India. Waste Kibisu silk was used to reinforce the composite procured from Aliya Silk Traders, Sadahali, India, just like in previous studies. For crosslinking, organic and fresh lemons were procured from the local vegetable market of Ropar.

3.3.1.2 Methodology

Six different samples were fabricated with various plasticisers, as mentioned in Table 3.4. The preparation of all the samples follows a similar approach, as shown in Figure 3.8. An equal proportion of WG and chopped WKS (length 20 ± 2 mm) were taken to prepare all the samples as this combination showed optimised properties in previous studies. To plasticise the wheat gluten, water was first heated up to 60 °C and kept the beaker on a hot plate magnetic stirrer to maintain the particular temperature. Then the other three major components, i.e. plasticiser, crosslinkers and dispersion agents, were added as per the weight fraction specified in Table 3.4. The components were then properly mixed using the magnetic stirrer at 400 RPM for 10 minutes. Afterwards, WG was slowly added in small proportions and mixed at 600 RPM with the water solution to avoid coagulation of the gluten particles. After 30 minutes of continuous stirring, it makes a smooth yellowish gluey paste which visually confirms the complete plasticisation process.

Before adding the WKSs into the gluey paste of WG, they were soaked in hot water (70 °C) for 30 minutes for softening. After adding the presoaked WKS with the gluey paste, it was thoroughly mixed using a hand and spread over a flat nonsticky surface with the hand lay-up method. The prepared sheets were then dried in a hot air furnace at 70 °C until dry. The prepared sheets hold an average thickness of 1.2 (± 0.3) mm. The prepared sheets were hot-pressed in a compression moulding machine at 120 °C and 120 bar for 10 minutes to acquire the standard sample thickness for various mechanical testing. The prepared samples were then oven-dried to remove any access oil and entrapped moisture. After the sheets were cooled to room temperature, different samples were prepared following ASTM standards for mechanical, thermal and chemical characterisations.

Table 3.4: *Constituents and their weight percentage to prepare composites of wheat gluten and waste Kibisu silk with different plasticisers.*

Full name	Assigned name	Constituents	%
Sample with glycerol as plasticiser	SGL (a)	Wheat Gluten, Waste Kibisu silk, Glycerol, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Glycerol, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter
Sample with castor oil as plasticiser	SCO (b)	Wheat Gluten, Waste Kibisu silk, Castor oil, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Castor oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter
Sample with linseed oil as plasticiser	SLO (c)	Wheat Gluten, Waste Kibisu silk, Linseed oil, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Linseed oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter.
Sample with mustard oil as plasticiser	SMO (d)	Wheat Gluten, Waste Kibisu silk, Mustard oil, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Mustard oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter
Sample with olive oil as plasticiser	SOO (e)	Wheat Gluten, Waste Kibisu silk, Olive oil, Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Olive oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter.
Sample with coconut oil as plasticiser.	SCCO (f)	Wheat Gluten, Waste Kibisu silk, Coconut oil , Sodium hydroxide (NaOH), Lemon extract	WG:WKS = 50:50 12% Coconut oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter.

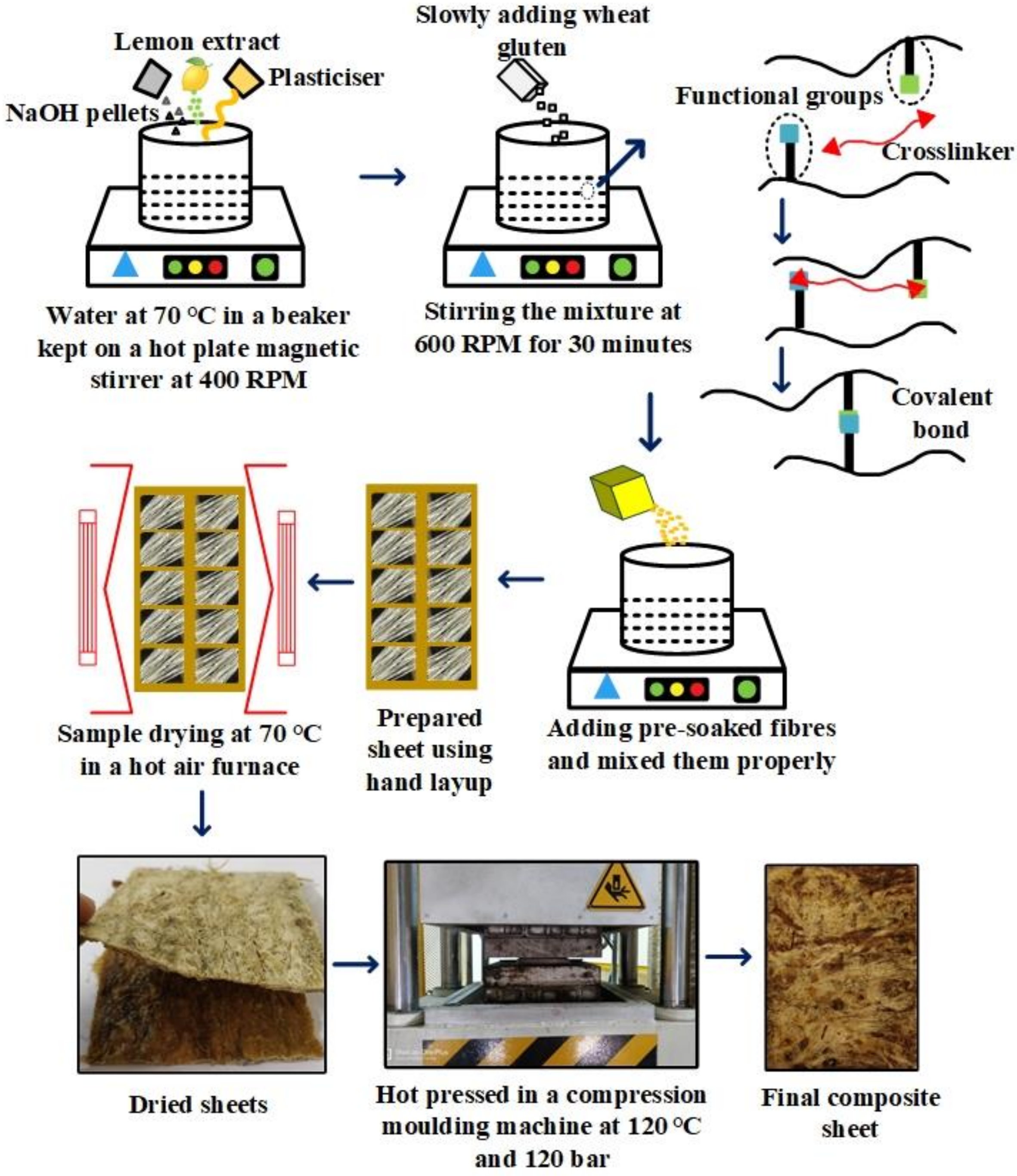


Figure 3.8. Methodology to prepare the composite sheets made of wheat gluten and Kibisu silk.

3.3.2 Characterisation

3.3.2.1 FTIR

FTIR was performed on Nicolet iS50 II FTIR spectrometer in the wavenumber range of 400–4000 cm^{-1}. Number of scans are 32 per minute, resolution 16, assemble ATR with a smart iTX diamond crystal.

3.3.2.2 Tensile testing

The tensile testing samples were prepared using ASTM D412. The samples were tested using the universal tensile testing machine (Tinius Olsen H50KS, capacity 50 kN) with a 2 mm/min crosshead speed using a load cell of 5 kN. A comparison of Young’s modulus (elastic modulus), ultimate tensile strength and elongation at break (% elongation) for each composite were studied.

3.3.2.3 Thermogravimetric analysis (TGA)

As the prepared composites can be used in different atmospheric conditions, finding the thermal stability for each type is extremely important. Therefore, all six composites were subjected to thermogravimetric analysis (TGA) to determine the percentage of weight loss (W_t) under changing temperatures. This thermal analysis of the samples was conducted on a Thermogravimetric Analyser instrument SDT-650, country of origin USA, model number SDT-650. The sample weight was kept at 5 mg, and the heating rate was 10 °C/min. The temperature range was from room temperature to 650 °C. The air supply rate for the test was 50 ml/min.

3.3.2.4 Scanning electron microscopy (SEM) fractographs

Before characterisation, the samples were cleaned with ethanol and dried at 50 °C for one hour. The cross-sections of the samples were studied using JSM – 7500F Field emission scanning electron microscope. The samples were platinum-coated using a fine coater machine JEOL JFC – 1600 Auto, Japan, for 15 seconds to make them conductive.

3.3.2.5 Bacterial degradation in Bio cabinet

Bacterial culture was performed using a laboratory strain of E. coli (Escherichia coli) in a media of LB broth and LB agar for solid and liquid media. The samples were kept in a Petri dish at 35°C in the respective medium in the presence of Escherichia coli for seven days, and the before-after weight of the samples was calculated.

3.4 Composite preparation with treated fibres

3.4.1 Materials and methodology

3.4.1.1 Materials

The WFS was procured from a local vendor in Karnataka, India, at INR 85/kg. The wheat gluten (WG), Organic fresh lemon, castor oil and sodium dioxide (NaOH) were procured from similar resources as described before.

3.4.1.2 Methodology

An alkali solution was prepared using 5% NaOH and distilled water for degumming the fibres. The fibres were soaked in the solution for 0.5, 1, 2, 4, 6, 8, 10, and 12 hrs, respectively as in Figure 3.10a. The fibres were withdrawn from the solution and washed in distilled water until clean and free from any NaOH residues. The treated fibres are semi-dried in natural sunlight and then kept in a hot air furnace at a temperature of 65 °C for 72 hours for complete drying. The weight ratio of wet and dry fibres varied between 1.5:1 to 1.3:1 as the sericin was gradually removed.

The fibres were chopped to a length of 20 (±3) mm for preparing the composite. A few fibres from each category were kept intact for the single fibre tensile test. The single fibre test specimens were prepared using the paper and glue method, as shown in Figure 3.9, according to ASTM 3822 standards. The gauge length was kept at 25 mm for all samples as per the ASTM standard, and five samples were tested under each category. The abbreviation used to represent different types of treated and untreated WFS are mentioned in Table 3.5.

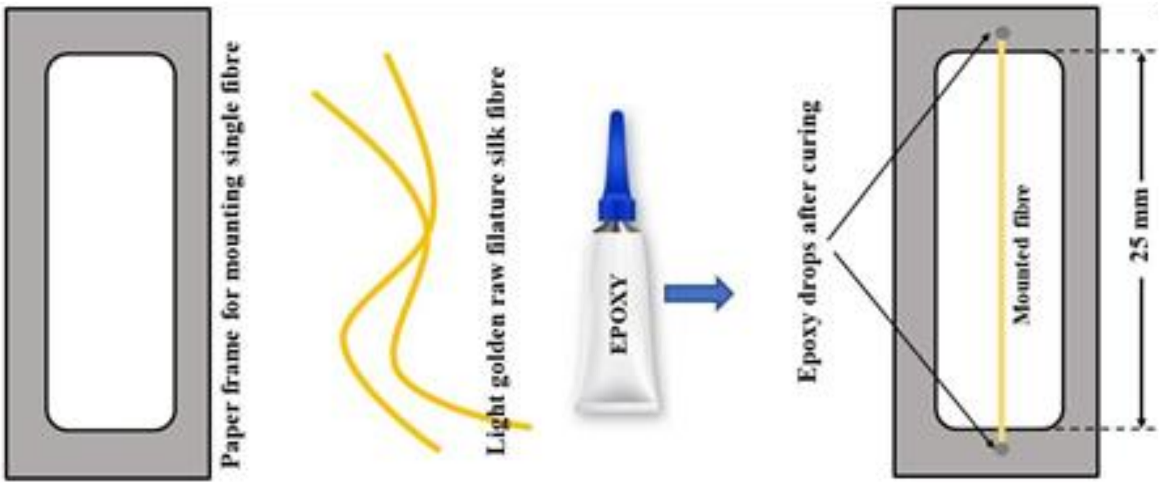


Figure 3.9. Schematic of sample preparation for the single fibre tensile test.

Table 3.5: *Abbreviation used to represent different types of treated and untreated WFS.*

Name	Abbreviation	Treatment time (hr)
Untreated fibres	UF-0	0
30 minutes of treated fibres	CF-0.5	0.5
1 hr of treated fibres	CF-1	1
2 hrs of treated fibres	CF-2	2
3 hrs of treated fibres	CF-4	4
4 hrs of treated fibres	CF-6	6
5 hrs of treated fibres	CF-8	8
6 hrs of treated fibres	CF-10	10
7 hrs of treated fibres	CF-12	12

For the fabrication of the composite, the WG was mixed with water in a 1:10 ratio. The water was preheated to 70 ℃ and kept on a hotplate magnetic stirrer to keep the temperature constant while mixing all the constituents. As a dispersion agent of WG, Sodium hydroxide (NaOH) pellets were mixed with water at a weight ratio of 2.5% of WG dry matter. Similarly, 2.5% (WG dry matter) lemon extract was added as a crosslinker to the hot water. For plasticising the WG, 10% (of WG dry matter) castor oil was added to the water as well and stirred for 10 minutes at 200 RPM. The WG was added slowly and gradually to the solution at 500 RPM to avoid cluster formation. After 30 minutes of continuous stirring, it forms a gluey paste of yellowish texture. The chopped fibres were added to the WG paste and mixed slowly with a stirrer until homogenous dispersion didn't occur.

The prepared mixture was then spread using the hand-layup process on a nonsticky surface and kept inside hot air woven at 70 ℃ until dry. The dried sample sheets had an average thickness of 0.9 (±0.25) mm. The prepared composite sheets were further compressed using hot compression moulding at 120 ℃ and 120 bar for 10 minutes, followed by 10 minutes of cold compression. The final fabricated composite sheets were used for various characterisation processes. All the composites were prepared using a similar method apart from the fibre types as per the treatment time. The graphical representation of the composite fabrication is shown in Figure 3.10b, where it represents the basic methodology to develop the composite sheet with hot compression moulding The composition of the prepared samples and the abbreviations used to refer to them are mentioned in Table 3.6.

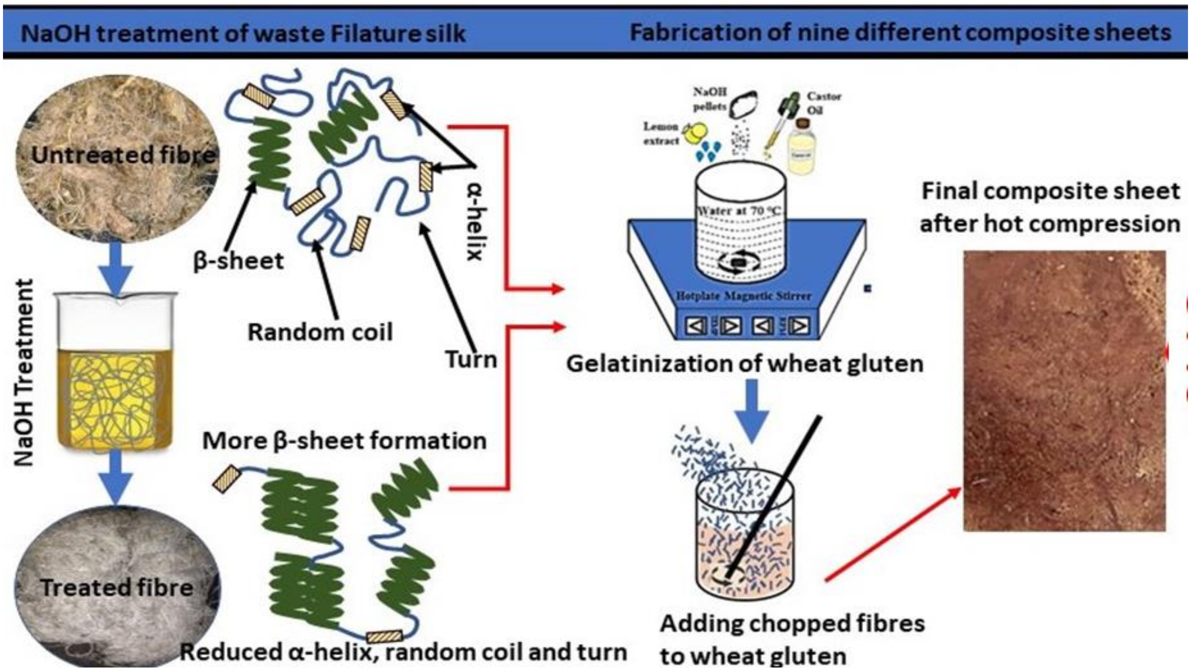


Figure 3.10. (a) Degumming of the waste Kibisu silk in NaOH solution causing more beta sheet formation (b) Preparation of the WG/WKS composite sheets with degummed fibres.

Table 3.6: *Sample composition and abbreviation used to represent different types of wheat gluten and filature/Kibisu silk-based composites.*

Name	Abbreviation	Constituents
Composite made with the untreated fibres	CUF-0	Wheat gluten:filature/Kibisu silk = 50:50 10% Castor oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter
Composite made with the 30 minutes of treated fibres	CTF-0.5	
Composite made with the 1 hr of treated fibres	CTF-1	
Composite made with the 2 hrs treated fibres	CTF-2	
Composite made with the 4 hrs of treated fibres	CTF-4	
Composite made with the 6 hrs of treated fibres	CTF-6	
Composite made with the 8 hrs of treated fibres	CTF-8	
Composite made with the 10 hrs of treated fibres	CTF-10	
Composite made with the 12 hrs of treated fibres	CTF-12	

3.4.2 Characterisation of the fibres and composites

3.4.2.1 X-ray Diffraction (XRD) analysis of the fibres

The degummed silk fibre's crystalline structure was examined using an X-ray diffractometer (Anton Par-PANalytical system, built Netherland) with Cu-Kα radiation source in the range of 10-60^o (2θ).

3.4.2.2 Fourier transform infrared spectroscopy (FTIR)

The functional groups and degree of interaction between the composite constituents were studied using Fourier transform infrared spectroscopy using Nicolet iS50 II FTIR spectrometer in the wavenumber range of 400–4000 cm^{-1}. Number of scans are 32 per minute, resolution 16, assemble

ATR with a smart iTX diamond crystal. As wheat gluten is more susceptible to moisture, all the samples were cleaned with ethanol and kept in a vacuum furnace at 60 ℃ for 24 hrs before every characterisation.

3.4.2.3 Scanning electron microscopy (SEM) analysis

The SEM micrographs were studied using JSM-7500F field emission scanning electron microscope. As the samples were nonconductive, they were platinum-coated by JEOL JFC-1600 Auto for 20 seconds prior to SEM analysis.

3.4.2.4 Tensile testing

The tensile strengths of the single fibres were assessed using the Instron 4301(3343) universal tensile tester at room temperature (32 ℃) and humidity (67.2%). Additionally, the strain rate was 1 mm/min, and a load cell of 1 kN was used. The mechanical characterisation in terms of tensile strength of the prepared WG-WFS composite sheets and mechanical characterisation was conducted using UTM Tinius Olsen H50KS of capacity 50 kN. The strain rate was kept at 2 mm/min, where the load cell was 5 kN. The samples were prepared following ASTM D412 standards, where each test was repeated four times, and average values were reported. The average values of Young's modulus, tensile strength, and percentage elongation for different samples are compared and reported.

3.4.2.5 Impact Strength

The impact strength of the composite was analysed using the Izod impact test using the Rockwell hardness testing machine by polymer digital impact tester (Fine Instruments Presto) according to ASTM D785-98 standards. The sample size was 63.5 mm × 12.7 mm × 3 mm with a v-notch at the middle. The impact energy was calculated in the Izod scale (J/m).

3.4.2.6 Thermogravimetric analysis (TGA)

The thermal properties of the prepared composites were tested by Thermogravimetric Analyser (TGA) (as mentioned in the previous section) in the room temperature range to 620 ℃. The initial weight of the samples was 5 mg, and the heating rate was 10 ℃/min.

3.5 Development of natural coatings for the composite

3.5.1 Materials

Agar Agar and gelatine powder were procured locally from the Ropar market. Commercial tea leaves were procured from Tata Tea, Munnar, Kerala, India. Castor oil was procured from the same source as described in the previous sections. Analytical grade 95% pure calcium chloride was procured from destiny chemicals, Vadodara, Gujrat, India.

3.5.2 Methodology

Two different types of coating were developed using natural constituents, as shown in Table 3.7. The first coating was developed using Agar Agar, as shown in Figure 3.11. This solution was prepared by stirring 2 mg/ml agar-agar solution with distilled water at 60-65 °C for 15 minutes at 600 RPM. It was followed by adding 1% castor oil (w/v) of the formed gel. The composite sheets were dip-coated as per the requirement and dried at 70°C. For stabilising the coating, it was coated with 2% (w/v) calcium chloride solution for 30 seconds and dried in a hot air furnace at 50 °C for 15 minutes [203].

Table 3.7: *Sample composition to develop Agar agar and Gelatine based natural coatings.*

Development of coating	Constituents
Agar Agar	Formation of gel, Agar Agar: Distilled water = 2 mg/ml (w/v), 1% castor oil (w/v) of the formed gel, 2% (w/v) calcium chloride solution in distilled water.
Gelatine	Tea leaf: water = 1:5 (tea water), diluted aloe vera extract: tea water = 50:50, 2% gelatine (w/v) in aloe-tea water solution.

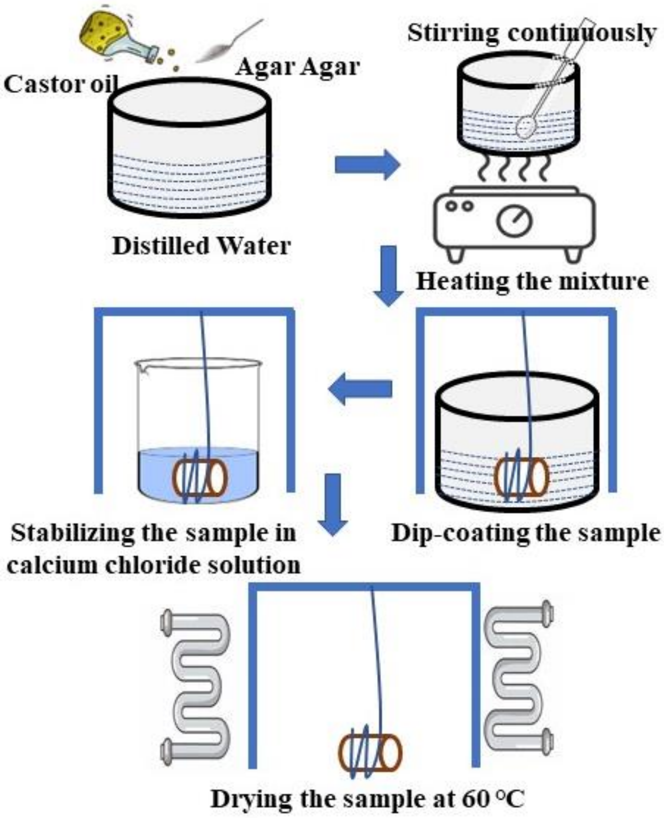


Figure 3.11. Stepwise process to develop Agar agar based coating.

For the second coating, the tea leaf was boiled in water with a ratio of 1:5 (w/v) and strained using filter paper. Aloe vera gel was collected naturally from plants and mixed with distilled water (50:50) to form an aloevera gel solution. The prepared tea water was mixed with natural aloe vera extracts at 60 °C in a 50:50 ratio for 30 minutes until it made a homogeneous mixture. For more thickening of the prepared mixture, 2% gelatine was mixed with the aloe tea water at 70 °C for 10 minutes till it made a semi-thick solution [204]. The composite sheets were then dip-coated as per the requirement and dried at 70 °C in a hot air furnace. The detailed methodology is shown in Figure 3.12. The developed coatings after complete drying are shown in Figure 3.13, where the Agar Agar-based coating is translucent in nature, and Gelatine-tea extract-based composite is chocolate brown in colour.

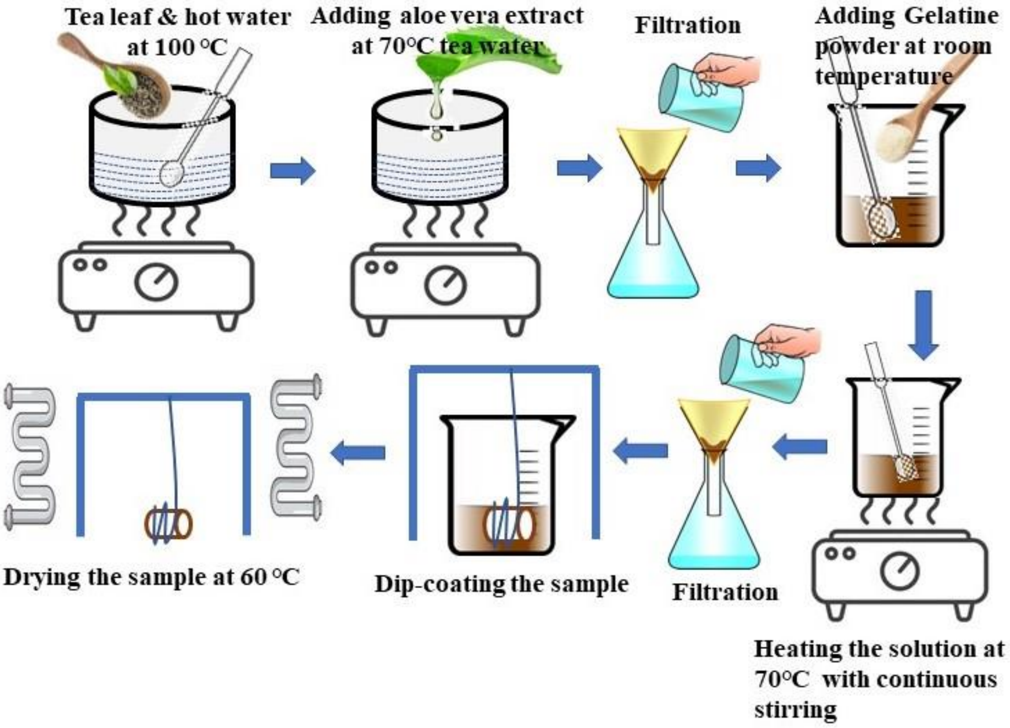


Figure 3.12. Stepwise process to develop gelatine-based coating.

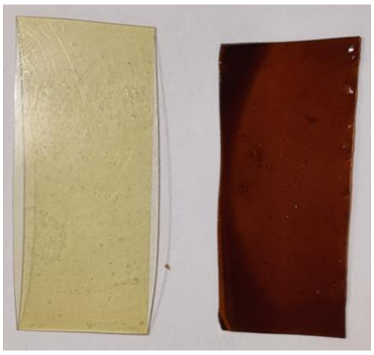


Figure 3.13. The final developed coating of Agar agar (Translucent) and gelatine (Coffee brown).

3.5.3 Characterisation

3.5.3.1 Biodegradability test in biocabinat

Bacterial culture was performed using laboratory strain of E. coli (Escherichia coli) in a media of LB broth and LB agar for solid and liquid media. The coatings were kept in a Petri dish in 35 °C in the respective medium in the presence of Escherichia coli for ten days, and the before-after weight of the samples was calculated. The SEM images of the coatings before and after degradation have also been added in this section for a clear understanding of the degradation.

3.5.3.2. Water absorption test

As the proposed composite will be used for the natural environment, it is essential to add a preventive coating to provide primary prevention from water penetration in the structure. To check the effectiveness of the developed coating water absorption test was performed using ASTM D 570-98. The samples were dipped into water for 48 hours, and the change in weight due to water absorption was calculated at different time intervals. The data shows the effect of different coating thicknesses on the water absorption of the composite. Water absorption is calculated at different time intervals using Equation 3.2. Where WA is water absorption (in percentage), W_1 is current weight and W_0 is the initial weight.

$$WA(\%) = \frac{W_1 - W_0}{W_0} \times 100 \quad \text{…………..……………………………………………………….} \quad (3.2)$$

3.5.3.3. Porosity of the developed coatings

The porosity of the developed coating was calculated by analyzing the SEM images of the coated surfaces using ImageJ software version 1.5.3 (open domain). The scale of the image and software were calibrated before the analysis and the calculated percentage of porosity was derived from it.

3.5.3.4 SEM analysis

The SEM micrographs of the cross-section of the coated surfaces were studied using JSM-7500F field emission scanning electron microscope. As the samples were nonconductive, they were platinum-coated by JEOL JFC-1600 Auto for 20 seconds before SEM analysis.

3.6 Summary

This chapter has presented an objective-wise description of the materials used in the study. The details on composite and coating development and standards used to carry out morphological, thermal, chemical, degradation and mechanical testing. The next chapter will investigate the developed composite’s morphological, thermal, chemical, degradation and mechanical properties.

CHAPTER 4

RESULTS AND DISCUSSION

In the current chapter, the mechanical, thermal, chemical, morphological, and degradation properties of the developed composites and coatings will be discussed in depth. The first section will cover the impact of fibre weightage on the physiochemical properties of the composite. In the next section, the effect of natural crosslinker on the properties of the composite will be verified. The third section will cover the effect of different natural plasticisers on the properties of the developed composite. This section also includes the bacterial cabinet degradation of the developed composites. The next section will cover the effect of fibre degumming on the properties of the developed composite. The last section of the chapter verifies the efficacy of the two developed biocoating. This chapter will optimise different parameters through different sections, leading us towards the most efficient wheat gluten and waste Kibisu silk (WG/WKS) composite with an all-natural coating as well.

4.1. Composite characterisation with different fibre weightage

4.1.1 Tensile testing

The tensile testing showed variation in Young's modulus, ultimate tensile stress and percentage elongation with a change in the mass fraction of fibres in the composite. It can be observed from Figure 4.1a that Type 2 composites having 50% WG and 50% F show remarkably higher Young's modulus compared to Type 1 and Type 3 composites. Similar trends were observed for ultimate force, where Type 2 samples have shown better properties, as shown in Figure 4.1b. It is observed that the composites having lower wheat gluten have shown low modulus due to a lack of binding material. A higher percentage of the binder holds the fibres together, which delivers better tensile modulus. However, too much wheat gluten causes agglomeration, leading to the irregular distribution of binder resulting in poor tensile properties. The tensile test also shows that the elongation of the composites decreases with an increase in the percentage of fibre in the matrix beyond 50%. Figure 4.1c shows that a maximum elongation has been achieved with Type 2 sample, which is another positive attribute of the developed composite. The tensile properties of these combinations are comparable with its contemporary options made up of different green fibre-reinforced wheat gluten composites.

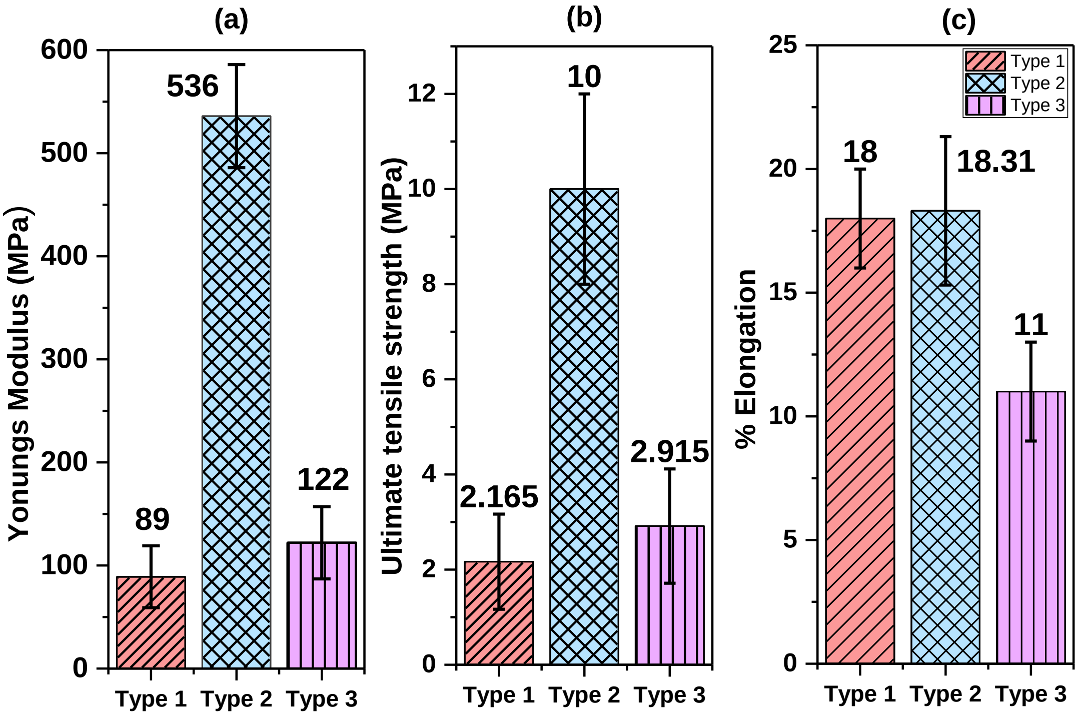


Figure 4.1. Variation in (a) Young’s modulus, (b) Ultimate tensile strength, (c) % Elongation of wheat gluten (WG) and waste Kibisu silk (WKS) composites with different mass fractions of fibres.

4.1.2 Thermogravimetric analysis (TGA)

Thermal stability of the developed composites is an essential property as it controls the end use of the product as well as the mechanical behaviour and processing of the composite. Figure 4.2 shows the TGA analysis for all the composites, which illustrates the percentage of weight (W_t) over continuous heating. It shows two stages of significant weight loss for all the samples. The first nine percent weight reduction ensued at around 100 °C, which indicates initial water loss and degasification. The combination of wheat gluten and lemon extract makes natural superabsorbent material absorbing ambient moisture instantly [89]. From the SEM images in Section 4.1.3, it is apparent that the samples are also porous. This might be the reason for the initial nine to ten percent weight loss of the samples due to the vaporisation of the absorbed moisture. Because of less percentage of wheat gluten present in Type 2 and Type 3 samples, moisture absorption is lower, which justifies better thermal stability during the first phase of weight loss in them. The second rapid decrease in the weight percentage occurs at around 180 °C for Type 1 and 267 °C for Type 2 and Type 3 samples. This is due to the degradation of the gluten followed by fibres. For Type 2 and Type 3 composites, the first 7% weight loss happens at 100 °C followed by 20% at 267 °C and 60% at 450 °C. Whereas the weight loss of Type 1 is 10% at 100 °C, 20% at 172 °C and 60% at 300 °C. This increase in W_t value is because of the highly loaded fibres and less gluten in Type 2 and Type 3 composites. The W_t value of a single untreated fibre has been previously calculated to be 20% at

320 °C. This also supports that the increased mass fraction of fibres in the composite increases the overall W_t of the composite.

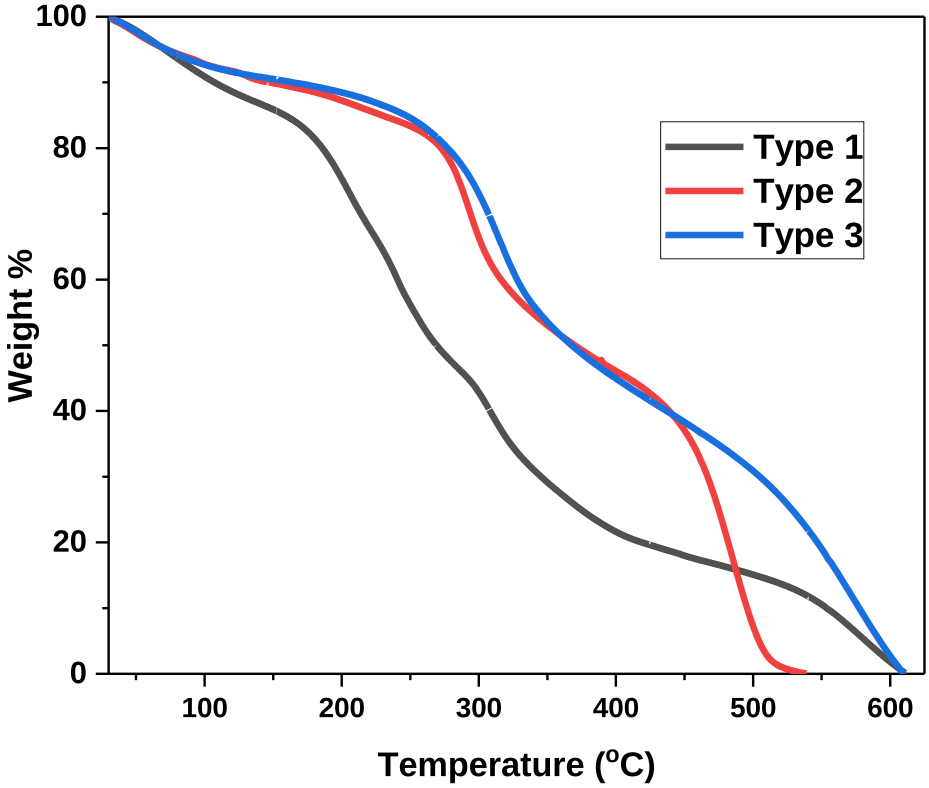


Figure 4.2. TGA analysis of Type 1, Type 2 and Type 3 Kibisu-wheat gluten composites of variable mass fraction of fibres showing the % weight (W_t) of the composites upon heating.

4.1.3 Fractographs by scanning electron microscopy (SEM)

After tensile testing, the fractured sections of Type 1, Type 2 and Type 3 composites were observed under SEM, and the fractographs are shown in Figure 4.3. A competent interface between fibres and matrix can be observed in Type 1 and Type 2 composites, as shown in Figure 4.3a and b, which may be attributed to a high percentage of WG providing better fibre-matrix bonding. This, in turn, results in a better elongation of Type 1 and Type 2 compared to Type 3. However, in Type 1 composite, the accumulation of wheat gluten throughout the structures made layer-to-layer adhesion irregular. The weak bonding in-between the prepared layers after compression moulding is the main reason for the premature failure of the Type 1 samples during tensile testing.

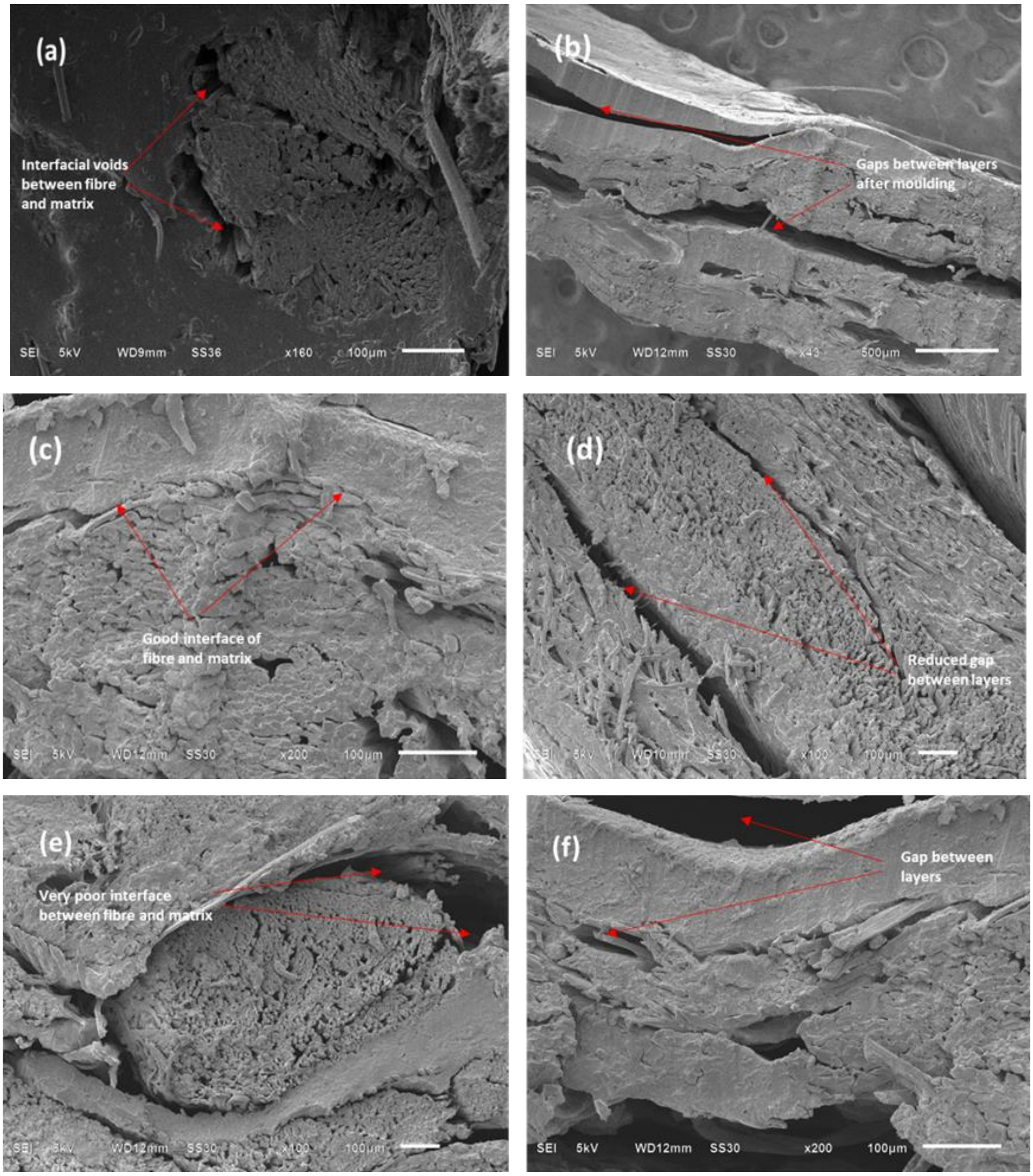


Figure 4.3. Tensile fracture SEM images of Type 1, Type 2 and Type 3 Kibisu-wheat gluten composite of variable mass fraction of fibre which shows, (a)-(b) Poor layer to layer but better fibre-matrix interface (c)-(d) better layer to layer and fibre-matrix interface.

In comparison, the SEM images of the Type 2 composite in Figure 4.3c to d show an improved adhesion between the fibre and the matrix. Improved adhesion leads to better bonding, likely the reason behind improved tensile strength in Type 2 compared to Type 1 and Type 3. It is perceptible that an increased amount of fibre and lesser wheat gluten in the prepared layers formed a smooth fibre matrix interface in Type 2 and Type 3 composites. The increased amount of fibre enhances the tensile property with a reduced accumulation of wheat gluten, which does not allow the development of any air pockets or air gaps in between the layers. Although from the SEM images, it is pretty evident that the interlayer gap is also there in Type 2 composites, however, it is much less than the other two types.

4.1.4 Biodegradability test using soil burial test

Biodegradability is usually studied by calculating the amount of mass degradation of any material over time. Biodegradation of any material happens when a microorganism develops over it and slowly degrades it due to enzymatic action. The ingredients used here are natural polymers with low crystallinity, leading to a reasonably high degradation rate as degradation increases with low crystallinity [205]. Figure 4.4a and b show the samples before and after degradation, and Figure 4.4c shows the set-up where the samples were buried for the entire period of degradation.

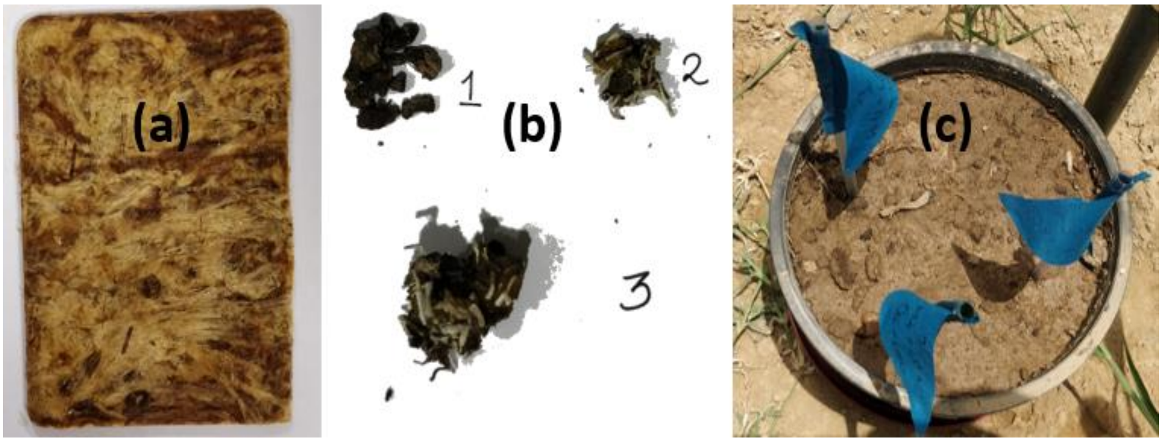


Figure 4.4. Biodegradation test for Kibisu-wheat gluten composites of Type 1, Type 2 and Type 3 under natural conditions (a) Sample before biodegradation (b) Remaining samples after one-week (c) Samples buried under the soil.

After one week, almost 90% of the composites were degraded, as shown in Table 4.1. It was observed that a thick and white layer of fungi developed on the remaining debris with a pungent smell, which indicates vigorous degradation. The reason behind faster degradation is the presence of hydroxyl group (-OH) in the natural polymer, which is easily degraded microbially. The hydroxyl group of wheat gluten initiates a hydrolysis reaction by absorbing water from the soil. This reduces the residual mass and increases the biodegradation of the composite [202]. It is also apparent that with increasing fibre percentage, the degradation rate decreases as the sericin layer above the waste Kibisu silk halts fungal growth to penetrate inside the fibrils. To calculate the percentage of degradation Equation 3.1 was used.

Table 4.1: *Rate of biodegradation for Kibisu-wheat gluten composites of Type 1, Type 2 and Type 3 under natural conditions showing a rapid degradation rate within two weeks.*

Name	Initial weight (gm)	After a week (gm)	% Degradation	After two weeks (gm)	% Degradation
Type 1	5.69	0.37	93.5	No trace found	100
Type 2	5.75	0.51	91.1	No trace found	100
Type 3	5.71	0.86	84.9	No trace found	100

4.1.5 Soil test

For the nitrogen test, cadmium metal was used to form nitrates $(NO_3^-)$to nitrites (NO_2^-). Nitrite ions in reaction with sulfanilic acid form an intermediate diazonium salt that forms an amber colour solution when coupled with the gentisic acid. For phosphorus, sodium molybdate and potassium pyrosulfate react with phosphate to form a phosphor-molybdate complex which, on reduction by ascorbic acid, forms a molybdenum blue colour. In the potassium test, the potassium ions combine with sodium tetraphenylborate to form a white precipitate of potassium tetraphenylborate, which causes turbidity. To determine the pH, the soil sample was mixed with an indicator solution (halochromic chemical compound) which changed colour according to the pH. The soil test shows that the soil quality before and after degradation did not change significantly. The soil test results before degradation are depicted in Figure 4.5, showing the soil solution's colour developed upon testing. The second soil test took place after the complete degradation of the composite samples, as shown in Figure 4.6. The colour developed due to the nutrients present in the soil remained unchanged in the second test, representing similar soil quality before and after degradation.

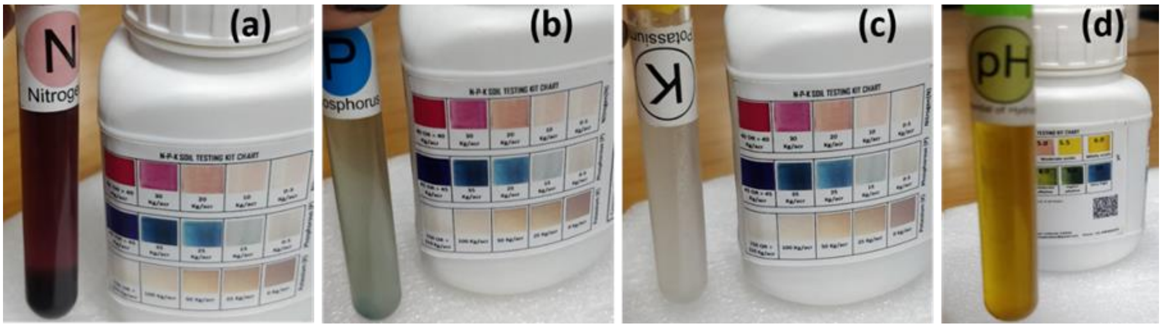


Figure 4.5. Soil test before biodegradation of Kibisu-wheat gluten composites shows, (a) Dark pink colour representing the presence of 40 or more than 40 kg/acre of nitrogen in soil, (b) pale bluish colour representing the presence of zero to 5 kg/acre of phosphorus in soil, (c) translucent whitish colour representing the presence of 100 kg/acre of potassium in soil, and (d) Greenish yellow colour represents the pH level of the soil to be 6.5 to 7.

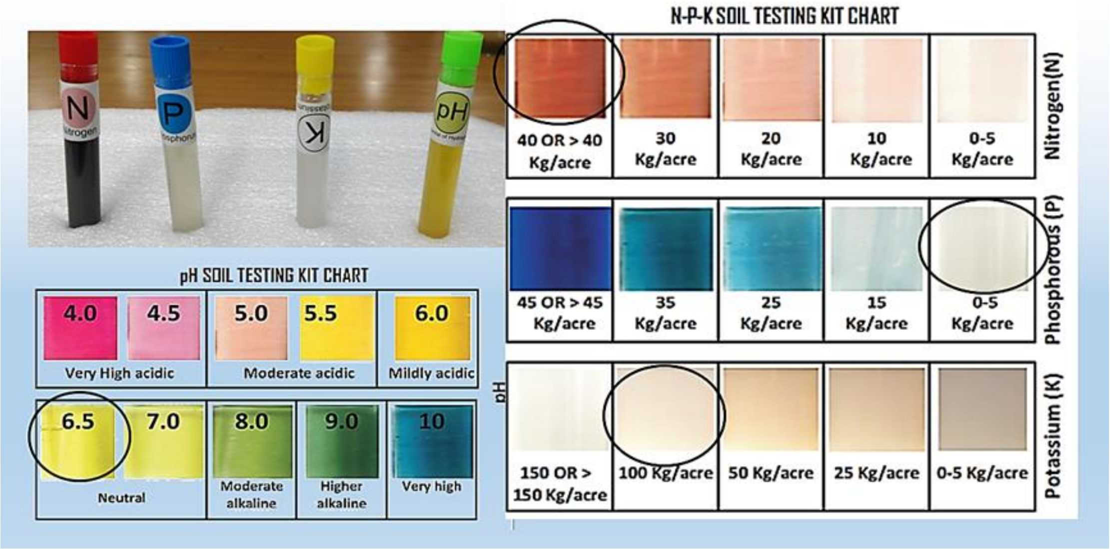


Figure 4.6. Soil test after biodegradation of Kibisu-wheat gluten composites under natural conditions showed no significant change in the essential nutrients of the soil.

4.2 Composite characterisation with the crosslinker

In the previous section, although the fresh lemon extract was used as a crosslinker instead of citric acid with glycerol as a plasticiser, the crosslinker's effect hasn't been thoroughly checked. In this section, the efficacy of lemon extract will be vividly verified in an all-natural environment where organic castor oil is used as a plasticiser instead of glycerol. The analysis of different characterisation data is discussed, therefore.

4.2.1 Fourier transform infrared (FTIR) spectroscopy

The effectiveness of the added lemon extract on the bond strength of the prepared composite can be studied from FTIR spectra formation [33]. Studies show that citric acid can greatly enhance the degree of cross-linking reaction for organic protein components. FTIR was employed to justify the cross-linking of WG before and after the addition of the crosslinker [60]. There are two purposes for adding sodium hydroxide with lemon extract. One is to develop sodium citrate in the arrangement, which will later react with the wheat gluten and oxidise the thiol group present in it [178]. Subsequently, they form disulfide bonds and change the gluten structure, thus upgrading the gluten yield remarkably [89]. In addition, upon heating, citric acid dehydrates itself and forms anhydride. This reacts with the hydroxyl group of the gluten amino acid and forms gluten citrate or modified WG [87]. The second reason is that sodium hydroxide is the best dispersion agent for WG. Mixing WG in a controlled alkali solution results in a high degree of deamidation while causing almost no hydrolysis of the peptide base of the gluten structure. Deamidation of gluten occurs because of the degradation of some essential amino acids during the reaction [206–208]. Also, the cross-linking among the lysinoalamine and lanthionine amino acids occurs during the alkali treatment of the wheat gluten protein [207]. All the possible reaction mechanisms responsible for WG's cross-linking are mentioned in Figures 4.7 and 4.8. As mentioned in Table 4.2, the peaks generated in FTIR spectra at different wavenumbers showed characteristic transmittance bands for different compound classes [209]. As shown in Figure 4.9, the peaks generated in FTIR showed a reduction in transmittance peak intensity near wavenumber 3300 to 3200 cm^{-1} which implies a reduction of hydroxyl groups after cross-linking [87]. Crosslinking enormously depends on the isoelectric point of any component. At the isoelectric point (IP), any material reaches its most stable state, which helps the material be unaffected by the surroundings as the net electron charge of any material at IP is zero [48,210]. The pH level of WG usually ranges between 2 to 3. But many researchers have reported the isoelectric point of WG ranges between 6.5 to 7.7 pH [89,178]. In this study, sample C1 couldn't reach its isoelectric point, making it weak due to unstable net electron charges. While in C2 samples, NaOH was added to increase the composite's pH level, but mostly it went beyond the isoelectric point. For enhancing the cross-linking and balancing the pH within the isoelectric range, in the C3 composite, the lemon extract was added to the mixture at an optimally designed proportion leading to better cross-linking. Improved cross-linking improves the interaction between the functional and proton donor groups. Improved interaction leads proton donors to give up their electrons and reduce the electron density.

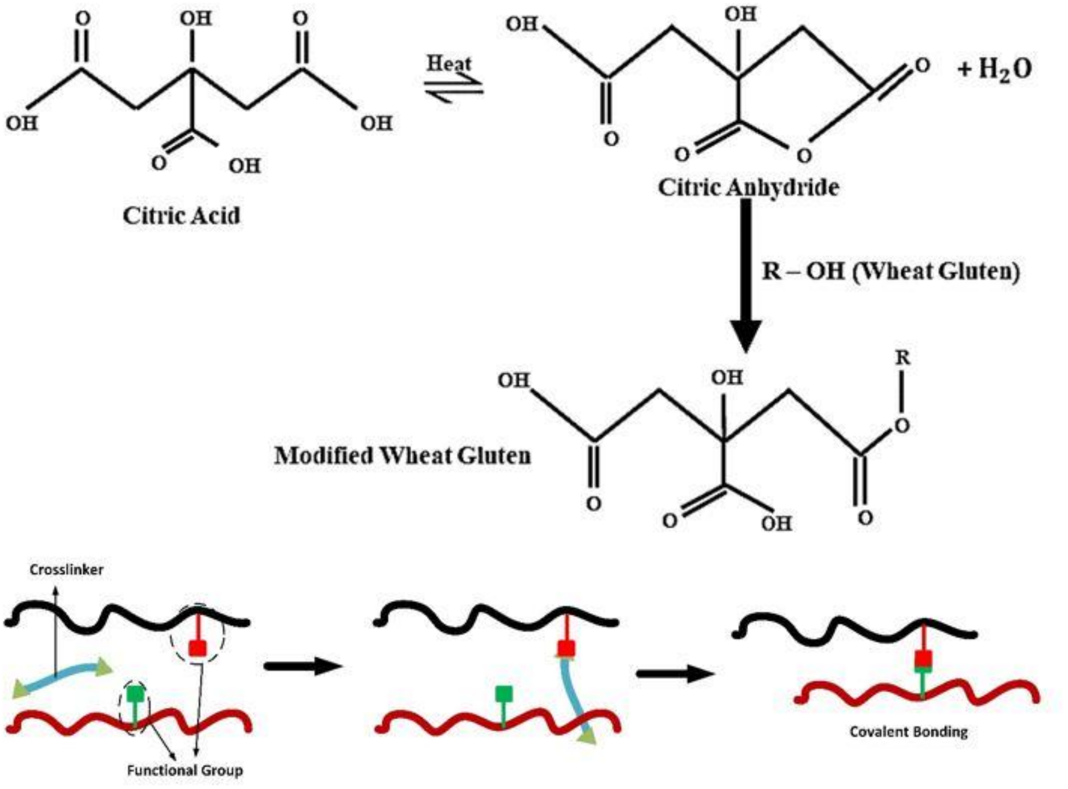


Figure 4.7. Reaction mechanism of Citric acid with WG

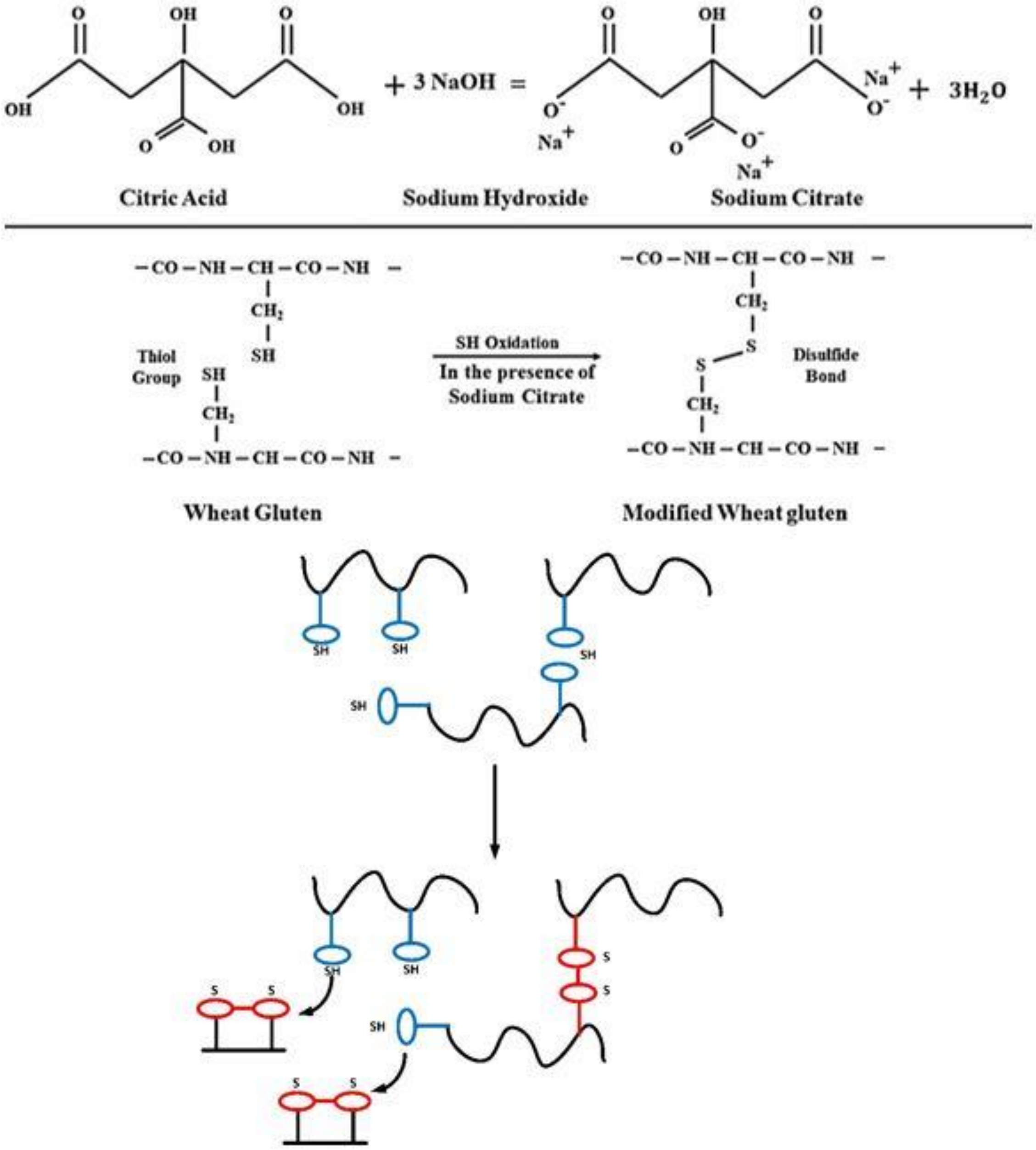


Figure 4.8. Formation of Sodium citrate and its reaction mechanism with WG.

As a result, the functional group’s vibrational energy also gets reduced, leading to producing peaks at lower wavenumbers in the FTIR spectra [82,87]. Table 4.2 represents different peaks generated for different functional groups of WG and WKS at different wavenumbers. The shifting of wavenumbers towards lower numbers from C1 to C3, shown in Figure 4.9, represents better interaction among the constituents in the presence of the crosslinker [33]. The peaks generated around 1000 cm^{-1} wavenumber are resultant peaks of WG and fibres both. The peaks shifts at 1066 and 800 cm^{-1} signify enhanced C-O stretching and C=C bending, respectively, indicating better interaction between the WG and WKS in the presence of the crosslinker, i.e. lemon extract and sodium hydroxide.

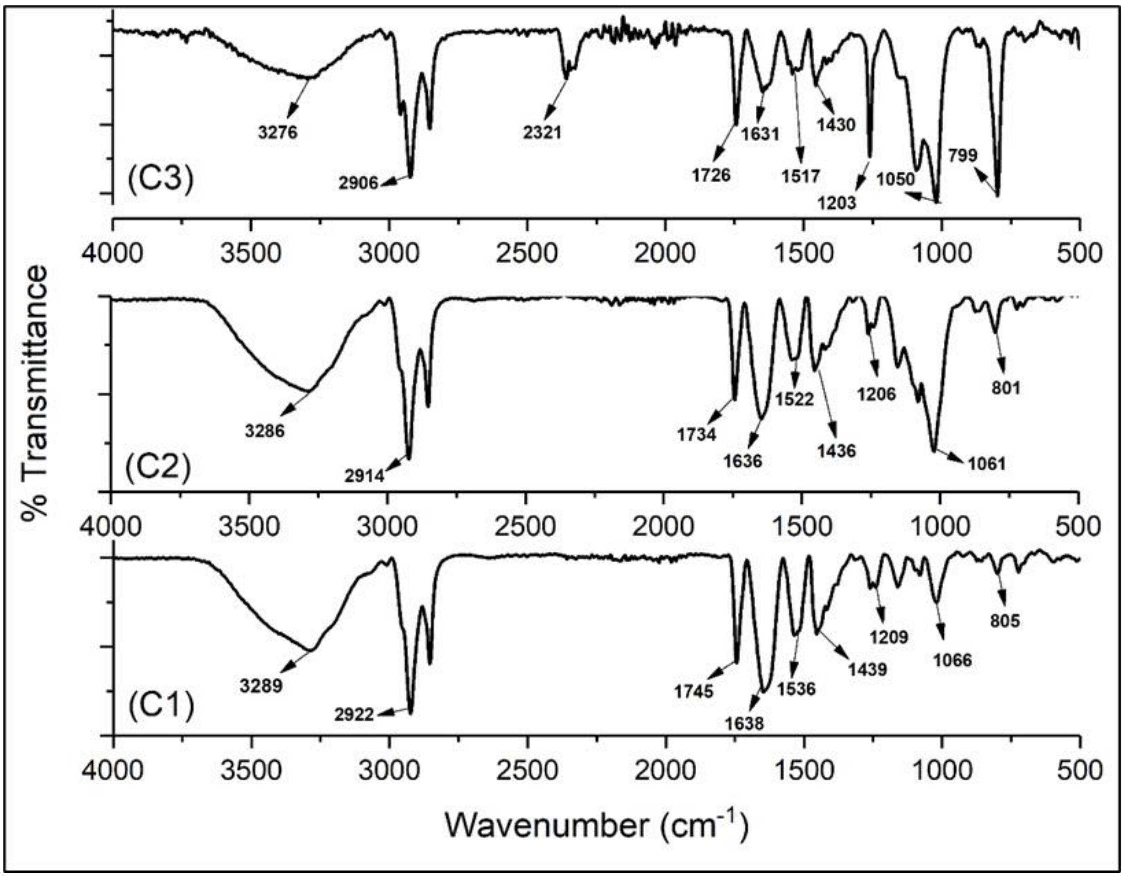


Figure 4.9. FTIR spectra analysis of (C1), (C2) and (C3) composite of wheat gluten and waste Kibisu silk.

Table 4.2: *FTIR spectra details of different functional groups and compound classes of wheat gluten and waste Kibisu silk.*

Absorption (cm^{-1}) range	Appearance	Group	Compound Class
3300-2500	Strong Broad	O-H Stretching	Carboxylic Acid
3000-2800	Strong Broad	N-H Stretching	Amine
2400-2000	Strong	O=C=O Stretching	Carbon dioxide
1750-1725	Strong	C=O Stretching	Esters
1650-1580	Medium	N-H Bending	Amine
1440-1395	Medium	O-H Bending	Carboxylic acid
1210-1163	Strong	C-O Stretching	Ester
1085-1050	Strong	C-O Stretching	Primary alcohol
840-790	Medium	C=C Bending	Alkene

4.2.2 Tensile properties

In this study, the prepared samples were tested for their Young’s modulus, ultimate stress and % elongation at the failure to evaluate the crosslinker (lemon extract) effect on the tensile properties of the prepared composites. The tensile properties of C1, C2 and C3 are shown in Figure 4.10. It can be observed from Figures 4.10a, and 4.10b that the C3 composite sheets prepared with lemon extract showed improved Young's modulus and ultimate stress compared to C1 and C2. The addition of a crosslinker improves the bonding between WG molecules, making them difficult to break [211]. Figure 4.10c indicates that the tensile strength of the composite increases with the addition of the crosslinker, but the elongation reduces. The cross-linking among the WG molecules increases tensile strength but simultaneously restricts the WG's easy movement, resulting in higher modulus but the lower elongation of the composite [46,212]. Too much cross-linking also leads to lower mobility, while optimised cross-linking shows improved mobility. Polymers with restricted mobility cannot stretch adequately under tensile loading [32,173]. This contributes to the fibres' load-bearing capacity, leading to decreased strength and mobility in C2 composites. The agglomeration of WG, as found in the C1 and C2 matrix (Figure 3.6), leads to poor adhesion of the matrix and fibre, resulting in early failure of the composite. The separated fibres from the agglomerated WG generate a stress concentration zone leading to crack initiation and quick propagation in the failure zone [213].

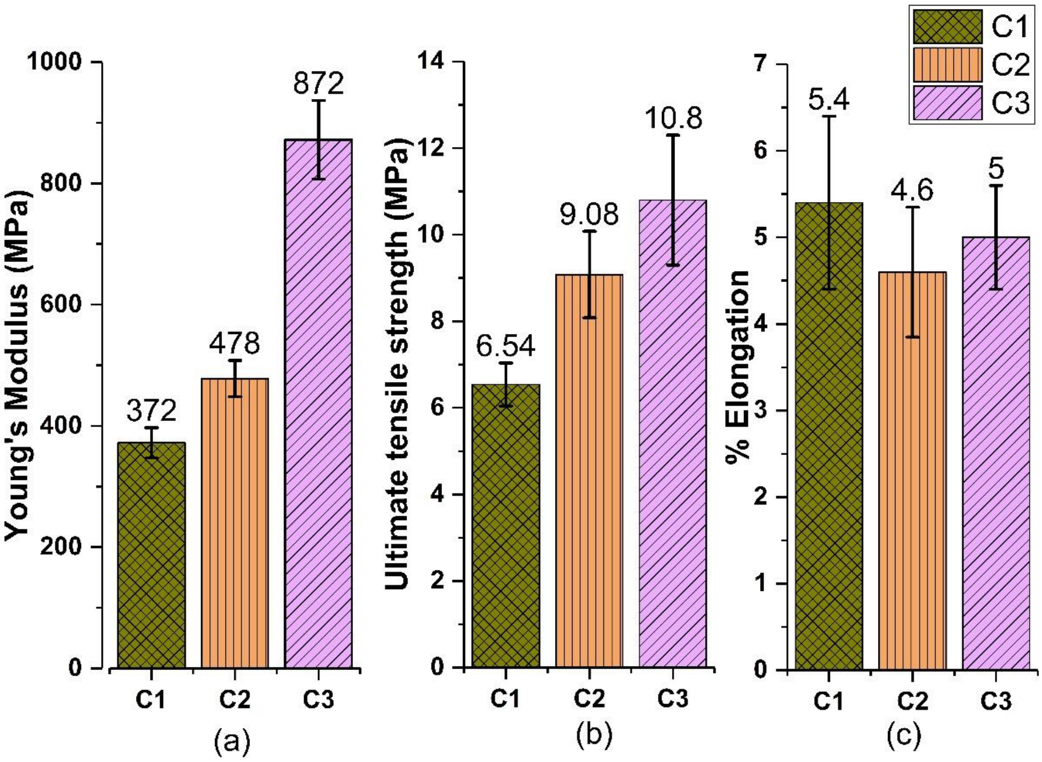


Figure 4.10. Variation in (a) Young's modulus, (b) Ultimate stress (c) % Elongation of wheat gluten (WG) and waste Kibisu silk (WKS) composites with and without natural lemon extract-based crosslinker.

4.2.3 Thermogravimetric analysis (TGA)

The thermal stability of the cross-linked and non-crosslinked composite sheets was compared by TGA. The first 3 to 7% weight loss occurred for all the composites due to the free bonded water and residual moisture. The second phase of significant weight loss ensues at around 217 to 250 °C due to castor oil evaporation. The next step of weight loss happens about 300 to 325 °C due to the breakage of covalent bonds in the amino acid group. The remaining portions having N-H, N-O, C-O, and C-C links break drastically above the temperature of 400 °C and decompose completely [46,133,214]. The TGA of the three different samples, C1, C2, and C3, are shown in Figure 4.11, which clearly shows the thermal stability of C3 being superior to the other two kinds. The initial phase of drastic decomposition starts at 217 °C for C1, 231 °C for C2, and 247 °C for C3. The second step of the major degradation peak of C3 is found at 470 °C, whereas for C1 and C2 it is found at 420 °C. It shows that the added lemon extract works effectively as a natural crosslinker and displays higher resistance to thermal degradation [46]. It is prominent from the Figure 4.11 that the overall thermal stability of the composite C3 remains better throughout the thermal decomposition process. Sample C3 showed better resistance to thermal decomposition by sustaining up to 762 °C at 100% decomposition, where C1 and C2 decompose at 579 °C and 717 °C. Better cross-linking leads to more energy to break the bonding, which justifies more thermal stability of C3 composite [78].

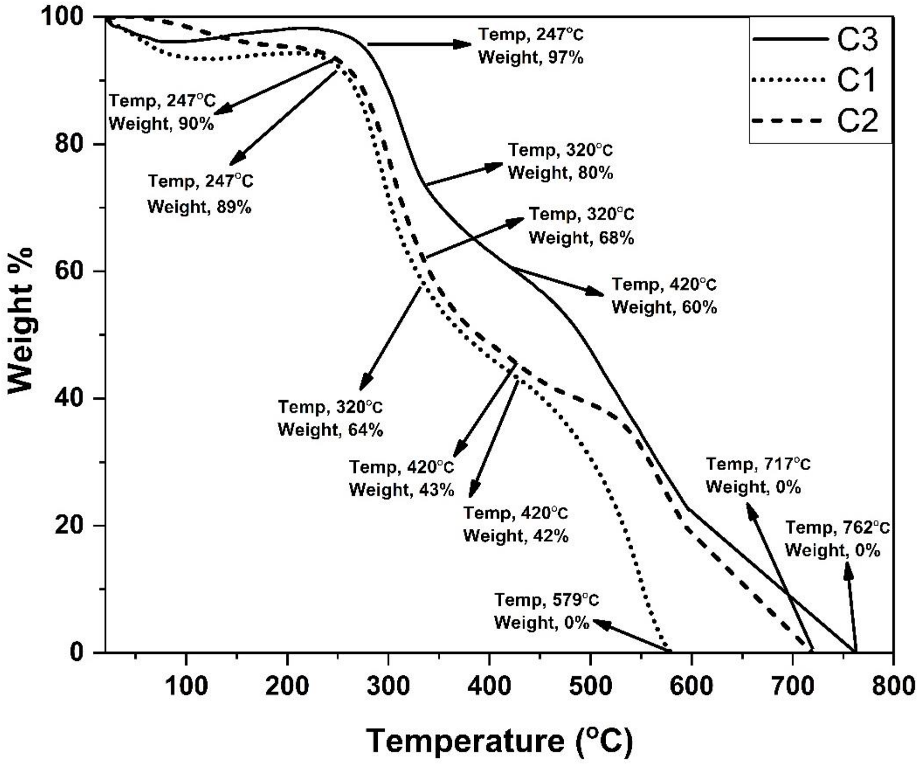


Figure 4.11. TGA graphs of C1, C2 and C3 waste Kibisu silk-wheat gluten composite with and without crosslinker showing better thermal stability for C3 composites with the added crosslinker.

4.2.4 Micrographs by scanning electron microscopy (SEM)

The cross-section of the compressed composite sheets C1, C2 and C3 was studied under SEM micrographs, as shown in Figure 4.12. The micrograph, shown in Figure 4.12a of sample C1, showed poor adhesion between the fibre and the matrix, which justifies the early failure of the same during tensile testing. The WG clustering throughout the composite sheet can be seen in Figure

3.6a, which led to poor bonding between the fibre and the matrix. The micrographs of sample C2 are represented with the help of Figure 4.12b, showing improved adhesion between the fibre and the matrix, which led to enhanced tensile properties compared to C1. The addition of NaOH reduces the clustering of WG in sample C2, as shown in Figure 3.6b, leading to better adhesion between the fibre and the matrix. It is perceptible that the addition of lemon extract improves the cross-linking of the WG as it showed almost no clustering of WG in the gluey paste developed earlier, as shown in Figure 3.6c. The micrographs of sample C3, as shown in Figure 4.12c, indicate outstanding adhesion between the fibres and the matrix attributed to a smooth gluey paste of the WG protein structure, indicating better fibre-matrix adhesion. The enhanced tensile properties of the C3 composite also justify better bonding between the major constituents of the sample. However, the SEM micrographs show the gap between fibre and the matrix in all the samples, but it is considerably less in C3 than in the other two sorts.

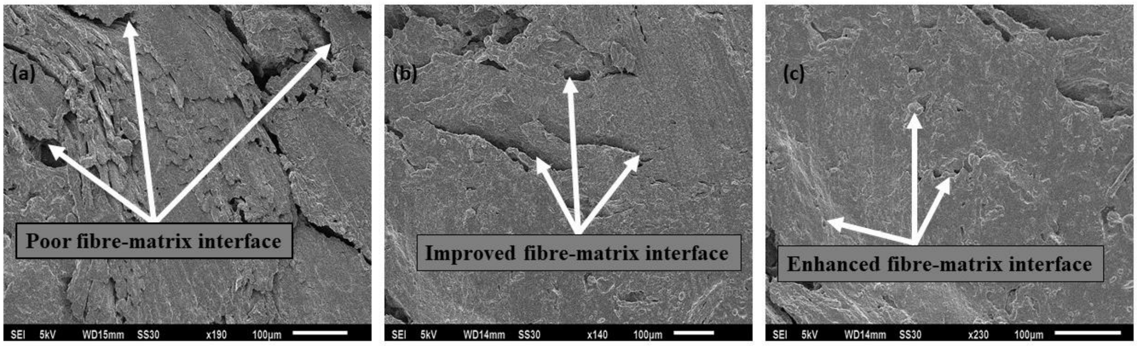


Figure 4.12. SEM micrographs of C1, C2 and C3 waste Kibisu silk-wheat gluten composite with and without crosslinker (a) C1 composite showed poor fibre-matrix interface in the absence of any crosslinker (b) C2 composite showed improved fibre-matrix interfaces in the presence of NaOH (c) C2 composite showed enhanced fibre-matrix interface in the presence of NaOH and lemon extract than the other two.

4.3 Composite characterisation with different natural plasticisers

4.3.1 Tensile properties

Better interaction between the polymer and plasticiser happens due to better linear chain-forming capacity by the hydrogen bonds. The more amylose content, the stronger the bonding and the better the composite properties. Regardless of the type of natural polymers, tensile strength decreases, and elongation improves with the addition of plasticisers. The intermolecular bond breaks between the chains of the natural polymer during the gelatinisation process in the presence of water and heat. Adding plasticiser to the polymer reduces the intermolecular force and changes the internal structure of the three-dimensional molecular grid. This is the main reason behind the increased flexibility with the enhancement of plasticiser in the composite. The mobility of the plasticiser increases as molecular weight decreases. Higher molecular weight means lower migration. Small molecules make better interactions with the polymer, thus lowering forces to hold the chains together. More interaction means lowering protein-protein intermolecular interaction over protein-plasticiser attraction. The molecular weights of the used plasticisers are mentioned in Table 4.3 [215].

Table 4.3: *The molecular weight of the different natural oils used as plasticisers.*

Name	**Glycerol**	**Castor oil**	**Linseed oil**	**Mustard oil**	**Olive oil**	**Coconut oil**
Molecular weight (g mol^{-1})	92	933	550	99.15	1382	554

In this study, the prepared samples were tested for their Young's modulus, ultimate stress and % elongation at the failure to evaluate the effect of different plasticisers on the tensile properties of the prepared composites. The tensile properties of SGL, SCO, SLO, SMO, SOO, and SCCO are shown in Figure 4.13. It can be observed from Figure 4.13 that composite sheets prepared with castor oil (SCO) showed improved Young's modulus and ultimate stress compared to other variations. Among all the synthetic plasticisers, glycerol has been used as a compatible plasticiser that stimulates better mechanical properties by interfering with the forces of the molecules. Natural polymers plasticised by glycerol (SGO) showed better flexibility than unplasticised films, as shown in Figure 4.13. The molecular weight of glycerol is the lowest among the other natural oil used, thus making it form most of the protein-plasticiser bond. It enhances flexibility, but low-strength bonding doesn't offer good Young's modulus or tensile strength. Olive oil contains monounsaturated fatty acids. The lipid particles of the olive oil embed themselves in the network, and the small particles closely connect to the polymer. The tensile strength of the composite may be decreased due to the large lipid globules of the olive oil, which reduces the cohesion between the fibre and matrix, thus breaking the samples faster with the applied load. The coconut oil-based composites also showed comparatively better flexibility, tensile strength and Young's modulus than olive oil. Being relatively polar, coconut oil tends to migrate to the surface of the composite very quickly, thus making it flexible. The excellent cross-link density of coconut oil with the natural polymer can justify the other tensile properties. Mustard oil contains a few fatty acids, and adding them to the polymers helps evaporate the water inside the polymer during the gelatinisation process. Having a smaller molecular weight than others, mustard oil migrates inside the polymer structure very easily, thus increasing elongation. But too much interaction lowers the intermolecular bonds, thus reducing Young's modulus and tensile strength of the composite. Having a higher molecular weight, it is difficult for castor oil to migrate in the polymer blend vigorously during gelatinisation. But it also offers optimised blending of the intermolecular hydrogen bond of polymer-polymer and also plasticiser-polymer. Under applied load, castor oil-based composites offer a good blend of mechanical properties with higher Young's modulus, tensile strength and moderate elongation. As the molecular weight of castor oil is higher, the migration stays limited, thus reducing the flexibility of the polymer chains. A similar trend can be seen in Linseed oil-based composite, where the flexibility remains low due to the average migration of plasticiser inside the protein structure. But it offers good Young's modulus and tensile strength. While more water penetration is a good indication of a faster degradation process, at the same time, it isn't feasible to develop food packaging or coating units using the developed composite. This problem can be tackled by optimising the percentage of plasticiser in the composite, where a perfect balance of desired sustainable and physiochemical properties can be achieved.

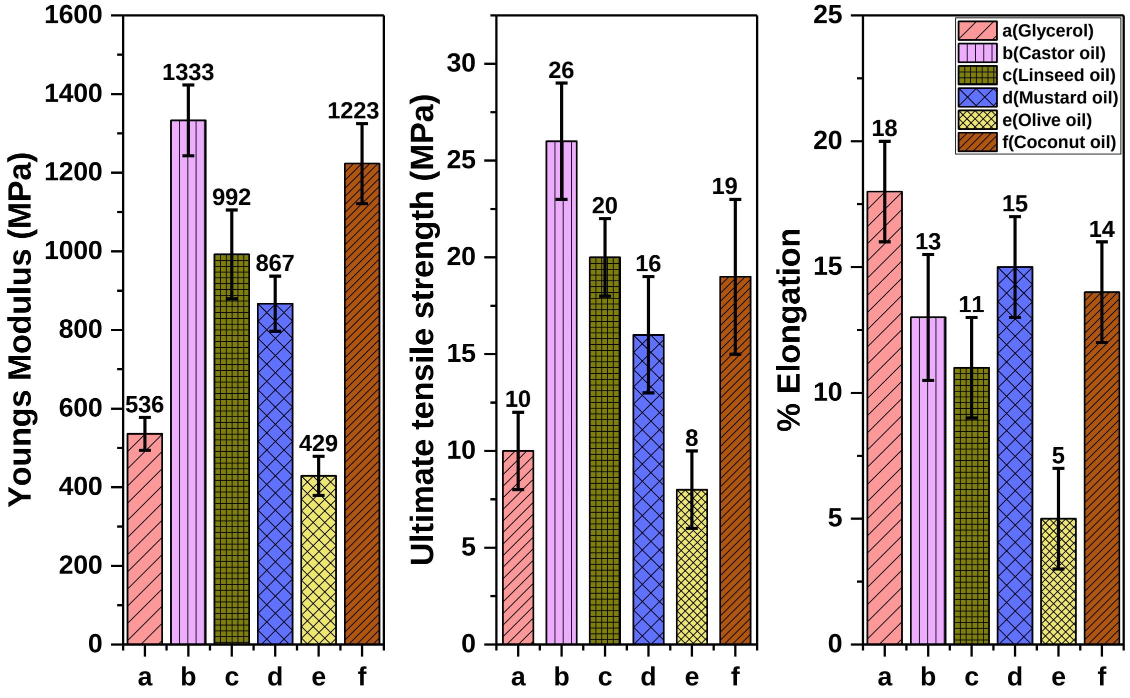


Figure 4.13. Variation in Young's modulus, Ultimate tensile strength, % Elongation of wheat gluten (WG) and waste Kibisu silk (WKS) composites with different plasticisers (a) Glycerol (b) Castor oil (c) Linseed oil (d) Mustard oil (e) Olive oil (f) Coconut oil.

4.3.2 Thermogravimetric analysis (TGA)

The TGA analysis demonstrates the thermal stability of the developed composite at elevated temperatures. Although the exact relationship between the molecular structure and adhesion between the polymers and plasticisers cannot be predicted, plasticisers promote higher amorphous region formation in the polymer, thus increasing flexibility and thermal stability [216]. All the TGA graph shows a three-stage degradation process. The initial weight reduction occurs due to the evaporation of the air and vapour trapped inside the composite. The evaporation of plasticisers almost takes 5 to 10% of the thermal degradation in the initial stage. The thermal stability of the composites with different plasticisers is attributed to the different thermal stability of the plasticisers. One of the major contributors is the boiling temperature of the plasticiser and how much penetration has occurred inside the polymeric chain during the plasticisation process. Figure

4.14 illustrates the second stage when a significant weight loss occurs at temperatures between 230 and 250 °C due to the composite's amino acid and hydroxyl groups breaking down. As most of the graphs overlap, an enlarged view of the TGA graph at 220 °C is shown in Figure 4.14a.

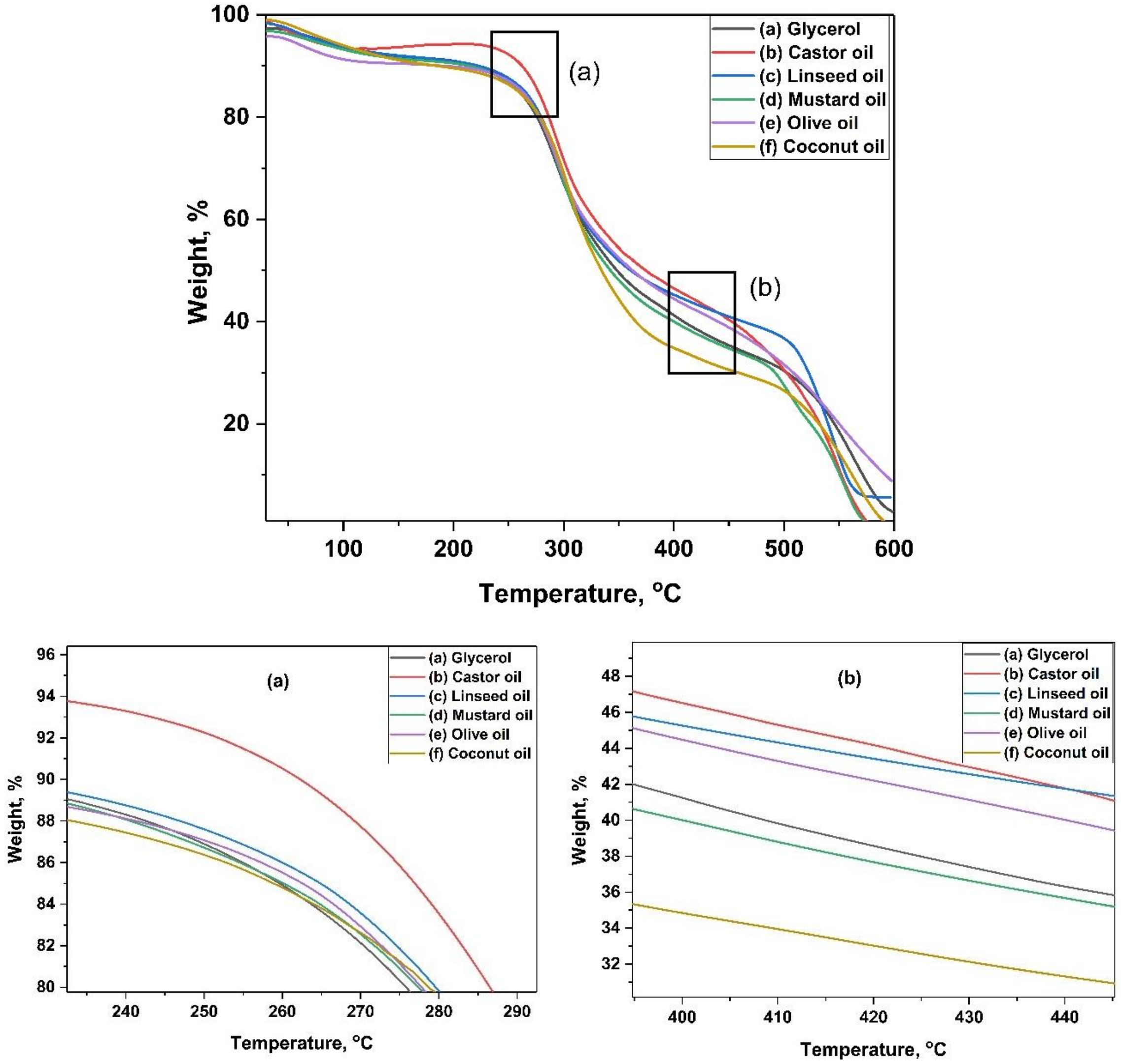


Figure 4.14. TGA graphs of waste Kibisu silk-wheat gluten composite with different plasticisers showing (a) thermal degradation at temperature 250 °C (b) thermal degradation at 420 °C.

Once the plasticisers evaporate, the newly formed hydrogen bonds break, and the composite starts to collapse drastically due to poor thermal stability. That's why a sharp drop in weight is seen after 250 °C to 400 °C. The final stage of thermal degradation is seen after 450 °C due to the rapid oxidation of the fibre and matrix residue at the higher temperature. Although the TGA graph shows close similarity among all the samples, at the first stage, SCO showed better thermal stability. Having a considerably sizeable molecular weight and boiling temperature (313 °C), castor oils fuse inside the polymeric structure of WG very definitely. As a result, It requires more heat to evaporate itself and leave the interstitial gaps of the polymeric chain. Having similar properties to castor oil, linseed oil (boiling temperature 316 °C) based composite SLO shows a similar trend. On the other hand, having the highest molecular weight, olive oil mostly ends up stacking at the surface level, making it very easy to evaporate at high temperatures. That's why SOO showed poor thermal stability at the first stage of thermal degradation. However, the other composites like SGO, SMO and SCCO show almost merging TGA graphs representing similar thermal stability. With comparatively smaller molecular weight and low boiling points, it is easier for the plasticisers to leave the polymeric chain at lower heating conditions. Similar trends can be seen at the end of the second stage of thermal degradation in Figure 4.14b.

4.3.3 Fourier transform infrared (FTIR) spectroscopy

Raw Kibisu fibres show FTIR bands around 3300 cm^{-1}, 2920 cm^{-1}, and 1500 cm^{-1}, which represent O-H stretching of COOH group, C-H stretching and N-H bending of the amino group, respectively. Wheat gluten shows band around 3300 cm^{-1}, 2900 cm^{-1}, and 1650 cm^{-1}, where the first two represents O-H and N-H stretching in the compound class of carboxylic acid and amide structure and the last band represents N-H bending from the amine group. It also shows a range of characteristic peaks between 1200 to 750 cm^{-1} that include C-O stretching –CO stretching, and –CC bending, which belongs to the compound class of ester, alcohol and alkene as mentioned in Table 4.4. The bands around 1000 cm^{-1} are a combination of fibre and gluten both The FTIR spectra of the developed composites are shown in Figure 4.15. Four significant peaks define the WG-based composites with different plasticisers. In the case of higher interaction, the proton donor groups interact effectively with different functional groups. It leads the functional groups to give up their electrons and reduce electron density. Along with reduced electron density, the vibration energy of the functional groups also gets reduced, which produces peaks of lower wavenumber in the IR spectrum. Upon hydrothermal plasticisation of protein, it rearranges hydrogen bonds and disulfide bonds. It led to the reduced energy of OH and NH stretching. Thus, the better the plasticisation effect, the more will shift towards lower wave numbers [217]. A comparable spectra shift can be seen in Figure 4.15, where the carboxylic group (OH stretching) and amine groups (NH stretching) shift to lower wave numbers for SCO, SLO and SMO composites. They closely resemble SGL in terms of the plasticisation effect on them. The appearance of more defined peaks in the range of 1200 to 750 cm^{-1} for SCO composite identifies the esterification of the wheat gluten influenced by the plasticiser. As the molecular weight is lower, glycerol and mustard oil can easily integrate inside the polymeric chains and form protein-plasticiser interaction more. That is why SGL and SMO form lower wave number peaks in the FTIR spectra. However, the increased hydrogen bond may also refer to more protein-protein interaction and plasticiser-plasticiser interaction. The weak hydrogen bonds forming between plasticiser-plasticiser and protein-plasticiser lead to early failure of the composite in tensile loading conditions. Having a medium molecular weight means integrating plasticiser inside the protein chains is low. It results in less plasticiser-plasticiser and protein plasticiser bonds. Thereby, SCO, SLO and SCCO deliver absorption peaks at higher wavenumbers than SGL and SMO. Having a higher molecular weight, SOO finds it challenging to migrate into the protein structure, making it less likeable as a plasticiser. The spectra of SOO showed the highest wavenumbers compared to other composites, as it missed the mark to develop more hydrogen bonds in the structure.

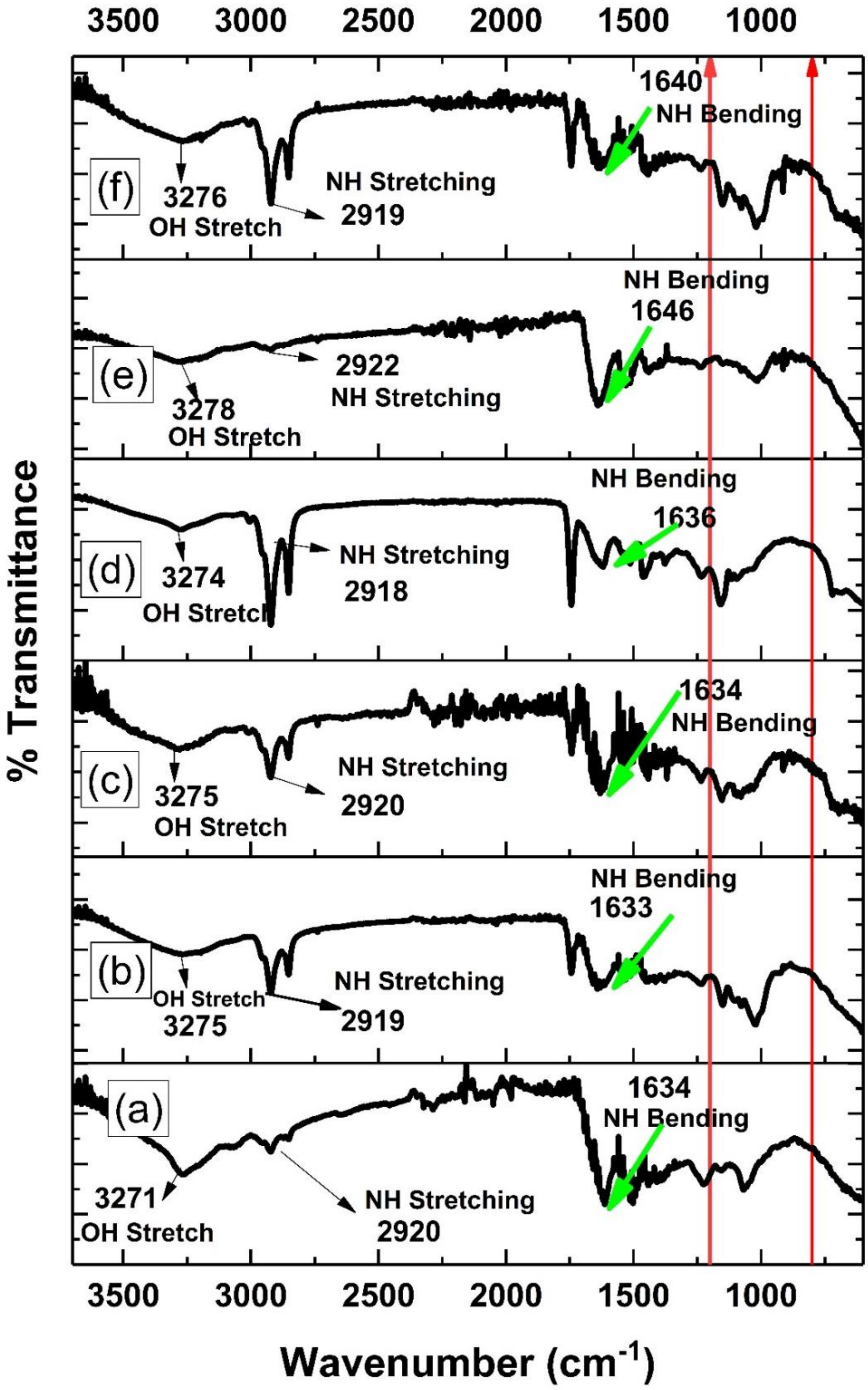


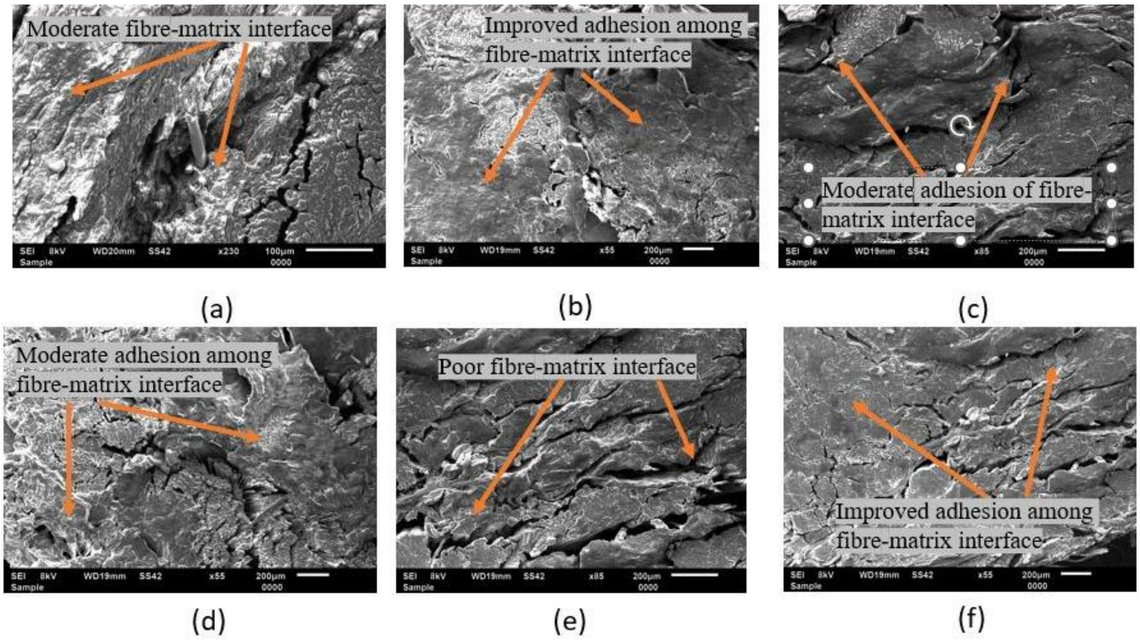
Figure 4.15. FTIR spectra of Composite sheet of wheat gluten, Kibisu silk and different natural plasticisers (a) Glycerol (b) Castor oil (c) Linseed oil (d) Mustard oil (e) Olive oil (f) Coconut oil showing formation of stronger peaks for castor oil plasticised composite.

Table 4.4: *FTIR spectra details of different functional groups and compound classes of wheat gluten and waste Kibisu silk.*

Absorption (cm^{-1}) range	**Appearance**	**Group**	**Compound Class**
3300-2500	Strong Broad	O-H Stretching	Carboxylic Acid
3000-2800	Strong Broad	N-H Stretching	Amine
2400-2000	Strong	O=C=O Stretching	Carbon dioxide
1750-1725	Strong	C=O Stretching	Esters
1650-1580	Medium	N-H Bending	Amine
1440-1395	Medium	O-H Bending	Carboxylic acid
1210-1163	Strong	C-O Stretching	Ester
1085-1050	Strong	C-O Stretching	Primary alcohol
840-790	Medium	C=C Bending	Alkene

4.3.4 Scanning electron micrographs (SEM)

The SEM micrographs in Figure 4.16 depicts the morphology of the cross-section of all the composites. The SGL, SLO and SMO micrographs showed a moderate fibre matrix interface at the fractured surface. In contrast, SCO and SCCO showed improved fibre-matrix interaction among the constituents of the composite, and SOO showed poor fibre-matrix interaction. As demonstrated in the earlier sections, under tensile loading conditions, the plasticisers with smaller molecular weight leach out from the polymeric chains breaking the hydrogen bonds, thus forming more cracks at the fractured surfaces.



(a) (b) (c)

(d) (e) (f)

Figure 4.16. SEM micrographs of waste Kibisu silk-wheat gluten composite with various plasticisers (a) Glycerol (b) Castor oil (c) Linseed oil (d) Mustard oil (e) Olive oil (f) Coconut oil , showing better fibre-matrix adhesion for castor oil-based composite followed by coconut oil.

However, the moderate molecular weight of the plasticisers finds it difficult to squeeze out from the intermediate space of the polymeric chain, thus providing better flexibility under high loading conditions. The vegetable oils with higher molecular weight find it extremely difficult to gelatinise the WG for not being easily sipped inside the structure. As a result, it shows poor flexibility of the developed composite. The smooth surface of Figure 4.16a, b, c, d, and f are probably due to the better plasticisation of WG with different vegetable oil-based plasticisers.

4.3.5 Bacterial degradation in Bio cabinet

The degradation of natural polymers and fibres mainly depends on different microorganisms. Microorganisms mainly degrade and detoxify the increasing amount of biowaste that enters the soil and water as an end product of different industrial processes. Although many bacterial strains are responsible for degrading natural compounds, *Escherichia coli (E.coli)* is recognised as a prevalent living organism in different media. The primary habitats of E.coli are soil, water, sediment and food. The growth of E.coli strains can be controlled with growth-limiting nutrients [218]. The current study uses LB broth and agar agar as growth controllers for solid and liquid media. The weight reduction after one week of bacterial degradation is shown in Table 4.5 and 4.6 for solid and liquid media. The degradation study shows that SGL and SMO showed maximum degradation after one week in solid media. The plasticiser's low molecular weight helped it quickly leave the polymer structure, followed by higher water sorption and faster microorganism growth. The next set of composites to show good degradation are SCO, SLO, and SCCO. They consist of plasticisers of higher molecular weight, making it difficult to leave the intermolecular space of polymeric chains. Thus the water penetrations are less, and the growth of microorganisms for decomposing the composite was slow. The least degradation was observed in SOO due to the poor migration of olive oil in or out of the structure having the highest molecular weight. Figure 4.17 depicts the visual degradation of the samples in solid media before and after one week. The SEM images of the degraded surfaces showed vigorous degradation of various samples in Figure 4.18.

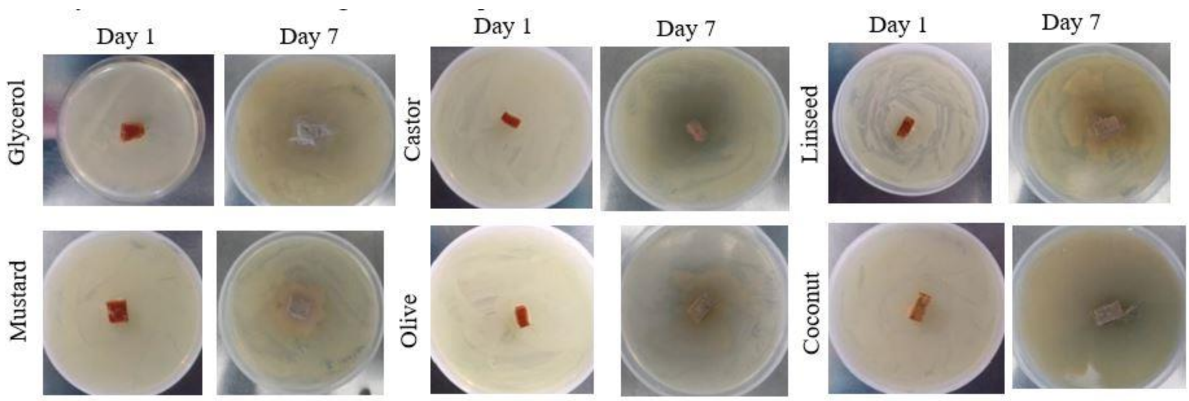


Figure 4.17. Degradation due to bacterial culture in solid media.

Table 4.5: *Weight reduction in solid media due to Bacterial degradation of wheat gluten and waste Kibisu silk composites with different plasticisers.*

Change in weight (gm) in solid media			
Sample	**Dry weight Day 1**	**Day 7**	**% Reduction**
(a) SGL	0.32	0.17	46%
(b) SCO	0.35	0.20	42%
(c) SLO	0.29	0.17	41%
(d) SMO	0.22	0.12	45%
(e) SOO	0.39	0.27	31%
(f) SCCO	0.30	0.19	36%

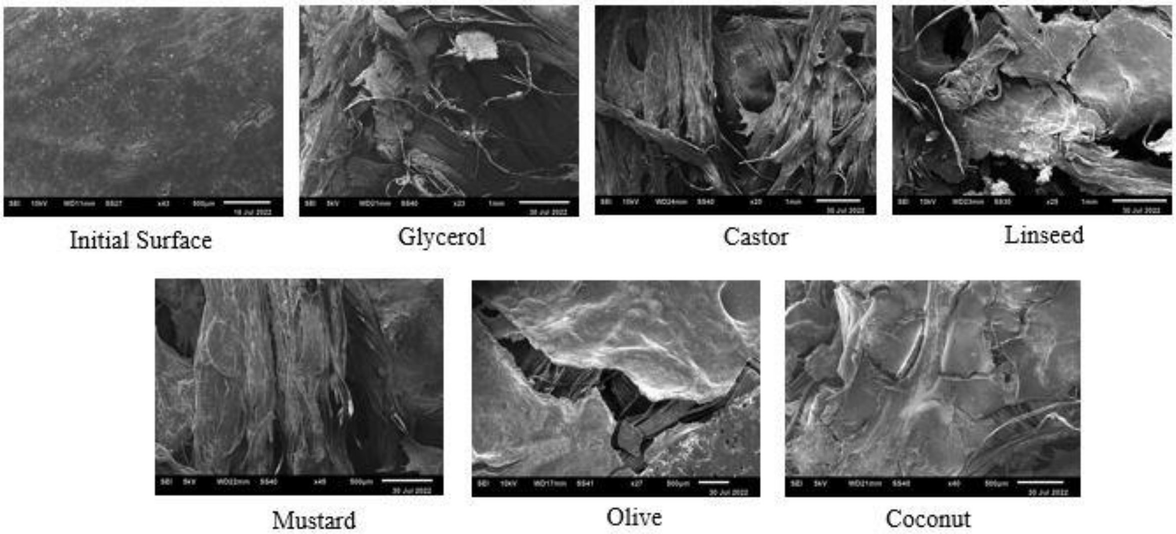
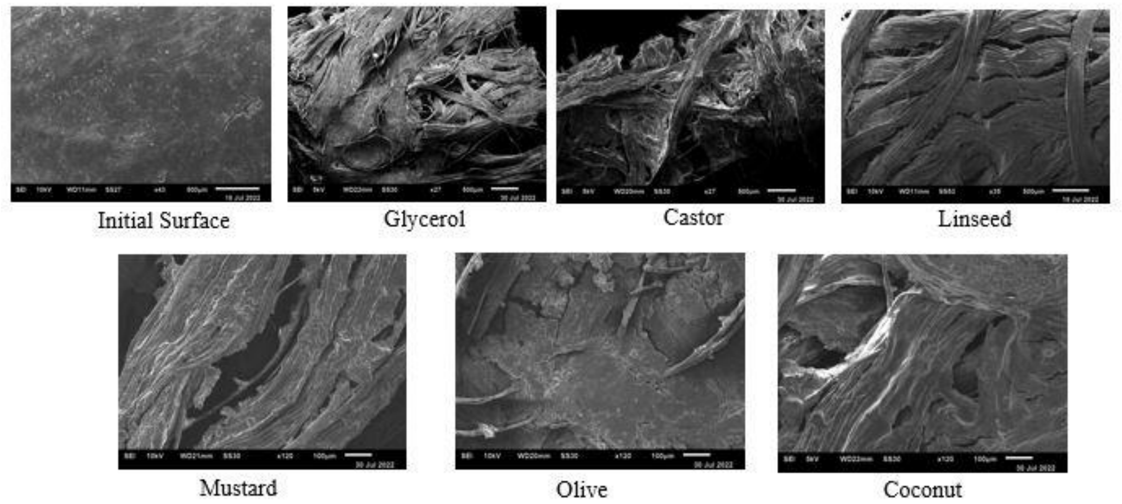

Initial Surface
Glycerol
Castor
Linseed
Mustard
Olive
Coconut

Figure 4.18. SEM fractographs of the degraded WG/WKS composites with different synthetic and natural plasticisers after seven days of bacterial culture in solid media showing strong degradation.

The initial surface and the surface morphology after one week showed how the bacterial growth of E.coli severely degraded the composites. The SEM micrographs also visually confirm the acquired data presented in Table 4.5. Similar trend can be found in the bacterial degradation of the samples in liquid media, as shown in Figure 4.19. The SGL and SMO showed reductions up to 56% and 53%, followed by SCO, SLO and SCCO at 44%, 43%, and 40%. The SOO stands lowest in terms of degradation in liquid media as well. The saturated olive oil on the surface of the composite structure worked as a barrier to stop the penetration of water inside the structure, delaying the growth of microorganisms into it. The Sem images in Figure 4.19 also verify the degradation data of Table 4.6 where SOO showed the least degradation and the rest of the composites showed impressive degradation.

Table 4.6: *Weight reduction in liquid media due to single-strain Bacterial degradation of wheat gluten and waste Kibisu silk composites with different plasticisers.*

Change in wet weight (gm) in liquid media				
Sample	**Dry weight**	**Day 1**	**Day 7**	**% Reduction**
(a) SGL	0.3267	0.70	0.31	56%
(b) SCO	0.3028	0.73	0.41	44%
(c) SLO	0.3149	0.74	0.42	43%
(d) SMO	0.2189	0.55	0.26	53%
(e) SOO	0.2876	0.67	0.45	33%
(f) SCCO	0.3528	0.65	0.39	40%



Initial Surface Glycerol Castor Linseed

Mustard Olive Coconut

Figure 4.19. SEM fractographs of the degraded WG/WKS composites with different synthetic and natural plasticisers after seven days of bacterial culture in liquid media showing vigorous degradation.

4.4 Composite characterisation with treated fibres

4.4.1 XRD analysis of the fibres

The XRD analysis was performed on all the silk samples before and after degumming to study the crystalline and amorphous structure change in the waste Kibisu silk (WKS)) for different conditions. It is shown in Figure 4.20 that the major diffraction peaks got generated at 21^{o} (2θ), which generally depicts the secondary β-structure or crystallinity of the fibres. It was observed that the raw silk fibre UF-0 showed a broad peak around 21.3^{o}, which indicated the natural pattern of amorphous silk. As degumming begins, the peak intensity increases gradually from CF-0.5 to CF-10.

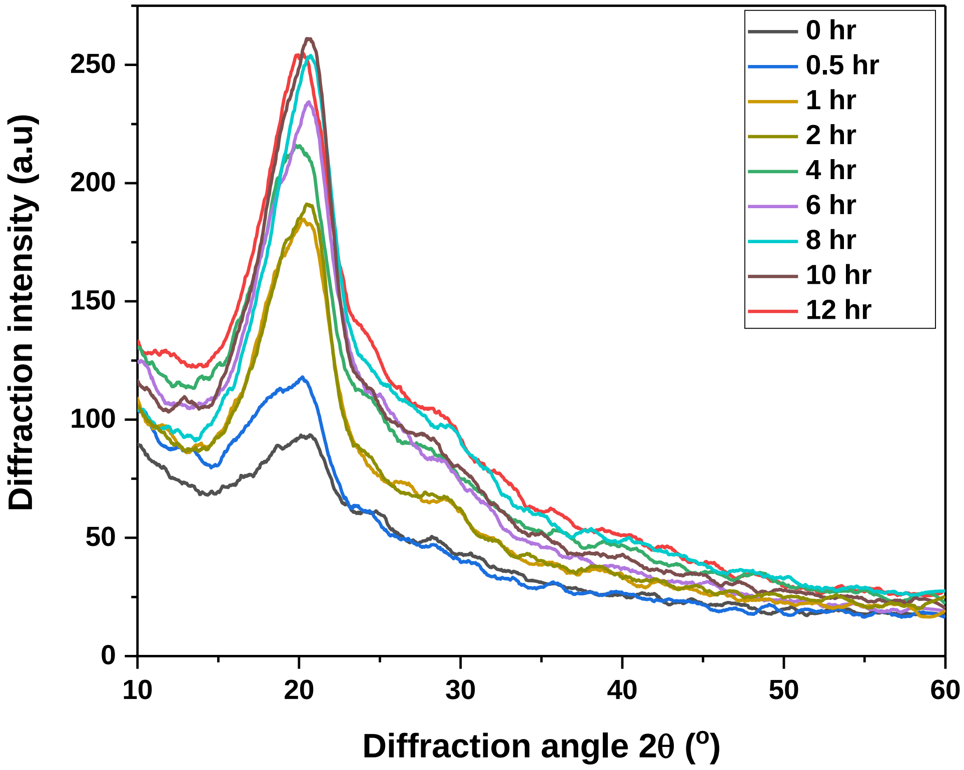


Figure 4.20. X-ray diffraction curves of the waste Kibisu silk before and after degumming in NaOH solution for different duration of time.

Table 4.7: *Change in percentage of crystallinity and amorphous region of waste Kibisu silk before and after degumming in NaOH solution for different durations.*

Sample name	% - Crystallinity	% - Amorphous
UF-0	38.5	61.5
CF-0.5	39.4	60.6
CF-1	40.4	59.6
CF-2	40.9	59.1
CF-4	43.7	56.3
CF-6	44.8	55.2
CF-8	48.4	51.6
CF-10	48.9	51.1
CF-12	47.0	53

The increase in peak intensity and height conveys the increase in the crystallinity of the fibres upon degumming. However, the peak intensity drops slightly for CF-12, imparting over-exposure to NaOH solution may have started to affect the core structure of the silks. Table 4.7 quantifies the percentage of amorphous and crystalline structure for all the fibres as per the XRD-generated data.

4.4.2 Fourier transform infrared (FTIR) spectroscopy

Building strong interfacial cohesion between silk fibroins and the adjacent polymer is challenging. Removing the sericin layer of silk by NaOH treatment improves the composite's overall properties.

FTIR shows the underlying factors of structural changes to understand the variation in mechanical properties of the prepared composites. Hence, FTIR spectra are used here to identify the structure of untreated fibre (UF), simple wheat gluten (WG), treated fibre (TF) and the composites prepared by them. The primary structure of the silk consists of amino acids of glycine, alanine and serine in a repetitive order 16. They take up to 90% of the protein in silk, and the last 10% consists of glutamic acid, valine and aspartic acids 35. They act like side chains and are primarily responsible for the elasticity and strength of the silk. The secondary structure of the silk is mainly dominated by β-pleated sheets connected to each other with hydrogen bonds 42. Apart from this, α-helix, turn, and random coils also play a dominating part in the secondary structure of silk.

FTIR spectra are widely sensitive to the secondary structure of silk fibroins, providing molecular validation of the change in silk structure 43,44. The signature absorption peaks for WKS are found around 1620 cm $^{-1}$ (Amide I), 1515 cm $^{-1}$ (Amide II) and 1260 cm $^{-1}$ (Amide III). These peaks are conformation peaks of crystalline β sheet structure, and the silk shows similar peaks in all degumming conditions until the core structure starts to break 36. The Amide I band of the silk fibroin (between 1600 to 1700 cm^{-1}) is mainly associated with C=O stretching vibration (70 to 85%) and is directly related to the backbone conformation. The Amide II band results from the N-H bending vibration (40-60%) and the C-N stretching vibration (18-40%). Amide III band is conformationally sensitive and very rarely traced with sharp peaks. The Amide II peaks are found where microsphere or aqueous formation takes place. The formation of microspheres confirms a more stable state of the fibroins. The stretched vibration peaks around 1600 cm $^{-1}$ confirm the presence of stronger C=O stretching. The definite peaks for the Amide I and Amide II region at 1630 cm $^{-1}$ and 1520 cm^{-1} indicate crystalline β-sheet conformation 45. As the degumming occurs, some subtle change occurs around 1000 cm $^{-1}$, as shown in Figure 4.21. The peak intensity increases for the treated fibres, indicating enhanced crystalline β structure formation 36.

Table 4.8: *FTIR absorption band of different compound classes present in Kibisu silk and wheat gluten composites* [48].

Absorption (cm^{-1})	Appearance	Group	Compound Class
3300-2500	Strong Broad	O-H Stretching	Carboxylic Acid
3000-2800	Strong Broad	N-H Stretching	Amine
1750-1725	Strong	C=O Stretching	Esters
1650-1580	Medium	N-H Bending	Amine
1550-1500	Strong	N-O stretching	nitro compound
1250-1020	Medium	C-N Stretching	Amine
1085-1050	Strong	C-O Stretching	Primary alcohol

Degumming generally increases the formation of crystalline β-sheets of WKS. Although lesser exposure to NaOH treatment does not affect the amorphous peptide formation of the silk but prolonged exposure of silk to alkali solution may affect the silk surface morphology. The alkali treatment removes the amorphous region and makes the silk more crystalline, thus reducing the silk elongation rate. Increasing the duration of fibre treatment reduces the elongation and creates more brittleness in the WKS and the composites. The gelatinisation of wheat gluten occurs in the presence of lemon extract, NaOH and castor oil. These components help to have better cross-linking in the WG and WG with WKS. Better cross-linking leads to better interaction between functional and proton donor groups. Enhanced interaction forces the proton donor group to make them give up their electron and reduce electron density. Reduced electron density also reduces the vibrational energy of the different functional groups, thus generating characteristic peaks at lower wavenumbers.

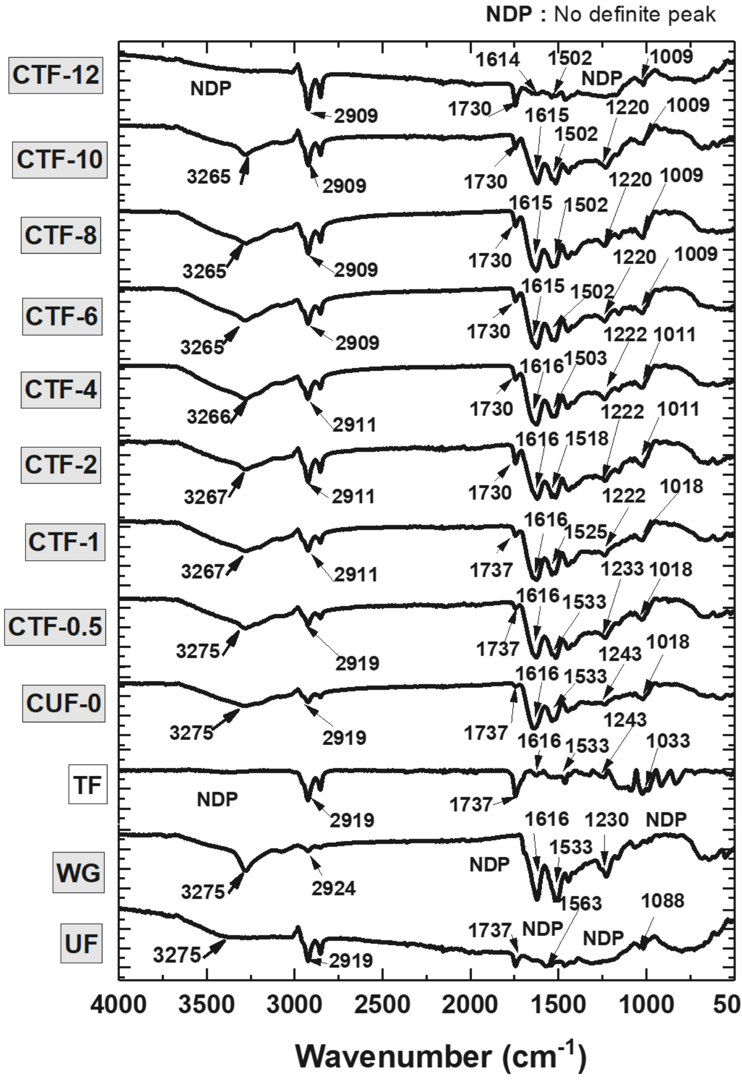
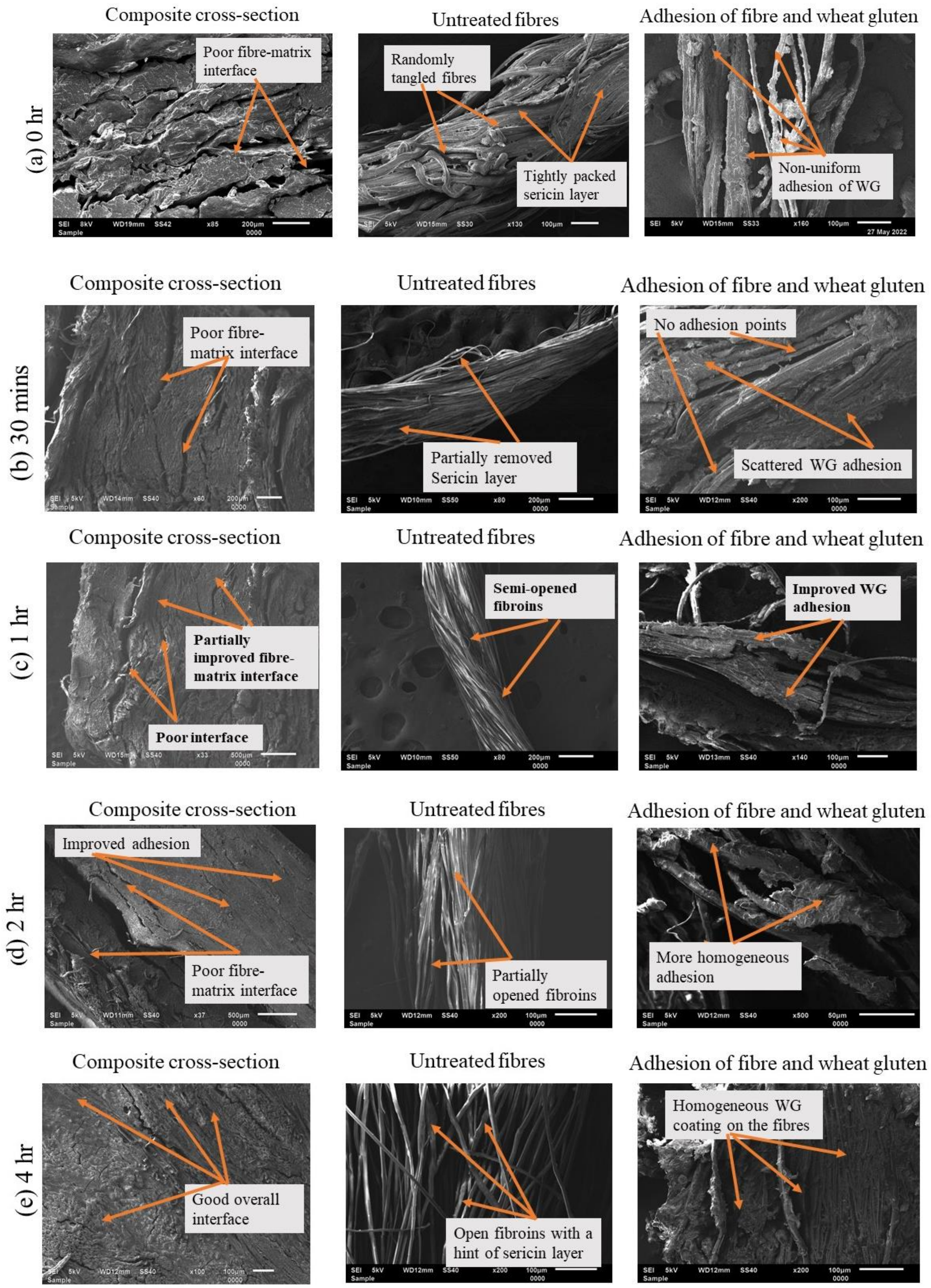


Figure 4.21. Comparison graph of FTIR analysis between treated-untreated waste Kibisu silk, wheat gluten and the composites prepared by them.

The signature peaks of lower wavenumber in FTIR studies indicate better interaction among the constituents of the matrix. The reduced intensity of WG to CUF-0 at 3200 cm^{-1} peaks represents reduced hydroxyl groups after cross-linking. The peaks between 1613 to 1632 cm^{-1} are assigned for β-sheets of WG protein structure. The β structure changes with the increase in hydration. The spectra generated around 1000 cm^{-1} are both the combined peaks for WG and WSF. The peaks and their absorption range are represented in Table 4.8 [209]. The tensile and thermal properties of the composites increase because of the presence of opened-up fibroins due to their ability to interact with WG protein to form complex protein structures. The reduced wavenumbers on and after CTF-4 indicated enhanced interaction among the constituents of the composite after the complete removal of the sericin layer. This data also supports the composites' enhanced tensile and thermal properties, as described in the upcoming sections.

4.4.3 Scanning electron micrographs (SEM)

The SEM micrographs showed the microlevel changes in the fibres and composites for all nine sets of samples. The micrographs shown in Figure 4.22 elaborate on the gradual removal of the sericin layer from the fibres, composite cross-section, and adhesion between the major constituents of the composites. Samples were collected from the fractured surface of the composites to study the behaviour of fibre-matrix bonding under applied load. Figure 4.22a shows CUF-0, which clearly shows the thick sericin layer and the heavily tangled fibroins in it. Fibre clustering renders poor mechanical properties. As the untreated WKS shows hydrophilic nature, the fibre-matrix adhesion was very poor for the prepared composite. The micrograph of the WKS collected from the fractured surface showed a lack of adhesion of WG to the fibre periphery. This slip of fibre from WG results in average mechanical properties. The treated fibres for the first three samples, CTF-0.5, CTF-1 and CTF-2, had medium to low traces of sericin present in them. The semi-open fibres and presence of sericin created uneven adhesin of fibre-matrix and interlayer voids, as shown in Figure 4.22b, c and d. The samples prepared by treated fibres from 4 hrs onwards had low to no traces of the sericin layer. The complete removal of sericin made the fibres straighter and more lustrous. This helped to develop a better composite structure that was almost free from voids and poor adhesion, as shown in Figure 4.22e to 4.22i. The CTF-4 and CTF-6 showed the most promising properties among all their counterparts. The interface is strongest for CTF-6 as it showed the least number of voids and a better interface among all the samples. A better fibre-matrix interface leads to better stress distribution, enhancing the prepared composites' load-bearing capacity under tensile and impact loading. Though the fibre matrix adhesion in CTF-8, CTF-10 and CTF-12 was also good, the fibroins' thinning due to extensive alkali exposure weakened the fibres. As a result, they could not carry the tensile and impact loads ensuing in easy failure, as seen in Sections 4.4.4 and 4.4.5.


(a) 0 hr
Composite cross-section
Poor fibre-matrix interface
Untreated fibres
Randomly tangled fibres
Tightly packed sericin layer
Adhesion of fibre and wheat gluten
Non-uniform adhesion of WG
27 May 2022
(b) 30 mins
Composite cross-section
Poor fibre-matrix interface
Untreated fibres
Partially removed Sericin layer
Adhesion of fibre and wheat gluten
No adhesion points
Scattered WG adhesion
(c) 1 hr
Composite cross-section
Partially improved fibre-matrix interface
Poor interface
Untreated fibres
Semi-opened fibroins
Adhesion of fibre and wheat gluten
Improved WG adhesion
(d) 2 hr
Composite cross-section
Improved adhesion
Poor fibre-matrix interface
Untreated fibres
Partially opened fibroins
Adhesion of fibre and wheat gluten
More homogeneous adhesion
(e) 4 hr
Composite cross-section
Good overall interface
Untreated fibres
Open fibroins with a hint of sericin layer
Adhesion of fibre and wheat gluten
Homogeneous WG coating on the fibres

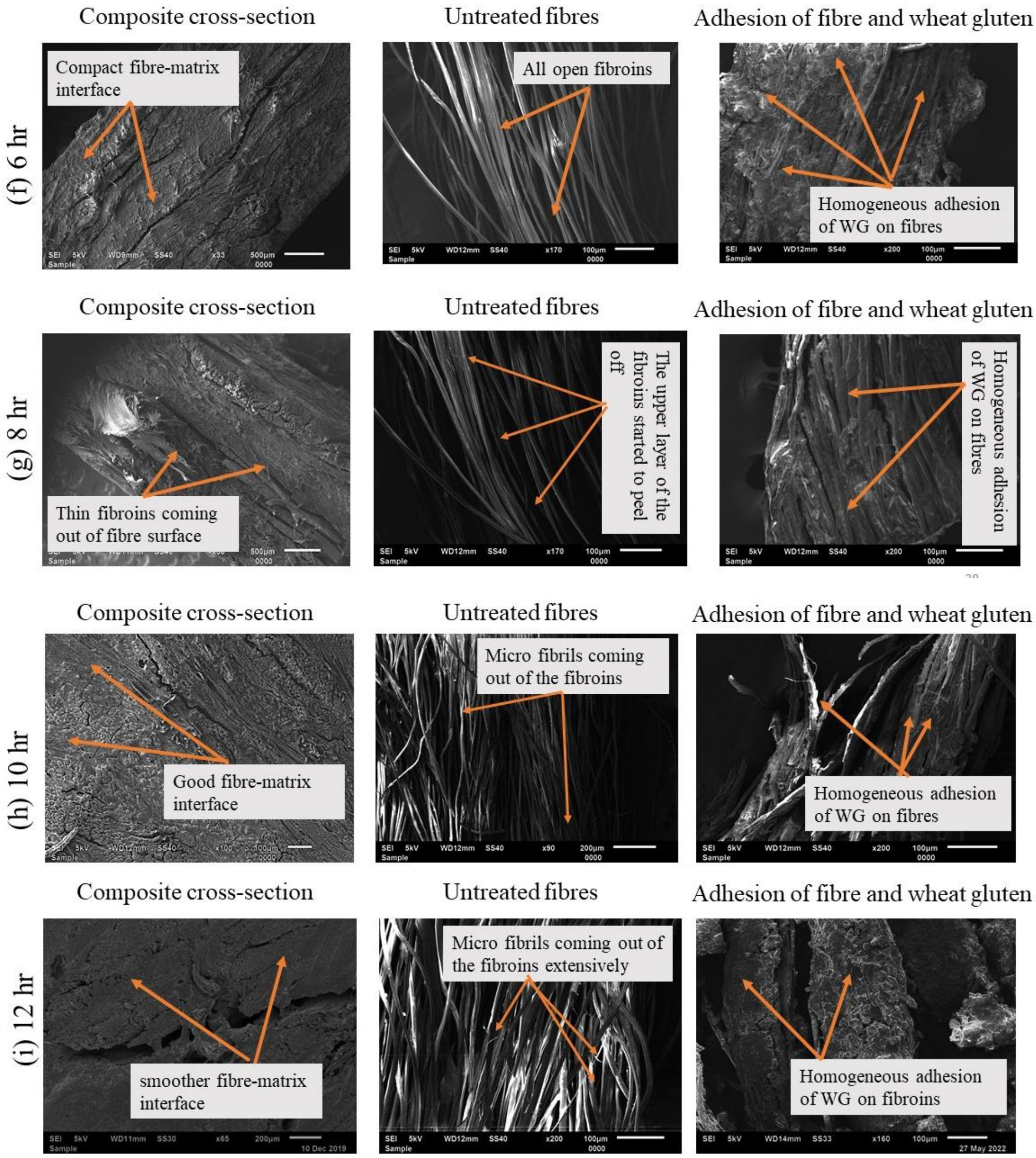


Figure 4.22. SEM micrograph of (a) CUT- 0 (b) CTF- 0.5 (c) CTF- 1 (d) CTF- 2 (e) CTF- 4 (f) CTF- 6 (g) CTF- 8 (h) CTF- 10 (i) CTF- 12 , analysing the microstructure of composite cross-section, fibres at different stages and fibre-matrix adhesion of waste Kibisu silk and wheat gluten.

4.4.4 Tensile properties

The tensile properties of the fibres and composites were compared among the nine sets of samples. The first sample, CUF-0, was prepared from untreated fibres where the sericin layer glued the fibroins randomly. The fibroins were badly tangled and unable to stretch themselves upon tensile loading resulting in the brittle behaviour of the composite [165]. As shown in Figure 4.24, the tensile strength is comparatively higher for CUF-0, but the elongation is critically low, supporting the brittle nature of the composite. The first four sets of treated fibre-based composites CTF-0.5, CTF-1, CTF-2, and CTF-4 showed a gradual increase in their tensile properties. The micrograph in Section 4.4.4 clearly showed the partially present hydrophilic sericin layer among the semi-opened fibroins, which delivers uneven sipping of wheat gluten into fibres[219]. This leads to the formation of internal voids in the composite, resulting in early failure upon tensile loading. As the sericin layer was completely removed after 6 hrs of NaOH treatment, the fibre matrix adhesion improved drastically, resulting in improved tensile properties. The 6 hrs of treated fibres showed improved elastic properties as they are entirely free from gluey sericin binding. It increases the tenacity of the fibres, which increases the interfacial adhesion of the fibre and matrix. Hence CTF-6 showed the best elongation among all the composites.

The CTF-8, 10 and 12 showed excellent Young's modulus but drastically reduced maximum tensile strength and elongation. Exposure of the fibres in NaOH solution for prolonged period results in forming more β-sheets, making them stiffer [220]. It also starts to peel the microfibrils, making the fibres thinner and weaker. It reduced the fibres' strength, which resulted in the samples' early failure. The same characteristic pattern can be seen in the tensile properties of the fibres, as shown in Figure 4.23.

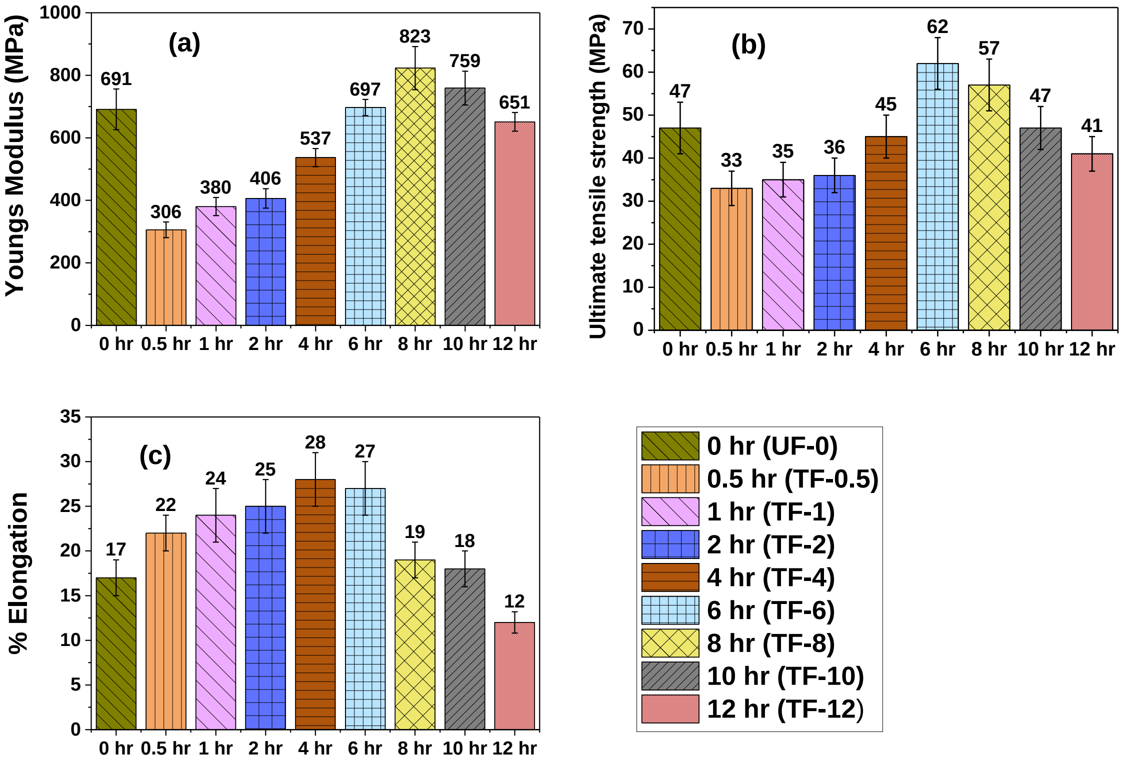


Figure 4.23. Comparison graph of the single fibre tensile properties of the waste Kibisu silk before and after treatment (a) Young's modulus (b) ultimate tensile strength (c) % elongation.

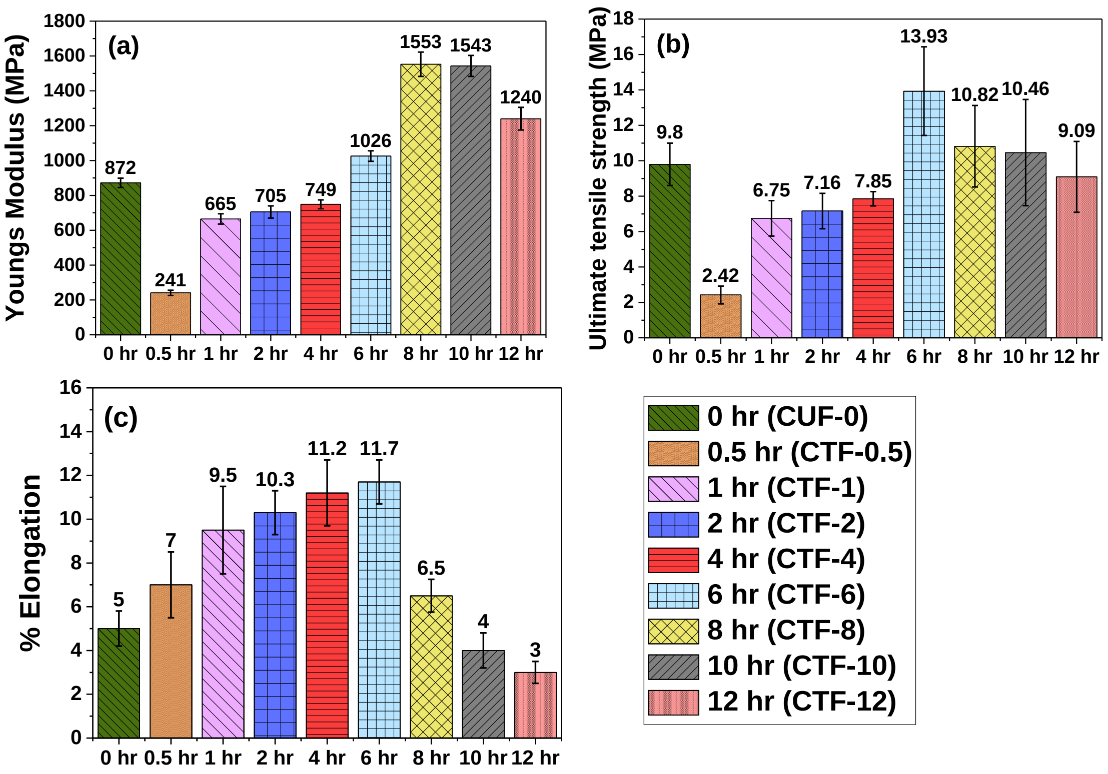


Figure 4.24. Comparison graph of tensile properties for the composite manufactured by wheat gluten and untreated and NaOH treated waste Kibisu silk (a) Young's modulus (b) ultimate tensile strength (c) % elongation.

Although the degumming process increases the crystallinity of the fibres, thus making them more stiffer but gradual removal of the sericin layer increases the fibres' elongation as they can move freely. It further verifies the composite's tensile behaviour influenced by the fibre's tensile properties as the degumming time increases. Because of all these reasons, Young's modulus for CUF-8 is maximum showing enhanced adhesion among the fibre and wheat gluten, but it lacks elongation. As fibre failure occurred drastically upon increased load, wheat gluten overpowered the composite's mechanical properties, thus making it more brittle. The same trend can be seen for CTF-10 and 12. The tensile comparison proved optimised properties for CTF-6 as it showed good Young's modulus, the best tensile strength and elongation among its peers.

4.4.5 Impact strength

During the impact test, impact energy gets dissipated in the form of matrix breakage, fibre breakage, matrix-fibre interface failure, delamination of layers, and crushing of the core structure of the composite elements. The impact test finds the damage tolerance of the composite as impact load reduces the design strength of the composite. Low-velocity Izod test primarily finds the damage resistance in terms of absorbed energy or impact energy which is directly proportional to impact strength. Figure 4.25 shows the impact energy for CUF-0 is 0.644, indicating higher impact strength than CTF-0.5 and CTF-1 composites. The sericin layer of the untreated fibres works like external support, increasing the composite's strength. But at the same time, sericin does not offer good fibre matrix adhesion with wheat gluten, being hydrophilic. The tangled fibres tightly packed under the sericin layer also fail to distribute stress under impact load. This results in the early failure of the composites. On the other hand, CTF-0.5 and CTF-1 show early failure due to randomly removed sericin layers. The semi-opened and semi-tangled fibres fail under impact load due to uneven stress distribution and non-homogeneous orientation of the fibroin. The impact strength increases CTF-2 onwards as the sericin starts to peel off entirely from the fibres and the fibroins start to untangle. NaOH-treated fibre removes the sericin layer, which activates alanine and glycine compounds. As both these compounds are hydrophobic in nature, it induces a higher affinity toward hydrophobic WG and develops a stronger bond 22,35. It increases the fibre matrix interface, thus increasing impact strength. The interface is most substantial for CTF-6 as it showed the best fibre and matrix adhesion and very few voids, as shown in section 4.4.3. The impact strength further reduces for CTF-8, CTF-10 and CTF-12 as the fibre diameter reduces, making them weak and breaking faster under impact.

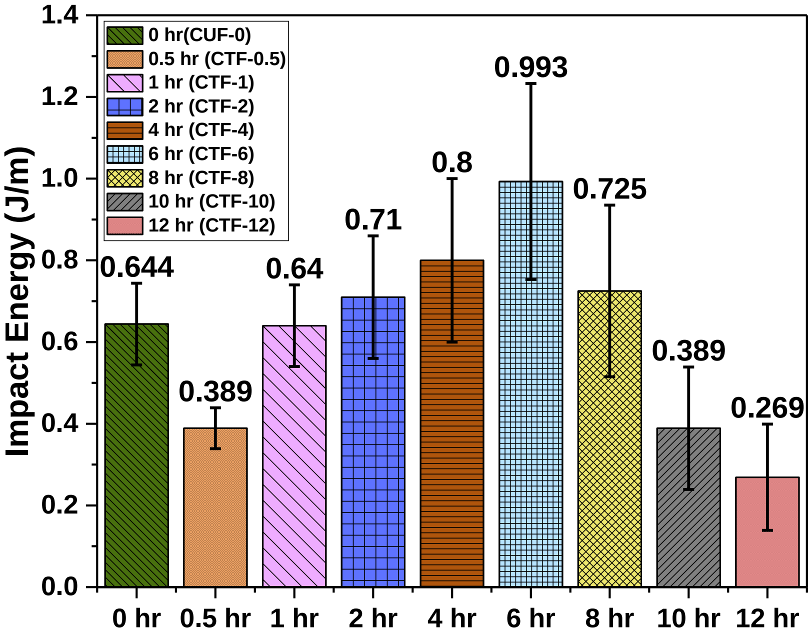


Figure 4.25. Comparison graph of impact energy for the composite manufactured by wheat gluten and untreated and NaOH treated waste Kibisu silk.

4.4.6 Thermogravimetric Analysis (TGA)

The TGA analysis shows the thermal stability of the composites at elevated temperatures. The first stage of 0 to 5% weight reduction occurs due to the evaporation of trapped moisture and air in the voids of the sample. The integral moisture absorption property of wheat gluten attracts a lot of residual moisture. This moisture and air evaporate upon heating, which occurs in the first stage of weight reduction. The subsequent 5 to 10% weight reduction occurs due to the evaporation of plasticiser in the composites, which is castor oil in this case. The next stage of drastic weight reduction occurs at the temperature range of 200 to 250 °C, as shown in Figure 4.26, due to the breakage of amino acid and hydroxyl groups present in the composite. As most of the graphs overlap, an enlarged view of the TGA graph at 220 °C is shown in Figure 4.26a.

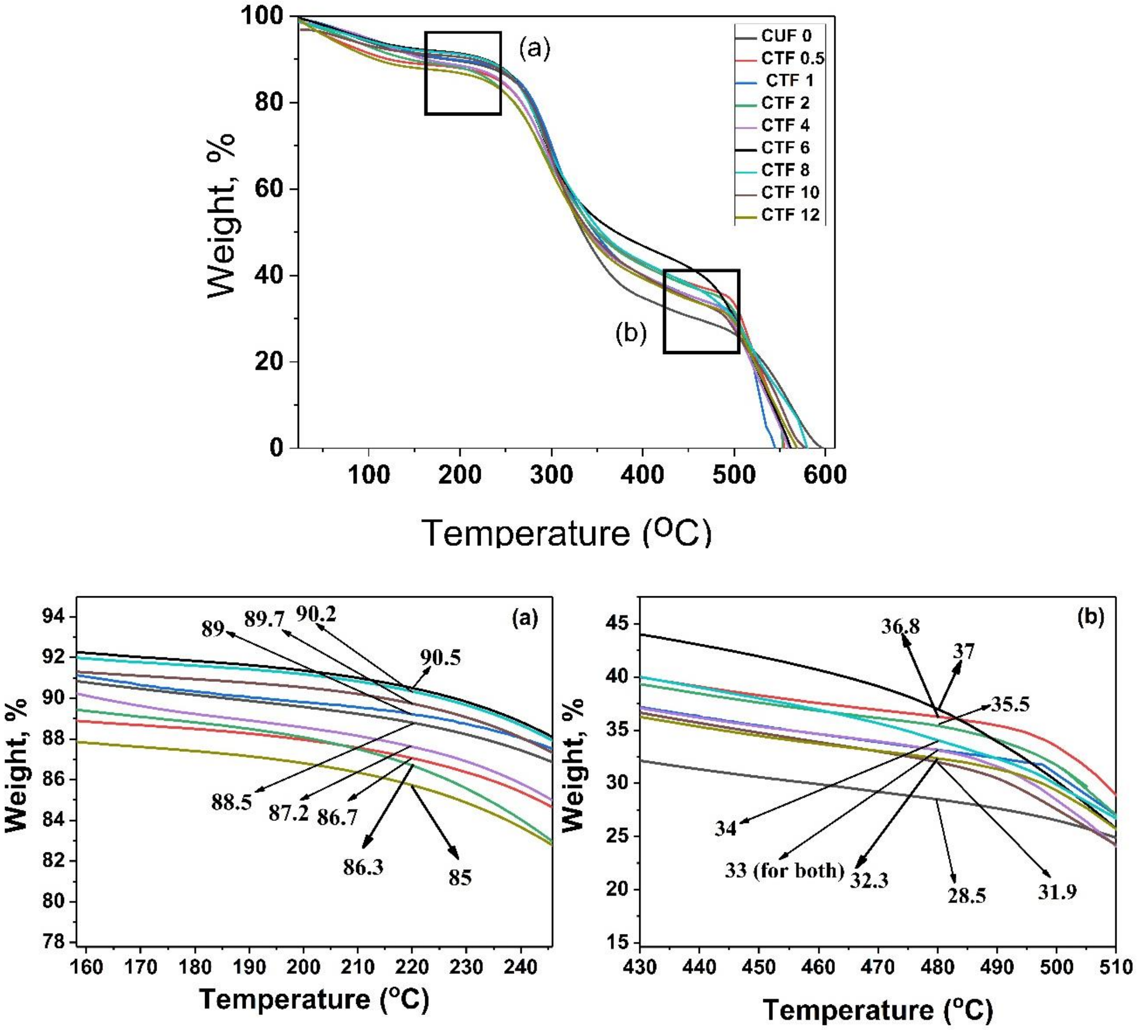


Figure 4.26. Comparison graph of TGA analysis for the composite manufactured by wheat gluten and untreated and NaOH treated waste Kibisu silk (a) enlarged view at 220 °C and (b) enlarged view at 480 °C.

The graph shows CTF-6 showing maximum stability of 90.5 weight percent at the said temperature. Better thermal stability represents better structural integrity and molecular interaction among the constituents of the composite. At 220 °C, the samples that showed good thermal stability after CTF-6 are CTF-8 with 90.2 percent and CTF-10 with 89.7 percent. It again proved to have good structural integrity, as mentioned in previous sections. The following composites, CTF-1, CUF-0, and CTF-

0.5, were protected by the amino acid bonds of the sericin layer from the extreme burning of the samples. But due to structural defects, it could not excel among its other counterparts. CTF-4 showed comparatively better thermal stability (87.2 weight % at 220 °C) because of better interaction among the constituents, as mentioned in previous sections. CTF-2 and CTF-12 showed poor properties, probably because of many voids in the CTF-2 structure and thinning of fibre, followed by poor strength for the CTF-12 composite. In the third stage of TGA, the drastic weight reduction occurs above 450 °C, where the remaining N-H, N-O, C-O and C-O links collapse and the sample completely decompose 37. Figure 4.26b shows the enlarged view at 480 °C, where the CTF-6 shows dominance with 37 weight percent over other samples. The CTF-0.5 follows it at 36.8 weight percent, CTF-2 at 35.5 weight percent, CTF-8 at 34 weight percent, CTF-1at 33 weight percent, CTF-4 at 33 weight percent, CTF-12 at 32.3 weight percent, CTF-10 at 31.9 weight percent, and CTF-0 at 28. 5 weight percent. They all are segregated with a very narrow margin showing good thermal stability for all the samples. At the last stage, all the composite samples completely decompose at the temperature range of 550 to 600 °C. Although CTF-6 showed elevated properties for the first three stages, it completely decomposes at 550 °C, which is lesser than CUF-0, CTF-8, CTF-10 and CTF-12. CUF-0. They survived more in the later stage, probably because of the presence of the sericin layer, and CTF-8, 10 and 12 survived because of better adhesion among fibre and matrix, which required more energy to burn out completely.

4.5 Natural coating characterisation

4.5.1 Water absorption test

Water resistance is one of the significant physical parameters to verify the efficacy of a coating. In the growing microbial safety and degradable coating market, Aagar agar is a significant component. Because of the inherent brittleness, Agar-agar is plasticised with castor oil for better flexibility and water resistance. The plasticising agents help overcome the biopolymeric composites' brittleness by reducing intermolecular forces. Gelatine coatings are known to bind the cell surface by fibronectin binding, making the surface hydrophobic in nature. The addition of tea extract and aloe vera adds to the anti-oxidation of the coating, thus preventing the samples from discolouration. The water absorption test results are shown in Table 4.9 and 4.10 for agar agar and gelatine-based coating, respectively. Agar agar plasticised with castor oil reduces the intermolecular forces, thus improving the coating's flexibility, elasticity and water barrier properties. The developed coating showed that as the coating thickness increases, the water absorption decreases drastically. For all the coating thicknesses (0.25 mm, 0.5 mm and 0.75 mm), after the initial ten minutes, the water absorption is found to be zero. As time progresses, single-layer coating samples start to swell gradually, whereas the double and triple-layer coating stops the water absorption, remarkably for an extended period. A similar trend can be found for gelatine-based coating as well. Gelatine binds the cell surface by fibronectin binding, which helps in protecting the substrate from water and vapour. At the end of forty-eight hours, the triple layer coating of agar agar absorbs 17% water, whereas gelatine coating absorbs only 11%. The added natural aloe vera with gelatine acts as a barrier to respiratory gases and fluids, increasing the water barrier of the samples [221]. The results showed that gelatine-based coatings were the most resistant to water than agar agar-based coatings.

Table 4.9: *Water absorption test of Agar Agar coating for 48 hours.*

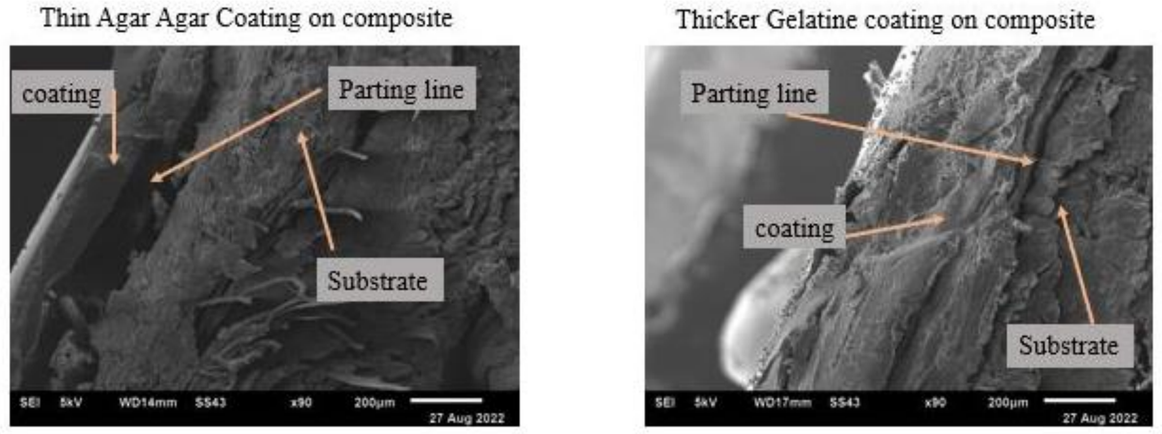
Type		**Single coating (0.25 mm)**	**Double coating (0.5 mm)**	**Triple coating (0.75 mm)**
Initial weight (gm)	In gm	25.4	26.5	27.8
Weight after water absorption for 10 min	In gm	25.4	26.5	27.8
	In %	0	0	0
Weight after water absorption for 30 min	In gm	26	26.8	27.9
	In %	2.36	1.13	0.3
Weight after water absorption for 60 min	In gm	28	27.2	28.3
	In %	10.23	2.6	1.8
Weight after water absorption for 6 hours	In gm	29.1	27.9	28.9
	In %	14.56	5.28	3.95
Weight after water absorption for 24 hours	In gm	31.1	28.7	29.6
	In %	22.44	8.3	6.47
Weight after water absorption for 48 hours	In gm	41.2	33.5	32.6
	In %	62.2	26	17

Table 4.10: *Water absorption test of Gelatine coating for 48 hours.*

Type		**Single coating (0.4 mm)**	**Double coating (0.8 mm)**	**Triple coating (1.2 mm)**
Initial weight (gm)		25	26	26.5
Weight after water absorption for 10 min	In gm	25.2	26	26.5
	In %	0.8	0	0
Weight after water absorption for 30 min	In gm	26.2	26.4	26.6
	In %	4.8	1.5	0.37
Weight after water absorption for 60 min	In gm	27	26.9	26.8
	In %	8	3.46	1.13
Weight after water absorption for 6 hours	In gm	27.8	27.4	27.1
	In %	11.2	5.38	2.26
Weight after water absorption for 24 hours	In gm	29	27.9	27.7
	In %	16	7.3	4.5
Weight after water absorption for 48 hours	In gm	38	31	29.6
	In %	52	19	11

4.5.2 Scanning electron micrographs of the cross-section

The SEM images of the cross-section of the coated surfaces are shown in Figure 4.27. The agar agar coating has created a parting line with the WG substrate, which represents poor adhesion of the coating. The poor adhesion is due to the anti-adhesion properties of the castor oil plasticised agar agar gel. Due to the length of fatty acid of the pendant structure, castor oil hampers the penetration of foreign particles inside the structure. At the same time, it reduces the adhesion of the coating on any substrate. For gelatine-based coatings, the adhesion is better as the gelatine combined with aloe vera gel creates fibronectin binding, which helps in creating better adhesion to the substrate surface.


Thin Agar Agar Coating on composite

Thicker Gelatine coating on composite

. **Figure 4.27.** SEM fractrographs of the agar agar and gelatine coating on the composite substrate

4.5.3 Porosity of the developed coatings

The porosity of the developed coatings is a significant index reflecting the performance of the coatings. The barrier effect against water vapour penetration of the coating depends on how much porous the surface is. The agar agar and gelatine film coatings show some degree of porosity or pore formation, especially if the film is relatively thick. The gelatine in water at concentrations forms low-viscosity solutions at temperatures above 40 °C. Once cooled, the solution becomes transparent, and rubbery, which forms a gel-type structure. The gel-type structure is formed because of the crosslinked intermolecular triple helix [222]. The gelation of agar agar is similar to gelatine and forms a transparent gelly structure below 40 °C. The temperature at which agar transforms from a sol to a gel state is primarily determined by the amount of methoxy groups present in the sample. Agar sols exhibit a thermo-reversible behaviour, forming physical gels with a significant time lag between the temperatures at which they melt (85 °C) and solidify (40 °C). These gels consist of antisymmetric double helices as their fundamental structural units [223].

While the formation of the sol-gel gelatine and agar agar mostly traps air bubbles inside, which increases the porosity of the coating. These pores can be irregularly distributed throughout the film or clustered in certain areas. The biocoated surface was studied for its porosity using the visualization technique of SEM. The visualization results of the coated surfaces indicate the presence of pores inbetween the globules of agar agar (Figure 4.28) and gelatine (Figure 4.29). The analysed SEM images with white marks show the interglobular spaces. As seen in Figures 4.28 and 4.29, agar agar and gelatine bio-coatings are porous in structure with a porosity of two and one percentage. Porous coating delays the formation of vapour films, increasing the resistance against vapour film formation on the surface. The porosity on the surface also allows the easy escape of the bubbles formed beneath the surface, increasing the product's shelf life.

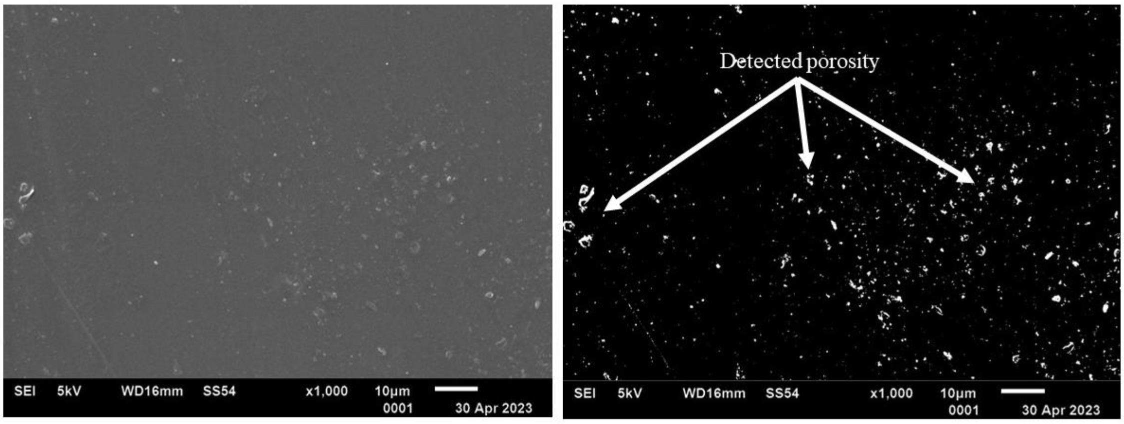


Figure 4.28. Porosity measurement by analysing SEM fractrographs of the agar agar coating on the composite substrate.

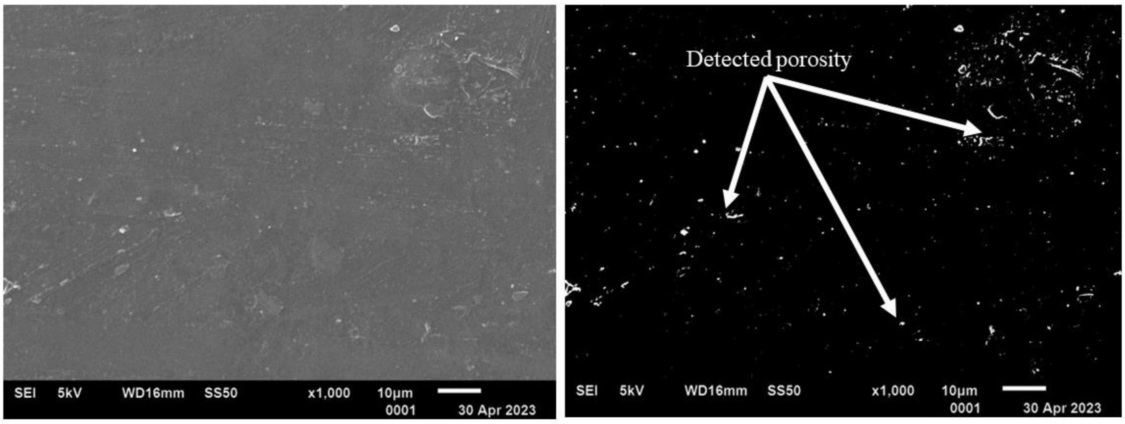
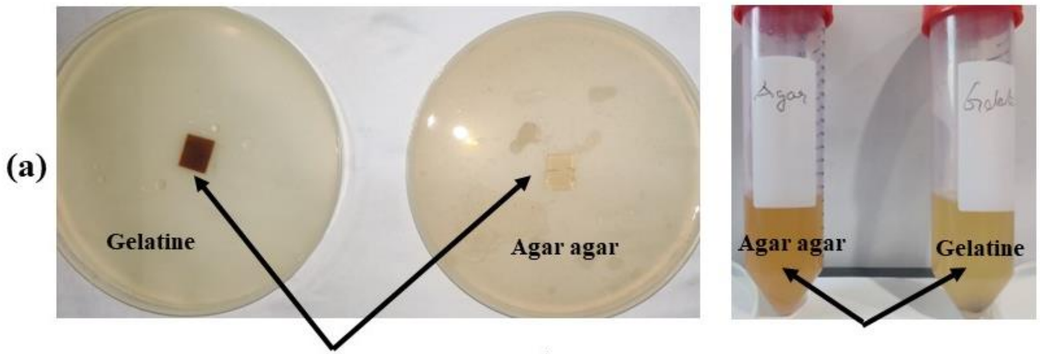


Figure 4.29. Porosity measurement by analysing SEM fractrographs of gelatine coating on the composite substrate.

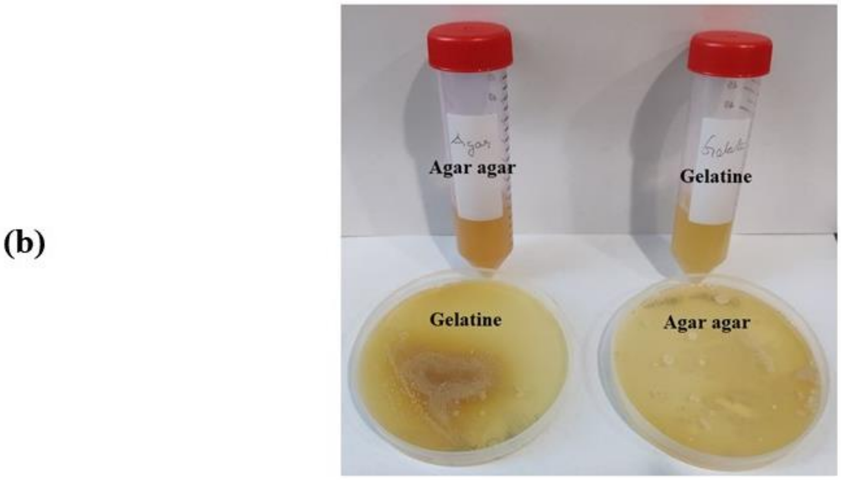
4.5.4 Biodegradability study

Figure 4.30a shows the initial phase of bacterial degradation of agar agar and gelatine coating in liquid and solid media. Exposure to accelerated climate enriched with bacteria changes the functionality of agar agar and gelatine coating. Microorganisms play a significant role in the degradation process of the coating. Introduction to the E.coli environment in a fixed humidity and temperature changes the film's morphological structure and mechanical properties due to the decrease in molecular size and number of sulfate groups.

The changes in the coating during bacterial degradation generates microcracks and embrittlement. This promotes extensive bacterial attack, which breaks the structure of the coating into micro fragments [101,224]. The Bacterial degradation study showed that after seven days in an E.coli environment, the samples broke the coating severely, and no trace of the coatings was found in the liquid media. Several degraded micro fragments were found in the solid media degradation, as shown in Figure 4.30b. The SEM micrographs in Figure 4.31 showed vigorous degradation in the solid media of both coatings.

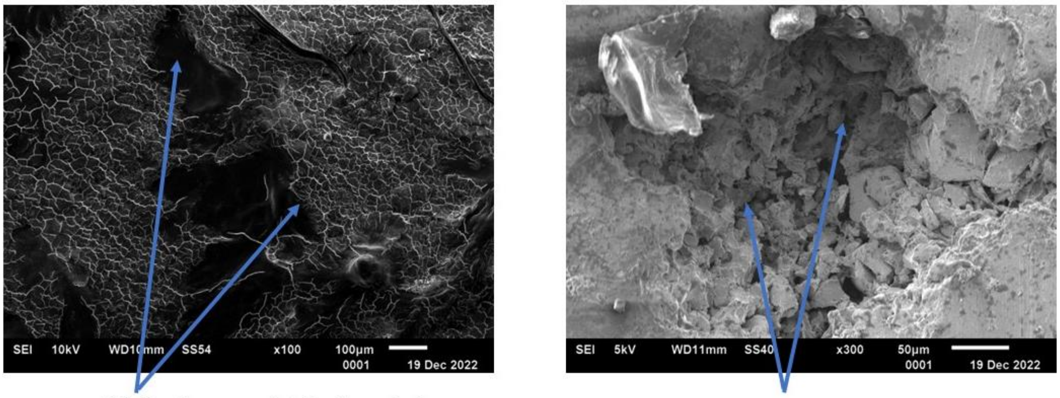


Coatings at Day 1 (Solid media) **Coatings at Day 1 (Liquid media)**



Coatings at Day 7 (in solid and liquid media)

Figure 4.30. Visual images of Agar agar and gelatine coating (a) before and (b) after seven days on solid and liquid media of bacterial degradation (E.coli environment) in the bio-cabinet.



(a) Coating completely degraded

(b) Vigorous degradation

Figure 4.31. SEM fractrographs of (a) agar agar and (b) gelatine coating before and after seven days in solid media of bacterial degradation (E.coli environment) showing vigorous defragmentation of the coatings.

4.6 Summary

The research reported in this chapter broadly consists of five parts.

- The fibre fraction has been optimised to develop the composite in the first part. The results showed that WG/WKS-based composites with fifty weight percentages of the fibres showed the composite's optimised physical and thermal properties.
- The second section carried out the influence of lemon extract as a natural crosslinker on the properties of the composite. Here the composite was developed with and without the natural crosslinker, where it has been found that natural crosslinkers enhance the physiochemical properties of the composite drastically.
- The third section studied the possibility of using different organic oils as a suitable plasticiser for WG-based composite. The study showed castor oil, linseed oil, coconut oil and mustard oil possess competitive properties like glycerol for plasticising the WG.
- The fourth section discussed the effect of degummed fibres on the properties of the composite. The fibres were degummed for a different time duration, and the best-suited degumming duration was selected for preparing the composite. The study showed that fibres degummed for six hours perform best in terms of the physiochemical and thermal properties of the composite.
- The fifth section discussed the properties of two developed coatings that are biodegradable. The agar agar and gelatine-based coatings were tested for water absorption, porosity measurement and biodegradability. It was found that gelatine-aloe vera-based coating provides a better water barrier for the composite substrate. However, both the coating

performed equally best in the biodegradation study. The cumulative results of the objective-wise characterisations are summarised in Table 4.11.

Table 4.11: *Objective-wise summarized results of the WG-WSF based natural composites and their coatings.*

Objective	Type	Common constituents	Variable	YM (MPa)	Ultimate tensile stress (MPa)	% Elongation	Thermal stability upto	Biodegradability
Composite with different fibre weightage	Type 1	10% glycerol, 2.5% NaOH, 2.5% lemon extract of wheat gluten dry matter	WG:KSF = 60:40	89	2.165	18	180 ℃	100 %
	Type 2		**WG:KSF = 50:50**	**536**	**10**	**18.31**	**267 ℃**	**100 %**
	Type 3*		WG:KSF = 40:60	122	2.195	11	267 ℃	100%
Composite preparation with the crosslinker	C1	WG:KSF = 50:50 12% Plasticizer	No crosslinker and dispersion agent	372	6.54	5.4	180 ℃	
	C2		Dispersion agent NaOH (5%)	478	9.08	4.6	217 ℃	
	C3*		**Dispersion agent NaOH (2.5%) and crosslinker lemon extract (2.5%)**	**872**	**10.08**	**5**	**247 ℃**	
Composite preparation with different natural plasticisers	A	WG:KSF = 50:50 12% Plasticizer (variable), 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter	Glycerol	536	10	18	220 ℃	100 %
	B*		**Castor oil**	**1333**	**26**	**13**	**246 ℃**	**100%**
	C		Linseed oil	992	20	11	220 ℃	100 %
	D		Mustard oil	867	16	15	210 ℃	100 %
	E		Olive oil	429	8	5	205 ℃	100 %
	F		Coconut oil	1229	19	14	200 ℃	100 %

Composite preparation with treated fibres	CUF-0	Wheat gluten: filature silk = 50:50 10% Castor oil, 2.5 % NaOH and 2.5% Lemon extract of wheat gluten dry matter	Untreated fibres	872	9.8	5	220 ℃
	CTF-0.5		30 mins treated fibres	241	2.42	7	215 ℃
	CTF-1		1 hr treated fibres	665	6.75	9.5	210 ℃
	CTF-2		2 hrs treated fibres	705	7.16	10.3	235 ℃
	CTF-4		4 hrs treated fibres	749	7.85	11.2	235 ℃
	CTF-6*		**6 hrs treated fibres**	**1026**	**13.93**	**11.7**	**250 ℃**
	CTF-8		8 hrs treated fibres	1553	10.82	6.5	230 ℃
	CTF-10		10 hrs treated fibres	1543	10.46	4	230 ℃
	CTF-12		12 hrs treated fibres	1240	9.09	3	210 ℃

			Water absorption (%) after						**Biodegradability after one week**
		Coating thickness (mm)	**10 min**	**30 min**	**60 min**	**6 hr**	**24 hr**	**48 hr**	
Development of natural coatings for the composite	**Gelatine based**	0.4	0.8	4.8	8	11.2	16	52	**100%**
		0.8	0	1.5	3.46	5.38	7.3	19	
		1.2*	**0**	**0.37**	**1.13**	**2.26**	**4.5**	**11**	
	Agar agar based	0.25	0	2.36	10.23	14.56	22.44	62.2	100%
		0.50	0	1.13	2.6	5.28	8.3	26	
		0.75	0	0.3	1.8	3.95	6.47	17	

* The optimised result out of the specific objective.

CHAPTER 5

CONCLUSIONS AND FUTURE SCOPE

5.1 Conclusions

This section broadly emphasises the concluding remarks for each section of Chapter 4. The different characterisations used to evaluate properties of WG/WKS reinforced composites with different fibre weightage, crosslinker, plasticiser, the degummed fibres of different time duration, and the properties of the developed coating will be discussed and concluded. The following conclusion based on the objectives of the thesis are as follows:

5.1.1 Composite with different fibre weightage

In this study, Kibisu fibre-reinforced wheat gluten-based biocomposites were successfully developed by hot compression moulding. The research reported here defines how the mass fraction of fibre affects the properties of the composite. It is palpable from the SEM images that the fibres and matrices have appreciable adhesion, which helps develop composites with better properties. It was found that the Type 2 composites made of 50%WG and 50%F showed almost five times higher Young's modulus, ultimate tensile stress and better elongation than Type 1 and Type 3 composites. The TGA results displayed that the sudden weight reduction of the composite samples upon heating starts at a moderately high temperature which authenticates the thermal stability of the samples. The most promising property of the developed composites is their higher biodegradation rate. They dissolved into soil entirely within two weeks of embedding them under natural conditions without any external interference. The soil test also showed no significant change in the quality of the soil after complete degradation. The convincing results after different characterisations make this a sustainable and environment-friendly composite that holds the potential to replace conventional thermoplastic composites to make biodegradable seedling pots and plates, disposable hospital trays, dustbins, etc.

5.1.2 Composite preparation with the crosslinker

The study revealed the potential to use natural lemon extract as a crosslinker for protein-based biocomposites with all-natural ingredients. As lemon extract is a vital source of citric acid, it can easily substitute synthetic citric acid as a crosslinker. The FTIR also supports the claims by showing decreased wavenumber from composites without any crosslinker to composites with crosslinker through its characteristic IR spectra. The thermal properties of the crosslinked composite showed outstanding stability at a higher temperature, which can be used as a key feature in replacing food-grade plastic usage. The mechanical properties also validate that the presence of crosslinker enhances the mechanical properties of the prepared sample with the added crosslinker. Adding lemon extract improved the Young's modulus to almost double for the composite with crosslinker compared to other samples without crosslinker. Crosslinking reduces the flexibility of the samples and increases stiffness, so elongation doesn't improve remarkably as the other mechanical properties of these samples. The composite with lemon extract as a crosslinker showed impressive results to be used as an all-natural composite having suitable mechanical and thermal properties under the influence of natural lemon juice as a crosslinker.

5.1.3 Composite with different natural plasticisers

It was found that the Type B composites made of castor oil as plasticiser showed better Young's modulus and ultimate tensile stress, but mustard oil-based composite showed better elongation than other organic plasticisers. The FTIR spectrographs show shifting peaks and the formation of new peaks in the sample having organic plasticisers, especially mustard oil, coconut oil and castor oil, showing polymer-plasticiser interaction in the composite. TGA results displayed better thermal stability for castor oil-based composite. SEM fractrographs show improved fibre-matrix adhesion among the constituents in the case of SCO composites made of castor oil. In bacterial culture, the degradation was moderately good for castor oil-based composites, though mustard oil-based composites showed more degradation.

5.1.4 Composite with treated fibres

In this study, the tensile, impact, thermal and morphological behaviour and molecular spectroscopy were systematically investigated for WG-WFS composites for different durations of fibre treatment in NaOH media. The XRD study showed increased crystallinity for the degummed fibres as the degumming duration increased. More crystallinity means straighter and lustrous fibres, which helped the composites perform better under tensile and impact loading. The FTIR analysis shows enhanced β-sheet formation as the fibre treatment time increases. It supports the enhanced mechanical properties of the treated fibres for extended hours. On the other hand, shifting peaks to the lower wavenumbers at CTF-6 justifies better interaction among the composite constituents. The surface morphology showed enhanced fibre matrix interaction as the surface of the treated fibres gradually got degummed. The CTF-6 showed the best surface morphology among other composites. The CUF-0 showed remarkable properties because of the support of the glue-like sericin layer. As the degumming progressed, the composites from CTF-0.5 to CTF-4 showed gradual improvement in their mechanical properties. The overall mechanical properties of CTF 6 excel all other composites in terms of ultimate strength, elongation and impact energy. However, Young's modulus of the CTF-8 stands highest as more alkali treatment tends to increase the β-sheet formation. The mechanical strength reduces further because of thinning of the fibre due to excessive NaOH exposure. The TGA analysis showed an almost similar trend for all the composites, but CTF-

6 performed marginally better than other composites in terms of thermal stability. Based on all the thermal and physiochemical observations, it can be concluded that the composite prepared by NaOH-treated WFS for six hours delivers the best combination of strength, elongation and thermal properties. This work also provides possibilities for conducting similar studies using other alkali solutions. The prepared sheets are used to develop 100% degradable nursery seedling pots and trays, which can be directly planted into the soil without discarding the pot.

5.1.5 Development of natural coatings for the composite

The water absorption test shows that agar agar and gelatine, both coatings, effectively prevent water penetration inside the composite. It can be seen that more the thickness of the coating, more it will prevent water absorption to the composite sheet. The comparison showed that gelatine coating prevented water penetration inside the composite better than Agar Agar coating in the long run by creating fibronectin binding on the cell surface. The SEM images showed the thicker Gelatine coating has better adhesion with the surface, thus protecting the composite from water absorption effectively. The thin layer of Agar Agar coating had poor adhesion with the composite surface resulting in more water sipping inside the layer. The porosity measurement showed the surface porosity of both the coatings are almost the same, adhering to the similarity in water absorption test results. According to the bacterial degradation investigation, the coating was severely degraded after seven days in an E. coli environment. There was no sign of the coatings in the liquid medium and micro fragments of both the coatings were found in solid media. The SEM images of degraded surfaces on the solid media showed deep craters of vigorous degradation of both coatings.

Based on the findings of this thesis, it can be concluded that waste Kibisu silk and wheat gluten with natural plasticisers and crosslinkers can make easily mouldable composite sheets which can be used for different purposes to reduce the usage of disposable plastics. Since the product is entirely biodegradable and non-toxic, it contributes toward clean and green manufacturing. The final result shows composite prepared with fifty percent fibre weight fraction of six-hour treated waste Kibisu silk and wheat gluten with 10% castor oil, 2.5% NaOH, and 2.5% lemon extract gives the most promising properties in terms of mechanical strength and thermal stability. The bio-coating of gelatine-aloe vera is best suited for effectively coating the developed composites to prevent moisture and water absorption.

5.2 Proposed applications

The products shown in Figure 5.1 represent biodegradable sampling pot and disposable plate replicating the similar counterparts available in the market, which is made of plastic. Since the product is entirely biodegradable and non-toxic, it contributes toward clean and green manufacturing. Hot compression moulding can easily mould these sheets into different shapes and sizes. They can be commercialised as hospital trays, ceiling tiles, sampling trays, nursery sampling pots and table tops in a protected environment.

Figure 5.1. Plate and pot developed using treated waste Kibisu silk-wheat gluten composite sheets representing the feasibility of preparing various biodegradable products.

5.3 Limitations of present work

Despite all the favourable properties of the developed composite, a few issues require further research in this field.

- Due to the hydrophilic nature of wheat gluten, the developed composite will be susceptible to ambient moisture, which may reduce the shelf life of the composite.
- Because of the brittle nature of wheat gluten, the composite sheets cannot acquire very complex shapes.
- This thesis has not explored the feasibility study of the water-vapour permeability of the coating.
- The effect of the percentage of added plasticiser on the physiochemical properties of the composite has not been studied.

5.4 Scope of future work

The present work leaves with a wide scope for future investigators to explore more on developing all-natural green composites. Some recommendation for future research includes,

- To enhance the shelf life of the developed composites, low-cost moisture resistance and anti-microbial and anti-fungal properties have to be incorporated into the composite.
- Future studies can be implied to enhance the flexibility of the prepared composites.
- Toxicity studies and time-dependent mechanical testing can also be performed on these composites to determine the shelf life of the developed product.
- FEM modelling on various aspects of processing for these composites may be performed using available simulation software.

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1. Bhowmik, P., Kant, R., Nair, R., & Singh, H. (2021). Influence of natural crosslinker and fibre weightage on waste kibisu fibre reinforced wheatgluten biocomposite. Journal of Polymer Research, 28(4), 1-14. https://doi.org/10.1007/s10965-021-02470-9

2. Bhowmik, P., Kant, R., Nair, R. & Singh, H.. The synergistic influence of lemon extract on the physio-chemical properties of Kibisu silk reinforced wheat gluten biocomposite. Polym. Bull. (2022). https://doi.org/10.1007/s00289-022-04267-2

3. Bhowmik P, Kant R, Singh H. Effect of Degumming Duration on the Behavior of Waste Filature Silk-Reinforced Wheat Gluten Composite for Sustainable Applications. ACS Omega. (2023). https://doi.org/10.1021/acsomega.2c05963

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1. Papiya Bhowmik, Ravi Kant, Harpreet Singh. "Green composites for sutainable applications" in "Sustainable Material, Design and Process" CRC Press (under review).

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P. Bhowmik., G. Arora, "Graphene/Metal Matrix Nanocomposites: Synthesis, Characterization, and Properties" in Metal Matrix Composites, (5th chapter-pages 40), CRC Press, 2022, ISBN 9781003194910.