Converting Poultry Feather Waste into Bioplastics using Green Modifying Agents

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Author

Marc Jirström

Supervisors

Mohammad H.A. Ibrahim

Hassan Amer

Baozhong Zhang

Examiner

Patric Jannasch

Abstract

Handling of problematic waste material, and the use of fossil-based and non-biodegradable plastics are two major sustainability challenges. By developing solutions that convert waste material to bioplastics, it is possible to address both these issues. Every year, large quantities of feathers from the poultry industry are either stored in landfills or disposed of by incineration. The company Bioextrax has developed an alternative, green approach that breaks down the feathers via microbial degradation, a process that generates keratin microfibers. During this project such microfibers have been used to synthesize a bioplastic film, using cheap and environmentally friendly reagents.

Since the microfibers do not form a coherent material on their own, glycerol and citric acid have been added as modifying agents. This thesis documents the development of a process to generate thin films from said reagents, by forming a pre-polymerized resin of glycerol and citric acid, subsequently mixing it with microfibers, and pressing the mixture into a thin film using a flatiron. The result is a flexible thin film that is insoluble in water. Analysis using TGA, DSC and FTIR shows indications of a one-phase system and increased thermal stability contributed by the microfibers. While the microfibers mixed with the resin can be processed into thin films using a simple approach, the main drawback is that control films are extremely difficult to produce without incorporating microfibers. This leaves much room for future development, while this project can serve as a stepping stone for future work.

Sammanfattning

Hantering av problematiskt avfall, och användningen av fossilbaserade och icke-biologiskt nedbrytbara plaster är två stora hållbarhetsutmaningar. Genom att utveckla lösningar som konverterar avfall till bioplast så blir det möjligt att lösa båda dessa problem. Varje år så genereras stora mängder av fågelfjädrar från slaktindustrin, dessa fjädrar hamnar antingen på soptippar eller så förbränns dem. Företaget Bioextrax har utvecklat en alternativ, grön nedbrytningsprocess som utnyttjar bakterier för att sönderdela fjädrar till mindre beståndsdelar; mikrofiber av keratin. Under detta projekt har sådana mikrofiber använts för att syntetisera en tunn film av bioplast, med hjälp av billiga och miljövänliga kemikalier.

Eftersom mikrofiberna inte kan forma fasta material utan tillsatser, så har glycerol och citronsyra använts för att modifiera materialet. Denna uppsats dokumenterar en process som har utvecklats för att kombinera tunna filmer, genom att först forma en förpolymeriserad vätska av glycerol och citronsyra, som sedan blandas med mikrofiber och pressas med ett strykjärn till en tunn film. Resultatet är ett flexibelt material som är olösligt i vatten. Analysmetoderna TGA, DSC och FTIR visar på indikationer att materialet består av en fas, och att mikrofiberna gör materialet mer värmetåligt. Processen för att forma tunna filmer är simpel, men den största nackdelen är att det inte är möjligt att använda samma process för att göra tunna filmer från enbart den förpolymeriserade vätskan. Detta innebär att det finns mycket utrymme kvar för fortsatt optimering av processen, medan detta arbete kan fungera som ett första steg i utvecklingen.

Biologiskt avfall till bioplast - med gröna processer

Fjädrar från slaktindustrin utgör ett mycket problematiskt avfall men kan omvandlas till bioplast med hjälp av billiga och gröna kemikalier. Under detta projekt har en helt ny bioplast utvecklats, baserad på mikrofiber från fjädrar i kombination med citronsyra och glycerol - två miljövänliga och billiga reagenser. Plasten är i formen av en tunn film, som är flexibel och olöslig i vatten i flera månader.

Eftersom fjädrar är mycket tåliga så tar det lång tid att bryta ner dem i naturen. Därför måste de miljontals ton som genereras varje år antingen förbrännas eller deponeras på soptippar. Med hjälp av en unik jäsningsprocess utvecklad av Bioextrax AB så bryts fjädrarna ned till små mikrofiber, som sedan kan användas som byggdelar i nya material. Detta projekt utforskar en sådan tillämpning, och en process utvecklas för att kombinera mikrofibrerna med glycerol och citronsyra för att skapa en helt ny, biobaserad och biologiskt nedbrytbar plast. Citronsyra och glycerol blandas och hettas upp för att bilda en transparent, flytande vätska som är ett förstadie till en hård och olöslig plast. Innan reaktionerna har gått så långt att en osmältbar plast har bildats, så blandas vätskan med mikrofiber och formar en torr, deg-liknande massa. Denna massa pressas sedan med hjälp av ett helt vanligt strykjärn till en tunn film, se figuren nedan.
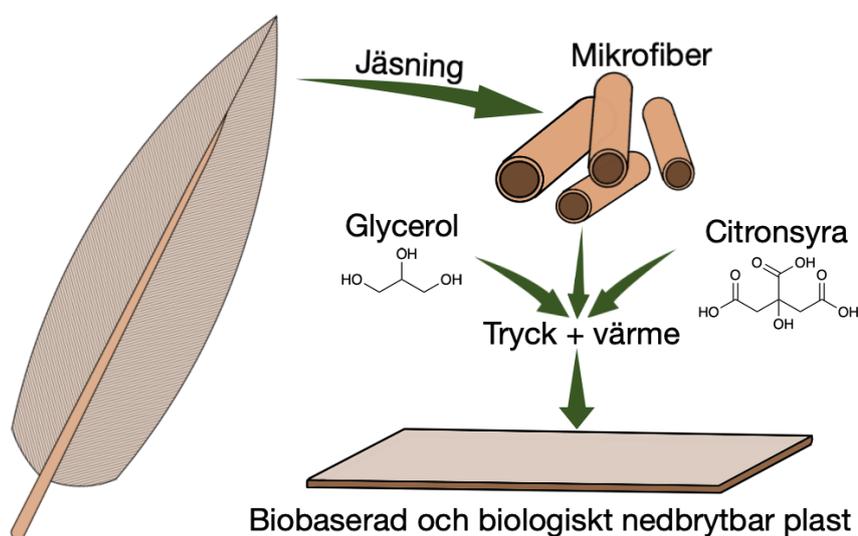
Processen verkar simpel, reagenserna är billiga och lättillgängliga, men de bakomliggande mekanismerna är komplicerade, och processen lämpar sig inte för att göra tunna filmer som inte innehåller mikrofiber. Det finns utrymme för framtida projekt för att bättre förstå de reaktioner som sker och hur mikrofibrerna fungerar i materialet. Resultaten kan användas som "proof-of-concept" och fungera som en språngbräda för fortsatt utveckling.

Acknowledgements

Abbreviations

IF   Intermediary filaments

CA   Citric acid

GLY   Glycerol

MF   Microfiber(s)

SHP   Sodium hypophosphite

DI   De-ionized

TGA   Thermogravimetric analysis

DSC   Differential scanning calorimetry

IR   Infrared spectroscopy

Contents

1   Introduction

In the aspiration to achieve a more sustainable way of living, societies around the world will have to tackle a multitude of challenges. Issues such as the handling of problematic waste material, and transitioning from fossil-based plastics to bio-based and plastics, will require technological development and innovative solutions. There is one type of solution that is two-fold in it's nature and can deal with both of these matters; first valorize the waste material by converting it to useful components, and then incorporate those components in new bio-based and biodegradable materials. This project utilizes such an approach to develop a new bioplastic from poultry feather waste, using sustainable processing methods.

Feather waste from the poultry industry is an abundant waste product, with millions of tonnes being generated annually around the world [1]. The feathers are resistant to biological degradation, and typically end up in landfills unless they are disposed of by incineration [2]. Not only is the handling of feather waste problematic from an environmental perspective, it also represents a missed opportunity of repurposing the waste to develop new products with unique features. In fact, bird feathers contain a high amount of keratin, a protein that forms durable structures. Such a property could allow for applications as a biodegradable and bio-based material, with potential applications in the packaging industry [3].

Before keratin from bird feathers can be used in materials, it needs to processed by breaking down large-scale structures, in order to generate smaller particles that are more easily processed. This can be done through various methods, most of which require high amounts of energy and/or large quantities of chemicals [4]. However, there is one approach which eliminates the need for expensive, toxic or environmentally harmful chemicals: microbial degradation of the keratin. Bioextrax AB, based in Lund, has developed a green process that converts feather waste into keratin microfibers, ready to use for material applications.

By themselves the keratin microfibers do not form materials with good mechanical properties. For this reason, modifying agents need to be added to the microfibers in order to improve crucial properties such as insolubility in water and mechanical strength. While there are many modifying agents that have been proven effective, the choice becomes limited when the the following requirements are made; the reagent should be bio-based, biodegradeable, cheap, non-toxic and readily available. Two reagents that pass these criteria are glycerol and citric acid. This project aims to explore the possibilities of synthesizing a cheap and green material from keratin microfibers, glycerol and citric acid.

1.1   Disposition

Chapter 2 reviews the structure, properties and applications of keratin in general, and also provides a more in-depth description of bird feather keratin. In **Chapter 3** the modifying agents are discussed, with a focus on possible reaction mechanisms and kinetics. The experimental work of the project is described in **Chapter 4**, and the results are analyzed and discussed in **Chapter 5**. Finally, **Chapter 6** provides a summary and a conclusion of the project.

2

Keratin - Properties and Applications

"Keratin" typically refers to one of nature's most ubiquitous groups of proteins, with fibrous structures and tough physiochemical properties [4, 5]. Being present in most vertebrates, all keratin is produced in epidermal cells, with the main function of protecting underlying tissue [3, 6]. In humans keratin can be found in nails, hair and the outermost layer of the skin. In other vertebrates it constitutes horns, hooves, fur, beaks and feathers [3–5, 7]. The protective abilities of keratin stems from the protein's high resistance to mechanical stress and insolubility under harsh conditions.

Compared to other proteins, keratin contains a high amount of the amino acid cysteine, which enables it to form strong disulfide bonds, allowing for resilient protein structures [8]. The unique chemistry of keratin's structure, and the resulting distinctive properties of keratin, have generated interest among researchers in the field of materials science. Additionally, the abundance of keratin in the form of waste material, such as bird feathers from the poultry industry, creates a need to valorize the waste as an alternative to incinerating it. For these reasons, efforts have been made to develop keratin-based products with potential applications in the industries of packaging material, drug delivery systems, cosmetics and more [3, 4].

2.1 Structure

The structure of keratin is typically discussed in terms of the protein's primary and secondary structure. The primary structure is the amino acid content and it's sequence, which makes out the polypeptide backbone of the protein. Interactions between amino acid residues in the protein's backbone shapes the chain into one of several three-dimensional conformations [9]. These conformations are referred to as the secondary structure of the protein.

2.1.1 Primary structure

For keratin, the amino acid content varies depending on the source, but in general keratin contains high amounts of the amino acids cysteine, arginine, threonine, leucine, glycine, valine and proline [3, 10, 11]. In particular the high content of cysteine, typically 7-20 % of the total amount of amino acids, grants keratin it's excellent mechanical, thermal and chemical stability [3, 12–14]. This is due to sulfhydryl groups (SH) in cysteine being able to form strong covalent sulfide bridges (S-S), as shown in figure 2.1. The bonding can be intramolecular or intermolecular, that is either between two cysteine residues in the same polypeptide chain, or between two different chains.

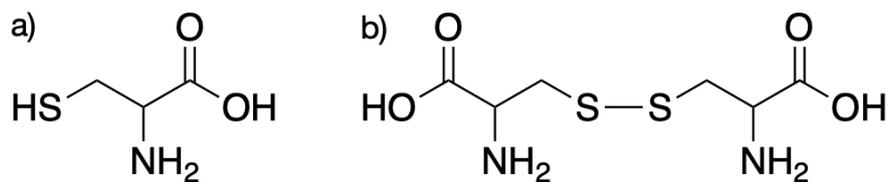**Figure 2.1**   **a)** Cysteine molecule. **b)** Sulfur bridge bond between two cysteine molecules.

Keratin can be classified as either "soft" or "hard" based on the cysteine content of the protein [3]. Soft keratin is typically found in epithelial tissue and contains a lower amount of cysteine and disulfide bonds. Hard keratin is the type that is found in nails, beaks and hooves, and contains a higher amount of cysteine [11].

2.1.2 Secondary structure

The most common way to classify keratin is based on it's secondary structure. The polypeptide backbone is shaped by hydrogen bonding between amino- and carboxyl groups in the peptide, which can result in various conformations [9]. The α-helix structure and the β-sheet structure are the two most common structures used to classify keratin. If the hydrogen bonding between amino acid residues is intramolecular, a single polypeptide chain can take the form of an α-helix (α-keratin). On the other hand, if the bonding is intermolecular, multiple chains can form a β-sheet conformation (β-keratin) [4, 5]. Figure 2.2 shows the general structure of α-helices and β-sheets. Ultimately, the secondary structure of a protein depends on the amino acid content, which differs between α- and β-keratin.

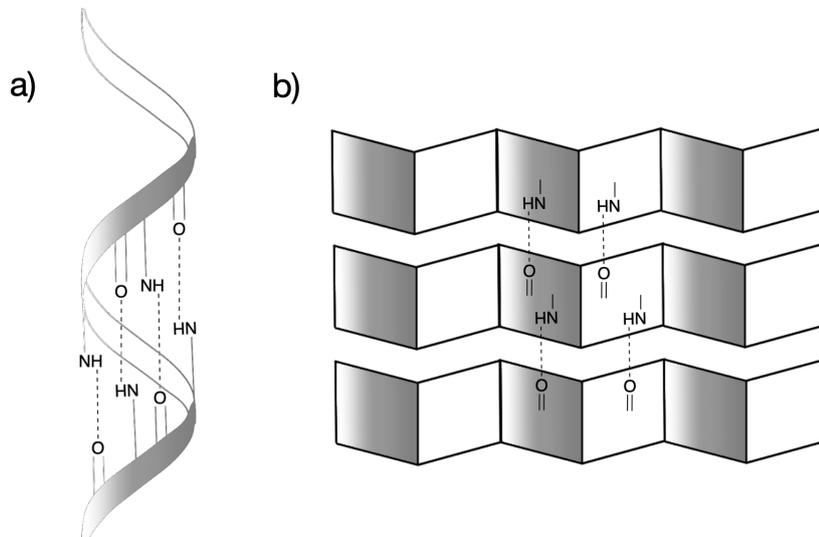**Figure 2.2** General schematic of hydrogen bonding between amino and carboxyl groups of polypeptide backbones, conforming into **a)** α-helix and **b)** β-sheet structure [5].

α-**Keratin**

Compared with β-keratin, α-keratin has a higher cysteine content [5]. The polypeptide chain is in the α-helix conformation, and can cross-link with another chain by forming disulfide bonds between cysteine residues. The arrangement of the two

chains into a dimer makes out the smallest molecular unit of α-keratin, and further cross-linking between multiple dimers results in larger intermediate filaments (IF) [15]. The IF are surrounded by an amorphous matrix of keratin, which has a higher cysteine content than the crystalline IF [3]. Sometimes referred to as "mammalian keratin", α-keratin is found in skin and wool [6]. The molecular weight is typically between 40-60 kDa.

β-**Keratin**

For β-keratin, there is no distinction between amorphous or crystalline portions of the protein. Multiple polypeptide chains cross-link to form β-sheets, the sheets twist and two sheets are connected to form IF [5]. The β-sheet formation provides more structural toughness and insolubility than the α-helix conformation [3]. In animals β-keratin is often found in hard protective tissue, such as claws, beaks and feathers. The molecular weight of β-keratin is significantly lower than α-keratin, typically around 10-20 kDa [6].

2.2   Feather Keratin

Poultry feathers from slaughterhouses typically contain around 90 % keratin [16]. The keratin is mainly in the form of β-keratin, with a small amount of α-keratin present during the development of the feather [5]. The cysteine content of feathers is around 7 % [15]. Disulfide cross-linking, hydrogen bonding and hydrophobic interactions between tightly packed β-sheets make feathers mechanically and chemically stable. The molecular weight of the keratin is typically around 10.5 kDa [3]. Hydrophobicity, low density and high mechanical strength are important functional properties of feathers, giving some avian species the ability to fly. Furthermore, feathers provide excellent thermal insulation [17].

The general structure of a feather is a herring-bone like composition of a rachis, barbs and barbules, see figure 2.3. The rachis is a cylindrical shaft, from which barbs protrude. The barbs support barbules, to which hooklets are attached [5]. The hooklets can interlock with eachother, giving feathers their characteristic smooth surface.
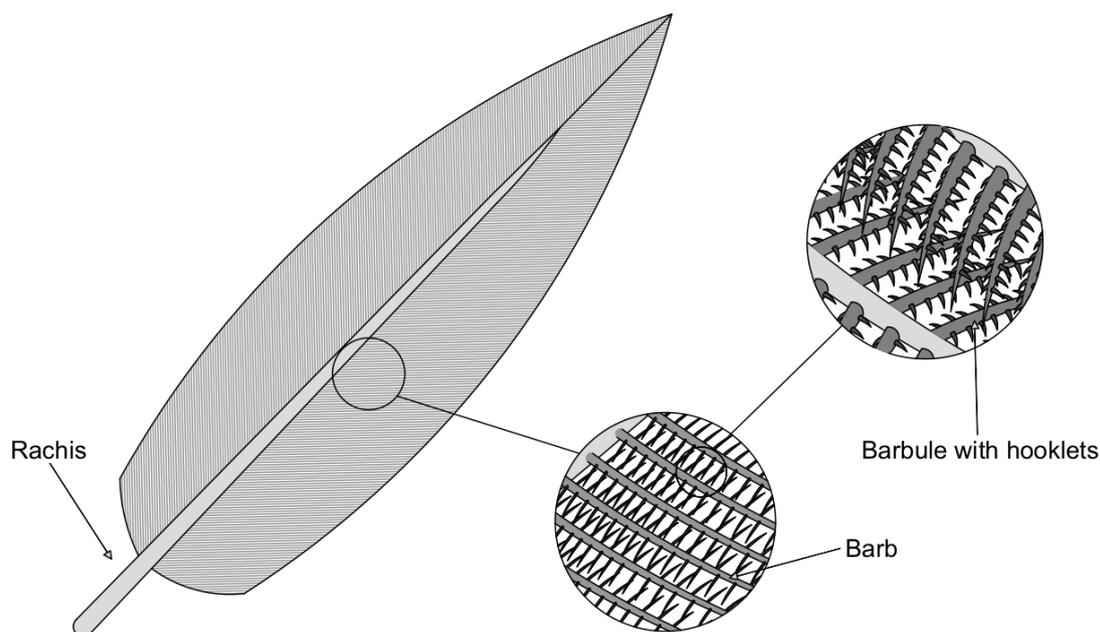
Figure 2.3 General structure of avian feathers, showing the rachis, barbs, barbules and hooklets [17].

Feather keratin is widely regarded as a highly problematic waste material. The same features that give feathers their highly stable physiochemical properties, desired from a materials science perspective, result in difficult challenges during disposal. Since feather keratin is highly insoluble, it is not easily biologically degraded, and must be disposed of through incineration [18].

2.2.1 Applications

Since feather keratin is abundant in the form of waste material, and with the combination of being biobased, biodegradable and possessing excellent physiochemical stability, extensive research has been conducted to find applications for the protein. Some of the most prominent potential uses are as bioplastics in the packaging industry, as a filler in other materials, as animal feed, as a component of pharmaceutical products, and for incorporation into textiles [4]. However, the raw native keratin, e.g. chicken feathers, often needs to be broken down into smaller pieces or solubilized completely, before it can be processed into materials.

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2.2.2 Processing keratin

In order to break down keratin, the sulfide bridges between cysteine residues must be broken. This can be done through oxidation, reduction or sulphitolysis [4]. If the properties of the native keratin are to be preserved, it is important that the method does not destroy or damage the secondary structure of the protein. Yet another challenge is to avoid using toxic, expensive and environmentally hazardous methods [2]. Researchers have employed various methods of processing keratin starting material, including the use of alkaline and acidic solutions, ionic liquids, deep eutectic solvents and microbes [4, 16].

Bioextrax's Method

The keratin used in this project is in the shape of microfibers (MF), which is the product of microbial degradation of bird feathers. The process, which is patented by Bioextrax, is a green alternative to other methods that typically rely on chemical solubilization. The generated microfibers are originally an internodal structure in the barbules of the bird feathers. The shape is cylindrical and hollow, which results in low density. The length of a microfiber is in the range of 30-150 µm, and the diameter is 2-6 µm. The composition of the microfibers is the same as for keratin in general; 91 % keratin, 1 % lipids and 8 % water. In addition to being bio-based, the microfibers are biologically degradable.

3

Modifying Agents

Since the required processing of keratin effectively cleaves sulfide bonds between cysteine residues, materials made out of purely regenerated keratin do not possess the same properties as the native protein. Such materials often exhibit poor mechanical strength, and dissolve quickly in water [4]. Researchers have therefore experimented with an extensive selection of modifying agents, in order to create insoluble and mechanically durable materials. Two common methods used to modify extracted keratin is to add a cross-linking agent and/or a plasticizing agent. The cross-linking agent forms new covalent bonds between two or more keratin molecules, which can make the material insoluble in water, and changes it's mechanical properties. Plasticizing agents can improve the flexibility of the material, by occupying free space between polymer chains and disrupting hydrogen bonding between them, which results in increased mobility of the chains.

A major challenge when modifying keratin materials is to find reagents that are effective yet non-toxic, cheap and environmentally friendly. Commonly used cross-linking agents, such as formaldehyde or glutaraldehyde, have proven to be effective under mild processing conditions [19]. However, these aldehydes are small molecules that are cytotoxic, which makes the resulting material unfit for various applications [20]. Conversely, using greener and safer cross-linking agents typically requires harsher processing conditions [21]. An increase in temperature may

be required in order for the greener, but less effective reagents to react with the keratin. The higher temperature may damage or denature the secondary structure of the protein. Therefore, it is necessary to optimize process parameters to achieve conditions that are as mild as possible, while still allowing for efficient reactions.

3.1 Selecting Modifying Agents

For this project, the choice of modifying agents is based on the following criteria:

- Reagents must be considered green, i.e. biobased and biodegradable.

- Reagents must be non-toxic and should be easy to handle.

- Reagents should be cheap and viable for large-scale production.

- The use of chosen reagents should be well-documented in scientific articles, which should serve as a starting point for the experimental work of the project.

The type of modifying reagents used in this project is a cross-linking agent, aswell as a plasticizer/cross-linking extender.

3.2 Cross-linking Agent

The chosen cross-linking agent for this project is citric acid (CA). The simple tricarboxylic acid is biobased, biodegradable, cheap and a popular choice among researchers [20, 21]. CA has a molecular weight of 192.124 g/mol. The melting temperature of CA is 153 °C, and the reagent starts to decompose at temperatures above 175 °C [22, 23]. The CA molecule is shown in figure 3.1.

3.2.1 Reaction

The functional groups of CA are three carboxyl groups and one hydroxide group. For a reaction to occur between CA and the functional groups of a protein, without
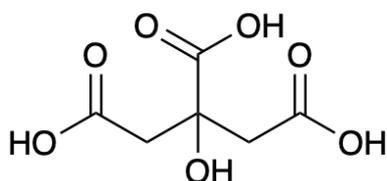
Figure 3.1 Citric acid molecule.

using a catalyst, it has been suggested that CA must first form a cyclic anhydride intermediate [20, 24]. This can occur at temperatures above 120 °C, as CA loses one molecule of water [25]. A cyclic anhydride is formed between the carboxyl group bound to the α-carbon (in the center of the molecule), and one of the other carboxyl groups in the molecule, see figure 3.2 **a)**. This implies that a maximum of two linkages are available for every CA molecule, since a minimum of two carboxyl groups are required to form the necessary intermediate. Hence, CA would function as a short linear cross-linking agent [21]. Figure 3.2 **b)** shows a general reaction between CA and keratin, with possible linkage to SH, OH and NH groups in the protein.
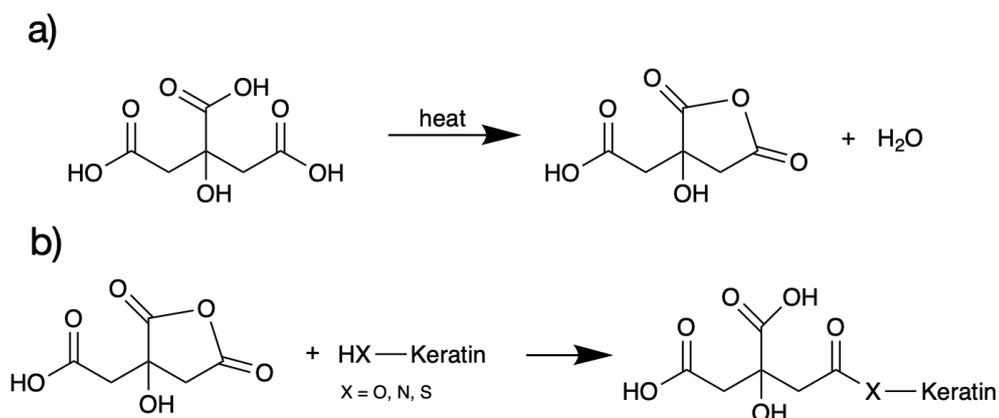
Figure 3.2 **a)** Formation of cyclic anhydride intermediate when citric acid is heated. **b)** Cyclic anhydride forms a covalent bond with one of the functional groups of keratin.

3.2.2 Effect on material properties

By cross-linking keratin fibers it is possible to synthesize a thermoset bioplastic, with improved wet strength compared to unmodified materials. It has been reported that for dry protein films cross-linked with CA, the tensile strength and Young's modulus improved significantly with increasing concentration of CA [26]. However, as the concentration of cross-linker increases, the elongation at break decreases. The linkage between polymer chains creates a stronger material, but also reduces the mobility of the chains, which reduces the elongation capacity. After immersion in water, all the mechanical properties of protein-based materials are typically worsened [21]. By cross-linking the material, mechanical properties under wet conditions, such as tensile strength, Young's modulus and elongation at break, improve significantly compared to the unmodified material under similar conditions. This is due to the cross-linking rendering the material insoluble in water, whereas non-cross-linked materials easily dissolve when wet.

Depending on the extent of cross-linking, the material may act as a hydrogel. As the degree of cross-linking increases, the structure becomes less mobile and swells less. By adjusting the degree of cross-linking it is possible to control the uptake of liquids, which can be useful for medical applications [4]. Furthermore, increasing cross-linking increases the time it takes to biologically degrade the material [27]. It is apparent that the extent of cross-linking is important to control, in order to modify some of the most important properties of the material.

3.3 Plasticizer and Cross-linking Extender

Films and other materials made from only keratin are often very brittle. In order to increase the flexibility of the material, plasticizers can be added. Plasticizers are often small molecules that can occupy free space in the polymer and disrupt some of the hydrogen bonding between polymer chains [28]. This allows for increased mobility of the polymer chains which increases the ductility and flexibility of the material. A commonly used plasticizer added to protein films is glycerol (GLY), which is the chosen plasticizer for this project [21, 26, 29]. GLY is an alcohol

with three hydroxide groups (OH), see figure 3.3. Similar to citric acid, glycerol is cheap, non-toxic, biobased and biodegradable. GLY has a molecular weight of 92.094 g/mol and a boiling point at 290 °C.
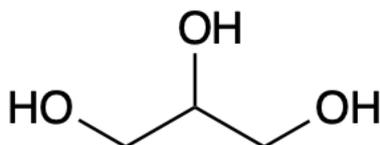
Figure 3.3   Glycerol molecule.

3.3.1 Effect on material properties

Protein films without added plasticizers may be too brittle to perform mechanical testing [29]. In general, plasticizers reduce brittleness, increase elongation, lower the glass transition temperature, and decrease Young's modulus of the material. Adding plasticizers can increase the solubility of the material, and increase the permeability of water vapor, which is typically undesired for applications as food packaging material [28]. Additionally, since the functional mechanism of plasticizers involves occupying free space within the polymer network without forming strong bonds with the polymer, the plasticizer molecule can desorb from the material. Therefore, it is necessary that the plasticizing agent is non-toxic and harmless to the environment. Besides choosing an appropriate plasticizing agent, the concentration of the plasticizing agent is an important factor which directly affects the properties of the material.

3.3.2 Crosslinking extender

While glycerol may act as a plasticizer in a keratin-based material, it can also act as an extender molecule in combination with a cross-linking agent. By reacting with CA through esterification, a larger branched network can form [30]. This can offset the relatively low cross-linking efficency of CA by enabling longer linkages between keratin molecules. In fact, it has been suggested that such an extension is necessary to achieve cross-linking between proteins [21]. Figure 3.4 shows how GLY, CA and keratin can react to form an extended cross-linking network.
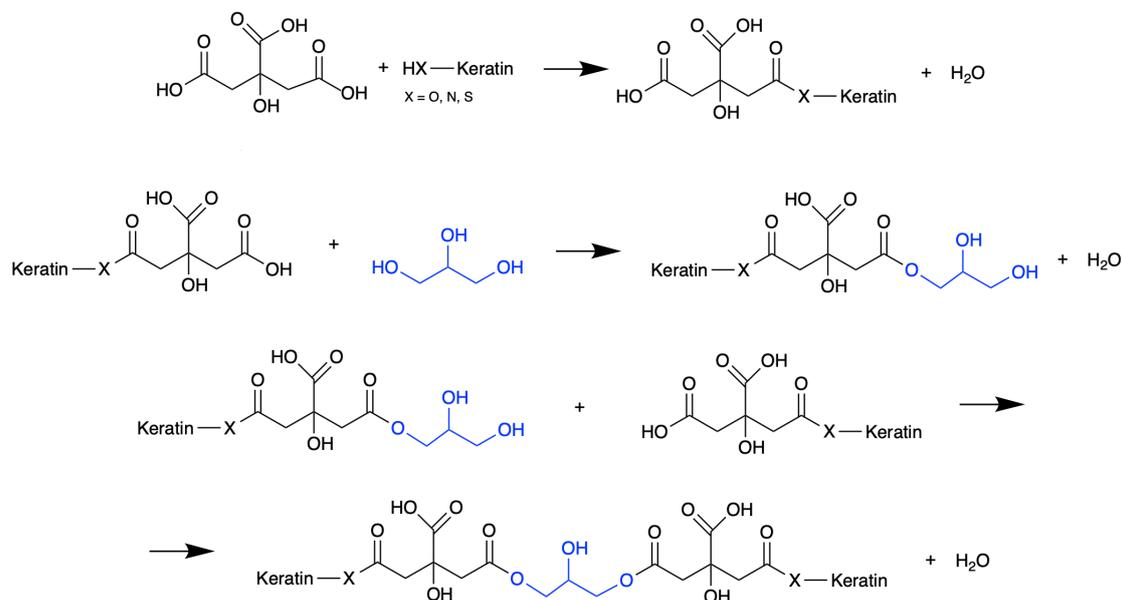
Figure 3.4 Reactions between CA, keratin and GLY, with GLY acting as a cross-linking extender.

3.4 Glycerol Citrate

The reaction between glycerol and citric acid, forming glycerol citrate, is believed to be an integral part of this project. Before adding keratin, the reaction conditions for glycerol citrate are investigated, to find the minimum requirements for a reaction to occur. The reaction between glycerol and citric acid has been studied and used to produce commercial biodegradable plastics [31]. The reaction is a seemingly simple condensation reaction, one molecule of water is lost when a carboxyl group of CA reacts with a hydroxide group of GLY to form an ester bond. However, there exist other possible outcomes that need to be taken in consideration. Furthermore, researchers describe the resulting polymer as a heavily cross-linked, branched polymer network, with the theoretical possibility that almost all COOH groups in CA have reacted with OH groups in GLY [23, 31, 32]. This implies that the previously suggested linear cross-linking mode, which is a result of the requirement for a cyclic anhydride intermediate, may be changed due to the incorporation of GLY.

3.4.1 Kinetics

Besides esterification between CA and GLY, two CA molecules can form an ester linkage together, and CA can undergo dehydration, as well as decarboxylation. Furthermore, the reactivity of different hydroxyl and carboxyl groups may differ. CA contains two carboxyl groups connected to β-carbons (β-COOH) and one carboxyl group connected to an α-carbon (α-COOH). GLY contains two primary hydroxide groups and one secondary hydroxide group. The functional groups are highlighted in figure 3.5.
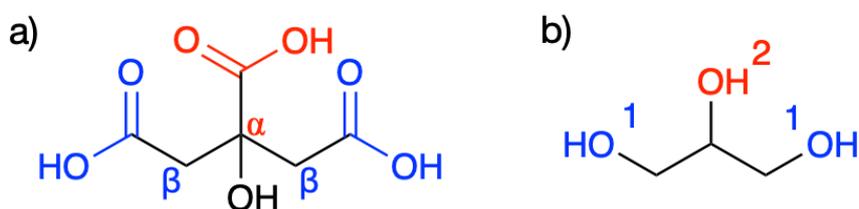
Figure 3.5 **a)** Citric acid's carboxyl groups "β-COOH" connected to β-carbon highlighted in blue, carboxyl group "α-COOH" connected to α-carbon highlighted in red. **b)** Primary alcohols (blue) and secondary alcohol (red) of glycerol.

The possible reactions between two CA molecules are esterification between either α-COOH or β-COOH of one molecule and the OH group of another. When CA reacts with glycerol the possible mechanisms are β-COOH and primary OH, β-COOH and secondary OH, α-COOH and primary OH, as well as α-COOH and secondary OH. Below 140 °C the reactions between β-COOH and primary alcohols, as well as the reaction between α-COOH and primary alcohols, are significantly more favoured than the other possible reactions [32]. This is illustrated in figure 3.6, where the linear relationship between the kinetic constants of the reactions are shown, at temperature 120 °C.

Lower reaction temperatures favor both the reactions between CA and GLY, and the dehydration of CA, while preventing decarboxylation of CA. Temperatures as low as 80 °C have been suggested to be sufficient for synthesis of glycerol citrate
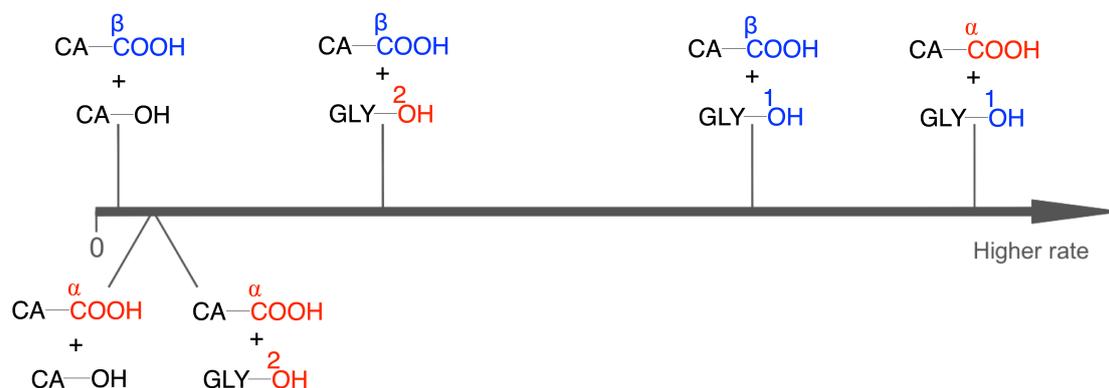
Figure 3.6 The linear relationship between the kinetic constant values of the possible reaction pathways, at 120 °C.

[31]. However, carrying out the reaction under lower temperatures may require days to form a sufficiently cross-linked polymer [23].

3.4.2 Glycerol–citric acid ratio

In order to make the material more flexible, there needs to be unreacted GLY present, acting as a plasticizing agent. This may require an excess of GLY when preparing the glycerol citrate resin. Resins have been successfully prepared using a 1:1-1.8 molar ratio of CA:GLY [31]. However, it is possible that an excess of glycerol may decrease the extent of polymerization, and result in an OH-terminated polymer. This can be demonstrated by calculating the average functionality, see equation 3.1.

$$f_{av} = \frac{2 \cdot n_A}{\sum N_i} \tag{3.1}$$

The variable n_A is the amount of the functional groups of the monomer that is limiting the reaction, or in the equimolar case it can be the amount of either functional group, and N_i is the amount of each monomer. If GLY is modelled as a trifunctional monomer with three equally reactive COOH groups, and GLY is

modelled in the same way with three equally reactive OH groups, then the equation can be used to calculate f_{av} for equimolar amounts and in the case of an excess of GLY. Equation 3.2 shows f_{av} for 1 mole CA and 1 mole GLY, whereas equation 3.2 uses 1 mole CA and 1.5 mole GLY as example.

$$f_{av} = \frac{2 \cdot 3}{1 + 1} = 3 \tag{3.2}$$

$$f_{av} = \frac{2 \cdot 3}{1 + 1.5} = 2.4 \tag{3.3}$$

The equations model how the average functionality decreases when the excess of one of the reactants increases. This implies that using an excess of glycerol would reduce the degree of polymerization. However, the model is a simplification; CA has four functional groups, and the OH and COOH groups in CA and GLY have different functional groups, as discussed previously. Nevertheless, the molar ratio of CA:GLY is likely to be an important parameter that influences the polymerization.

4   Experimental Work

4.1   Materials

Citric acid (100.0 %, VWR Chemicals), glycerol (99.9 %, VWR Chemicals), sodium hypophosphite monohydrate (Alfa Aesar), and sulfuric acid (95 %, Fischer Scientific) were used as received. Keratin microfibers (Batch nr. MF-XF 17+22) were supplied by Bioextrax.

4.2   Glycerol Citrate Polymerization

The initial experiments investigated the reaction between GLY and CA without incorporating MF. Different reaction temperatures, durations and vessels were tested and evaluated before moving on to the addition of MF.

4.2.1   Pre-polymerized resin formation in glass tubes

CA and GLY were added to 15 mL glass tubes with CA:GLY molar ratios 1:1 and 1:2. The tubes were heated using a block heater and jacketed casings at 120 °C for 3 and 6 hours. Samples were checked on every 30 minutes, to note the extent of condensation. After heating, samples were cooled to room temperature and de-ionized (DI) water was added to fill the remaining volume of the tubes. After 5 minutes the samples were mixed using a vortex mixer.

Adding catalyst

In order to investigate the possibility of pre-polymerization at lower temperatures, samples were prepared with catalytic amounts of SHP or H_2SO_4. These samples were heated for 3 hours at 90 °C and checked on every 30 minutes. After cooling down to room temperature, DI water was added and the tubes were stirred. All resin samples prepared in glass tubes with jacketed heating are summarized in table 4.1.

Table 4.1   Preparation of pre-polymerized resin samples in 15 mL glass tubes with jacketed heating.

CA	GLY	Molar ratio	Temp.	Duration	Catalyst
(g)	(mL)	CA:GLY	(°C)	(h)	
2.63	1.00	1:1	120	3	-
2.63	2.00	1:2	120	3	-
2.63	2.00	1:2	120	6	-
2.63	1.00	1:1	120	6	-
1.32	1.00	1:2	90	3	-
1.32	1.00	1:2	90	3	H_2SO_4 (50 µL)
1.32	1.00	1:2	90	3	SHP (50 mg)

Adding microfiber

Three samples were prepared according to the previous process, with an addition of 100 mg MF in each sample. The samples were heated at 120 °C for 1-3 hours. After cooling down to room temperature, DI water was added to the tubes.

4.2.2   Using larger surface area and higher temperature

To increase the surface area of the reaction mixture, and prevent water condensation inside the vessel, three different kinds of vessels were used for the reaction;

aluminum foil sheets, glass petri dishes and silicone rubber forms. Additionally, the temperature was increased to 140 °C and samples were heated in a fan oven.

Glass petri dishes

Initially, two samples were prepared in glass petri dishes (6 cm diameter). The molar ratios of CA:GLY was 1:1 and 1:2.262. After heating, the samples were cooled to room temperature. The samples that were prepared in glass dishes were scraped off using a spatula, and pieces of the sample were immersed in DI water.

Aluminum foil

In an attempt to reduce the adhesion between the polymer and the vessel, aluminum foil was chosen as reaction vessel. Samples with different GLY amounts were prepared; 0.25, 0.50 and 1.00 mL. The CA content was kept constant at 0.50 g. After heating, the samples were cooled down to room temperature and the aluminum foil was peeled off.

Silicone rubber

To further minimize the adhesion between the polymer and the vessel, conventional silicone rubber baking forms (6 cm diameter) were used as reaction vessels. Samples were prepared according to the same method as used for aluminum foil experiments.

Glass tube control samples

In order to assure that the choice of reaction vessel caused polymerization, rather than heating temperature being the only factor, control samples were prepared in glass tubes and heated in the fan oven. After heating, samples were poured onto aluminum foil and cooled to room temperature. Resin samples prepared in a fan oven using various containers are summarized in table 4.2.

Table 4.2   Preparation of pre-polymerized resin samples in glass petri dishes, silicone rubber forms, or on aluminum foil sheets, heated in a fan oven at 140° for 3 hours.

CA	GLY	Molar ratio	Container
(g)	(mL)	CA:GLY	
4.00	4.00	1:2.62	Glass dish
5.26	2.00	1:1	Glass dish
0.50	0.25	1:1.31	Aluminum foil
0.50	0.50	1:2.62	Aluminum foil
0.50	1.00	1:5.24	Aluminum foil
0.50	0.25	1:1.31	Silicone rubber
0.50	0.50	1:2.62	Silicone rubber
0.50	1.00	1:5.24	Silicone rubber
0.50	0.25	1:1.31	Glass tube
0.50	0.50	1:2.62	Glass tube
0.50	1.00	1:5.24	Glass tube

Measuring the extent of the reaction

Silicone rubber trays were chosen as reaction vessels. Six samples were prepared. The ratio of CA:GLY was kept constant at 0.50 g CA and 0.25 mL GLY. The samples were heated in a fan oven at 120 °C for 1-6 hours. The exact weight of CA and GLY was measured. During mixing, some of the resin was lost due to adhesion to the spatula, this portion of the resin was assumed to be a homogeneous mix of CA and GLY, and was deducted from the total weight. The amount of CA and GLY in each sample was calculated, and weight measurements were made before and after heating.

4.3   Thin Film Formation and Incorporation of Microfibers

In order to generate thin films a method was developed that utilized a conventional flatiron to press and heat a resin-MF mix between two aluminum foil sheets. The temperature of the flatiron was measured with a thermometer to 160 °C and was kept at the same setting throughout all experiments. The resin was prepared in silicone rubber forms in a fan oven. While mixing the resin with the MF, the silicone rubber form was heated on a hot plate at 100 °C to increase the viscosity of the resin and allow for even mixing. For all films made with the incorporation of MF, control films without MF were made using the same temperatures, durations and amounts of CA and GLY.

4.3.1   Finding the conditions for thin film formation

Four parameters were varied to find conditions that allowed for thin film formation. The amount of GLY, the amount of MF, the heating duration of the resin, and the duration of the pressing with the flatiron were varied. The CA content was kept constant at 0.50 g, and the heating temperature of the fan oven was maintained at 120 °C.

Glycerol and microfiber content

Three different amounts of GLY was added when preparing resins; 0.25, 0.50 and 1.00 mL. For MF the amounts used were 50, 100 and 150 mg. The resins were heated for 3 hours. The MF was added portion-wise to the resin and was mixed using spatulas. After mixing, the MF-resin paste was pressed between aluminum sheets for 5 minutes. After cooling down to room temperature, the sheets were peeled off.

Resin heating duration

The heating duration of the resin was varied between 2-6 hours. The MF content was kept constant at 100 mg and also the GLY content at 0.25 mL. Films were prepared using the previous process.

Pressing duration

A resin-MF mix with 2000 mg CA and 1.00 mL GLY resin heated for 5 hours, and 400 mg MF was prepared and divided into five pieces that were pressed between aluminum sheets for durations of 1, 5, 15, 20 and 30 minutes. Table 4.3 summarizes the films made with resin and MF.

4.4 Methods of Analysis

Besides measurements of weight loss, dissolution in water, and purely qualitative measurements, additional quantitative measurements were carried out at the Centre for Analysis and Synthesis at Lund University. This analysis was done after all of the laboratory work was completed.

4.4.1 Thermogravimetric analysis

Thermogravimetric analysis (TGA) was carried out using a TGA Q500 from TA Instruments. Initially samples were heated to 120 °C and kept at isothermic conditions for 20 minutes to evaporate any moisture. Samples were then heated from 50 to 600 °C at a rate of 20 °C/min. The samples were heated in a pure nitrogen environment with a flow rate of 50 mL/min. Weight loss and the first derivative of the weight loss were recorded, as well as the onset degradation temperature, and the maximum degradation rate. Eight samples were analyzed; citric acid, glycerol, microfibers, glycerol citrate heated for 5 and 6 hours, and MF-resin films pressed for 1, 15 and 30 minutes.

4.4.2 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was done using a DSC Q2000 from TA instruments. Samples went through a heat/cool/heat cycle from -20 to 250 °C, with a heating rate of 20 °C/min and a cooling rate of 10 °C/min. The atmosphere was pure nitrogen with a flow rate of 50 mL/min. The samples analyzed were pure MF, glycerol citrate heated for 6 hours at 120 °C and a MF-resin film pressed for 30 minutes.

Table 4.3   Preparation of MF films with varying CA:GLY ratio, amount of MF, heating duration and pressing duration.

CA (g)	GLY (mL)	Molar ratio CA:GLY	MF (g)	Temp. (°C)	Heating (h)	Pressing (min)
0.50	1.00	1:5.24	50	120	3	5
0.50	1.00	1:5.24	100	120	3	5
0.50	1.00	1:5.24	150	120	3	5
0.50	0.50	1:2.62	50	120	3	5
0.50	0.50	1:2.62	100	120	3	5
0.50	0.50	1:2.62	150	120	3	5
0.50	0.25	1:1.31	50	120	3	5
0.50	0.25	1:1.31	100	120	3	5
0.50	0.25	1:1.31	150	120	3	5
0.50	0.25	1:1.31	100	120	2	5
0.50	0.25	1:1.31	100	120	4	5
0.50	0.25	1:1.31	100	120	5	5
0.50	0.25	1:1.31	100	120	5	1
0.50	0.25	1:1.31	100	120	6	5
0.50	0.25	1:1.31	100	120	5	15
0.50	0.25	1:1.31	100	120	5	20
0.50	0.25	1:1.31	100	120	5	30

4.4.3 Infrared spectroscopy

Infrared spectroscopy (IR) was performed using an Alpha II model from Bruker. The instrument was in attenuated total reflectance mode. Obtained spectras were averaged over 24 scans from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Samples

were analyzed at three different positions and the results were averaged. The samples analyzed were CA, GLY, MF, glycerol citrate heated for 5 and 6 hours, and MF-resin films ironed for 1, 15 and 30 minutes.

5

Results and Discussion

5.1 Glycerol Citrate Polymerization

The work needed to produce a resin with desired properties proved to be immense. The reaction was not as straightforward as initially hypothesized, and properties like adhesion and viscosity turned out to be crucial parameters that needed to be understood and controlled before adding MF. Generating the resin and then handling it was not a simple task, and much effort went into finding appropriate vessels and reaction conditions to obtain a resin that was easy to work with.

5.1.1 Pre-polymerized resin formation in glass tubes

After only a few minutes of heating, citric acid and glycerol started to form a transparent liquid. The viscosity of the liquid was dependent on the ratio of CA and GLY, samples with 1:2 molar ratio CA:GLY were significantly less viscous than samples with 1:1 molar ratio. As expected, a higher temperature lowered the viscosity of the resin. After heating at 120° C for a few minutes, both molar ratios resulted in transparent liquids that could be poured from the tubes. However, when cooled down to room temperature, the 1:1 molar ratio resin showed minimal flow, while the 1:2 sample flowed very slowly.

After 30 minutes of heating, there was noticeable condensation of water on the

inner walls of the glass tubes. Since water is the byproduct of the esterification of GLY and CA, this served as an indication that polymerization had been initiated. During the remaining 2.5 hours of heating, there was condensed water present at all times. The stages of the resin preparation are shown in figure 5.1.
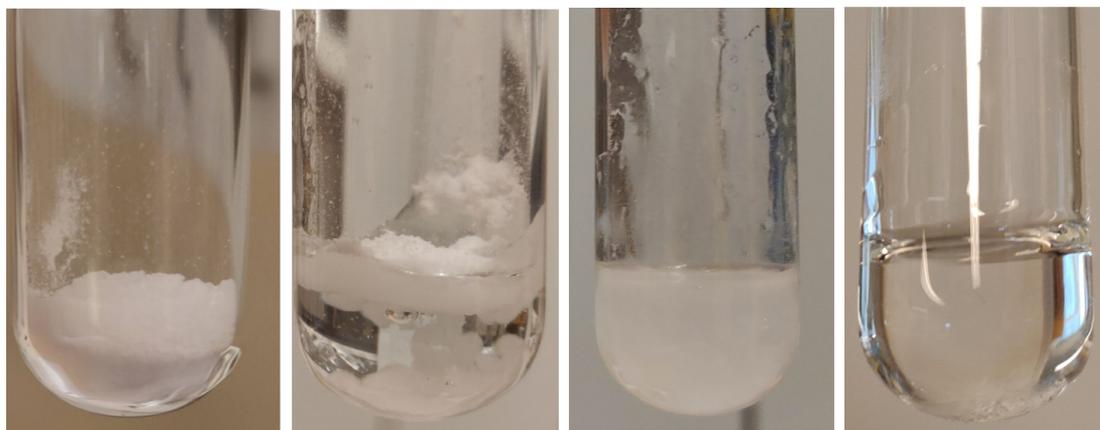

Figure 5.1 From left to right: CA, CA and GLY, CA and GLY mixed, CA and GLY mix after heating at 120°C for 30 minutes.

Upon adding water to the resin samples, there was no immediate dissolution of the resin. However, after a few minutes of rigorous mixing the resin was completely dissolved. This was the case for both molar ratios used, however the resin with a higher amount of GLY was more easily mixed due to it's lower viscosity. The dissolution of the resin indicated that the degree of polymerization was low.

Resins heated for 6 hours behaved very similar to the resins heated for 3 hours. There was still condensation on the inner walls of the glass tubes after 6 hours of heating, and the resins dissolved after a few minutes of stirring. Even though the condensation on the walls of the tube indicated that the reaction was taking place, it also served as an indicator that the removal of water was insufficient. The resin in the glass tubes had a small surface area and the inside of the tube walls could act as a surface for condensation, resulting in some of the condensed water dripping back down into the resin. Since the ester reaction is an equilibrium reaction, the rate of water removal could be the limiting rate for the reaction [23, 31]. Hence, the glass tubes may have limited the polymerization of the resin. This

meant that choosing a vessel that limits the evaporation of water could prevent the polymer from curing inside the vessel, however it may not reach a desired degree of polymerization. Conversely, curing the resin may have required a vessel that allowed for faster evaporation of water.

Adding catalyst

The control sample with no catalyst, heated at 90 °C, showed minimal amounts of condensation after 1 hour. The same was true for the sample with 50 mg SHP added. However, the sample with 50 µL H_2SO_4 showed significant condensation. Acids are common catalysts used for esterification, and the results indicated that the reaction temperature could be lowered if an acid catalyst was added.

Adding microfiber

When MF was added to the CA and GLY, the formed resin was highly viscous and could not be poured from the tubes. Longer heating time resulted in a deeper brown colour of the resin. During the heating there was condensation in all of the samples, which indicated that esterification reactions were taking place. However, the resin dissolved in water almost immediately. The heating duration made no noticeable difference. Since the reaction mixture could not be poured from the tubes, and the polymerization seemed to be very limited, it was deemed necessary to switch to using vessels and heating methods that allowed for more efficient evaporation of water.

5.1.2 Using larger surface area and higher temperature

At this stage, no water insoluble polymer had been obtained, meaning that the extent of cross-linking had been insufficient. To shift the equilibrium reaction further to the product of water, the temperature was increased and various reaction vessels were used. The idea was to increase the surface area of the resin and reduce the surface area that water could condense onto. Additionally, the first sample that was prepared this way used an equimolar ratio of CA and GLY to further favour the cross-linking reaction.

Different reaction vessels

Transitioning from heating in glass tubes in a block heater to flatter vessels in the fan oven yielded immediate results. The first two samples prepared in glass petri dishes and heated at 140° C for 3 hours resulted in hard and solid polymers. It was impossible to remove the polymers from the glass dish in one piece, so small pieces had to be scraped off using a spatula, and were then immersed in 50 mL DI water. The sample with highest molar ratio of GLY, 1:2.62 molar ratio CA:GLY was smooth and transparent, whereas the 1:1 sample showed bubble formation inside the polymer. When the 1:2.62 sample was immersed in water, the pieces looked like a swollen hydrogel. The pieces from the 1:1 sample were harder and more difficult to scrape off, and seemed to swell very little. These pieces were still insoluble more than two months later, without changes in appearance. The pieces from the 1:2.62 sample however, were completely dissolved after 4 weeks. The insolubility of the samples was an indication that cross-linking occurred. As expected, the sample with the equimolar ratio showed signs of increased cross-linking, since it was insoluble for a longer duration and showed less swelling. This was the first indication that confirmed the hypothesis that an excess of GLY could reduce the degree of polymerization, as discussed in chapter 3. Nevertheless, since cross-linking was proven possible using higher amounts of GLY, the rest of the samples prepared used an excess of GLY to be used as a plasticizer in the final product.

In order to maintain a high surface area yet also reduce the adhesion between polymer and reaction vessel, extra thick aluminum foil was used. For the samples prepared on aluminum foil sheets, it was possible to peel off only one. The sample with the lowest amount of GLY (1:1.31 CA:GLY ratio) could be peeled off in one piece. The sample was a hard, brittle and transparent polymer with a smooth surface. Small pieces of the sample were cut off and immersed in 50 mL DI water. The pieces still remained insoluble after more than two months. The sample with 0.50 mL GLY had a hard surface but was more flexible, though it could not be peeled off. The sample with 1.00 mL GLY did not solidify. Again, these results demonstrated the importance of using an appropriate ratio of CA:GLY.

Since the resin still adhered quite strongly to the aluminum foil, silicone rubber was chosen as a reaction vessel due to the material's inert nature. This was by far the least adhesive material for the resin, and samples were significantly easier to dislodge. The sample with 0.25 mL GLY formed a solid and hard polymer, still insoluble in water after more than a month. The 0.50 mL GLY sample could be peeled off but parts of it had not solidified. This sample was stable in water for a few days at most. Again, the sample with 1.00 mL did not solidify.

None of the samples prepared in glass tubes solidified when cooled down. When the heating process was finished, there was still condensation on the inner walls of the tubes, suggesting that water was not being removed efficiently. It could therefore be concluded that the choice of reaction vessel was important for two reasons; it affected the rate of water removal and also the ability to dislodge the polymer from the vessel in one piece. All reaction vessels that improved the evaporation of water resulted in successful cross-linking, and yielded polymers that were insoluble in water. Figure 5.2 shows cross-linked samples made in glass, aluminum and silicone rubber vessels. The highly inert silicone rubber causes the resin to spread out less, which decreases the surface area of the resin and thereby increases the required heating duration. Despite this, the silicone rubber forms were the chosen vessels used for future experiments, due to the ease of dislodging the polymers.
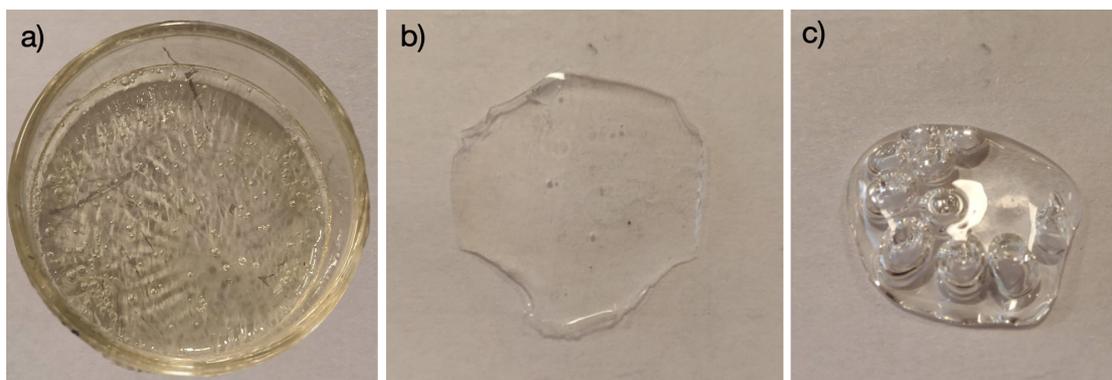

Figure 5.2   Cross-linked polymer prepared in **a)** glass petri dish, **b)** on aluminum foil, **c)** in silicone rubber form.

Resin weight measurements

The amount of weight lost during heating for pre-polymerized resin samples is shown in figure 5.3. The results indicated that there was a continuous loss of weight even up to six hours reaction time. Samples heated for 1-3 hours had not solidified and dissolved quickly in water. The sample heated for 4 hours was partially solid and dissolved in water after a few minutes. Samples heated for 5 and 6 hours were solid and insoluble in water for at least one month.

The weight loss difference between the 4 hour sample and the 5 hour sample was small, yet the difference in insolubility was great. This experiment was carried out without using replicates, which would be needed to establish the weight loss with more accuracy. It was believed that if the degree of polymerization was too high, it would not be possible to mix the resin with the MF due to it's high viscosity, and also there would not be a sufficient amount of functional groups to react with the MF. After six hours of heating, the resin was still liquid before it cooled down to room temperature, and this was chosen as the upper limit for the resin heating duration.

5.2 Thin Film Formation and Incorporation of Microfibers

Since the goal of the project was to produce thin films, there was a need to alter the synthesis method. For this purpose, a flatiron was used to mimic the conventional pressing method used when curing polymers. The idea was to press a mixture of MF and resin between sheets of aluminum foil to generate thin films. From previous experience it was clear that mixing the microfibers with the resin could be difficult if the resin was cooled down and it's viscosity increased, therefore the mixing was done while the resin was heated. This method proved to be efficient, it was possible to obtain evenly mixed pastes using almost all samples prepared for these experiments. However, pressing pre-polymerized resins without added microfibers proved to be extremely difficult. When pressed, the resins spread out over the sheets and made them near impossible to peel off. The resins, regardless of heating duration and CA:GLY molar ratio, did not form any cohesive pieces of
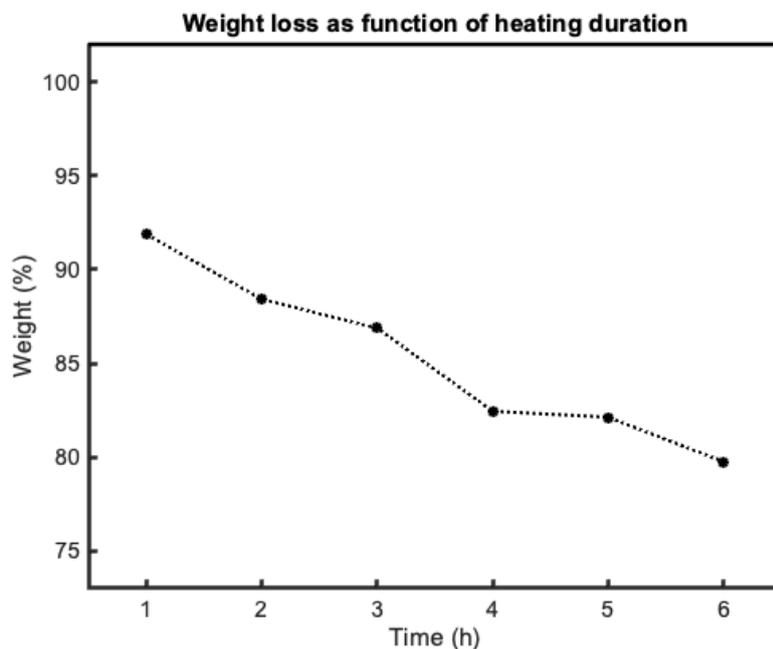
Figure 5.3 Weight loss of resin samples prepared in silicone rubber baking forms, with 1:1.31 molar ratio CA:GLY. Samples were heated in a fan oven for 1-6 hours at 120 °C.

polymer. This made it impossible to produce control samples in the shape of thin films, since MF played a key role in enabling film formation.

5.2.1 Glycerol and microfiber content

The GLY and MF content was proven to be crucial for thin film formation. As previously mentioned, without MF it is extremely difficult to form a cohesive film. On the other hand, the sample prepared with 150 mg MF and 0.50 mL GLY made the mixing difficult, and after pressing the sample there was a significant amount of MF that was not incorporated in the resin-MF film. The sample prepared with the highest amount of GLY and the lowest amount of MF, 1.00 mL and 50 mg respectively, was indeed easy to mix, but when pressed the resin spread out and was not contained by the MF. The sample prepared using 100 mg MF and 0.25 mL GLY yielded the best results in every aspect; mixing was easy and the pressed film showed minimal leakage of resin. After cooling down, the film was easy to

peel off, see figure 5.4. These results demonstrated that GLY and MF content should be carefully optimized. Since the addition of MF were necessary for thin film formation, this led to the assumption that the adhesion between MF and the resin is very strong. This could have been a result of extensive hydrogen bonding.

Figure 5.4   MF-resin thin film formed with 100 mg MF, 0.50 g CA and 0.25 mL GLY. The mixture was pressed for 5 minutes.

5.2.2 Resin heating duration

With the MF and GLY content kept constant, it was observed that the resin heating duration also played a key role in thin film formation. Only resins heated for 5 or 6 hours yielded films that could be peeled off in one piece. The resins heated for a shorter duration leaked out of the MF-resin paste during pressing. It could be that an increased content of MF would allow for thin film formation even with resins heated for a short duration, due to the interaction between MF and resin where MF seemed to contain the resin during the pressing. Regardless, the optimal conditions for thin film formation were determined to be the following: 100 mg MF added to resin prepared with 0.5 g CA and 0.25 mL GLY having been heated for 5 hours.

5.2.3 Pressing duration

For these experiments, a larger batch of MF-resin mix was prepared and split into five pieces in order to make the resulting samples as uniform as possible. The effect of pressing duration was significant in various regards. Upon immersion in DI water, the samples pressed for 1 and 5 minutes dissolved quickly. The samples pressed for 15 and 20 minutes fractured into smaller pieces, which still remained intact more than two months later. The sample pressed for 30 minutes was not fractured, and also remained intact, however it became very soft and flexible. The pressing duration also had an effect on the flexibility of the film in the dry state, with increased rigidity as the pressing duration increased. Immediately after pressing and cooling down, the films pressed for 15 minutes and more were hard and seemed brittle, but after storing them for a day in ambient conditions, they became more flexible. In fact, they could be folded multiple times. This indicated that the films had absorbed some moisture which acted as a plasticizer and increased the flexibility. Figure 5.5 displays a film pressed for 1 minute and another pressed for 30 minutes. Visually the increased heating duration resulted in a darker film due to the MF being burnt to some extent, and the resulting film was also thinner.

5.2.4 Qualitative analysis of films

The final films, pressed for different durations, were brittle immediately after processing but became flexible after storage under ambient conditions. This indicated that the films had absorbed moisture, effectively plasticizing the material. Using a longer pressing duration resulted in reduced flexibility, which could be a result of increased cross-linking in the resin and/or cross-linking between the resin and the MF. Since the film pressed for 30 minutes was the most stable in water, while still maintaining good flexibility in it's dry state, this film was deemed to be the best sample produced.
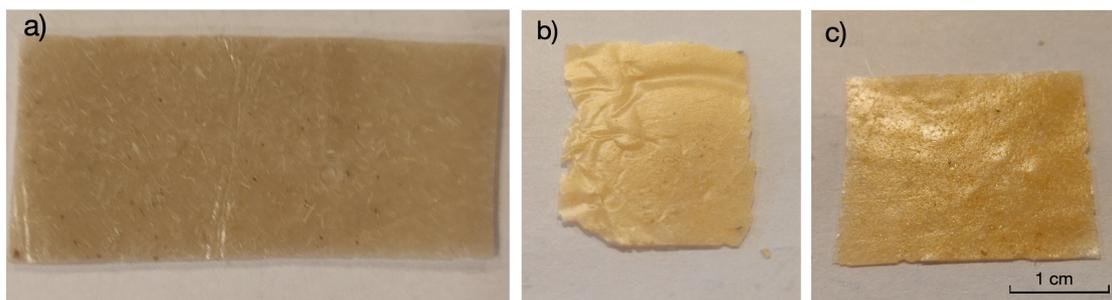
Figure 5.5 MF-resin films pressed for **a)** 1 minute, **b)** 15 minutes and **c)** 30 minutes.

5.3 Quantitative Analysis

For the quantitative analysis there were three sets of samples analyzed; the pure reagents (MF, CA and GLY), three thin films pressed for different durations, and two glycerol citrate samples heated for different durations. Since it was impossible to create pressed control films without MF, glycerol citrate heated in a fan oven were used instead.

5.3.1 Thermogravimetric analysis

Figure 5.6 shows the weight loss and derivative weight loss for the samples, aswell as the onset degradation temperature and the peak degradation rate. Both CA and GLY are fully decomposed at 300 °C. Glycerol citrate heated for 5 hours shows a stepwise degradation, possibly due to the reaction of residual CA and GLY, resulting in a weight loss due to the evaporation of the generated water. Conversely, glycerol citrate heated for 6 hours shows a different degradation profile where the material degrades in one step, suggesting that very little unreacted CA and GLY is present. This result explains why mixing resin heated for 6 hours with MF was difficult, the glycerol citrate resin was polymerized to such an extent that it had solidified and had fewer functional groups available for interaction with the MF.

For the glycerol citrate heated 6 hours, and the MF-resin films, the thermograms indicate that there is little unreacted CA and GLY in the sample. There is no

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degradation at temperatures lower than 350 °C. Despite the initial excess of GLY it seems like all of the GLY has been incorporated into the polymer, hence the effect as a plasticizing agent may be negligible. Indeed, the pressed films were quite brittle immediately after processing, and only became flexible after a day's storage in ambient conditions. This suggests that the plasticizing mechanism is a result of absorbed water.

Pure microfibers have the highest onset degradation temperature and also the highest peak degradation rate. The degradation profile is broad, since the protein contains a wide variety of chemical compounds that start to degrade at different temperatures. All MF-resin films show higher thermal stability than the pure glycerol citrate samples, however it can not be concluded that this is due to the incorporation of MF. The resin used for the MF-resin films was heated for 5 hours, but during pressing the resin could have reached a higher degree of polymerization than the glycerol citrate sample heated for 6 hours.

The MF-resin films contained approximately 14 wt% MF. The degradation curves do not show any clear separation between the degradation of the glycerol citrate and the incorporated MF, however the degradation rate shows a broader peak than the glycerol citrate curves, indicating that MF residue degrades last. The film pressed for 15 minutes showed slightly lower thermal stability than the film pressed for only 1 minute, whereas the 30 minute film showed significantly better thermal stability. This can be explained by changes in the MF during heating. During pressing, the keratin in the MF starts to denature and more functional groups become accessible. After 15 minutes, the protein has been degraded and hence the thermal stability is worsened. However, after 30 minutes, the protein has degraded but the additional pressing duration has allowed for increased interactions between the resin and the MF, either via covalent bonding or hydrogen bonding. This would also explain why the film pressed for 15 minutes dissolves in water within minutes, whereas the 30 minute film is stable after 3 months.
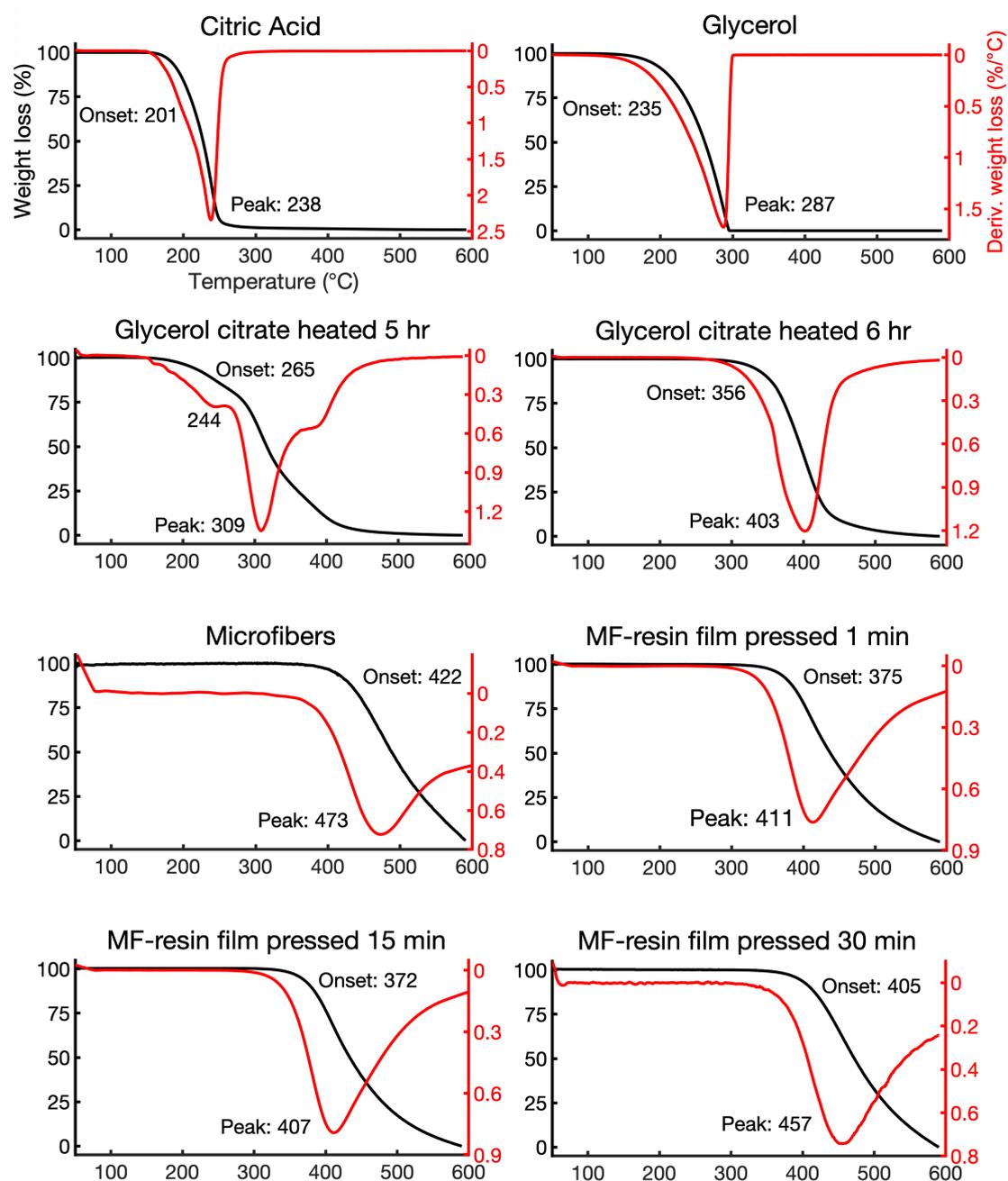
Figure 5.6 Weight loss and derivative weight loss for CA, GLY, MF, glycerol citrate samples and pressed MF-resin films.

5.3.2 Differential Scanning Calorimetry

Three samples were analyzed using DSC; pure MF, glycerol citrate heated for 6 hours, and the MF-resin film pressed for 30 minutes. Figure 5.7 shows the thermograms for the three samples. Since the TGA measurements showed no degradation below 350 °C for these samples it is assumed that the samples are fully intact during the measurements. It is likely that the samples contain some moisture, especially the MF-resin film which has showed signs of absorbing moisture.

The three thermograms each show a distinct endothermic event, possibly a glass transition. The pure microfibers show the highest temperature for the event, at 191.5 °C. The glycerol citrate undergoes an endothermic transition at 77.1 °C, and the MF-resin film at 99.3 °C. Incorporating MF increased the temperature where the endothermic event occurs, and only one such event is visible in the thermogram for the MF-resin film, rather than two separate events at 77.1 °and 99.3 °C corresponding to the ones observed in the other thermograms.

If the events described are glass transitions, it would be expected that addition of MF would increase the glass transition temperature. Regardless whether the MF covalently bind to the resin or rather interact via hydrogen bonding, the mobility of the polymer chains would reduce, resulting in an increased glass transition temperature. Since there is only one such thermal event occurring for the MF-resin film, this could serve as an indication that the system is a one-phase system. If two transitions were visible, corresponding to the transitions in the pure MF and the resin, it would be an indication of a two-phase system.
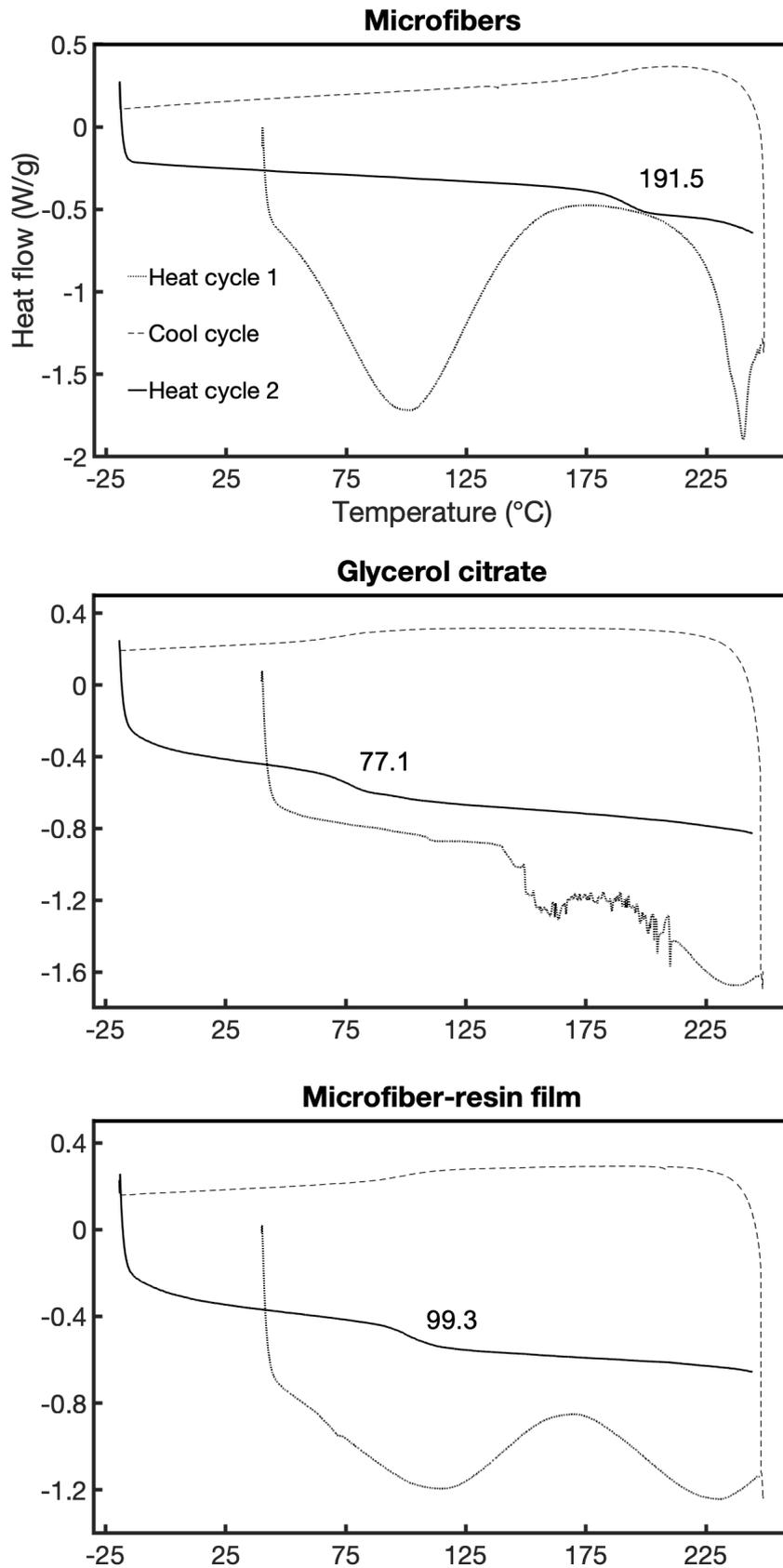
Figure 5.7 DSC thermograms for pure microfibers, glycerol citrate heated for 6 hours, and MF-resin film pressed for 30 minutes. The thermograms show the three heat/cool/heat cycles, as well as the glass transition temperatures.

5.3.3 Infrared spectroscopy

For each sample analyzed, three different measurements were taken. For the solid polymeric samples the measurements were performed at different places on the sample. For all samples, the variation in the spectra differed only a few percent. For the MF-resin films this indicates that the mixing between MF and resin is even. The fingerprint region (below 1500 cm^{-1}) contains molecular fingerprints and varies little between polymeric samples. Therefore, the analysis is focused on the functional group region above 1500 cm^{-1}. Figure 5.8 shows absorption spectra for CA, GLY and glycerol citrate samples.
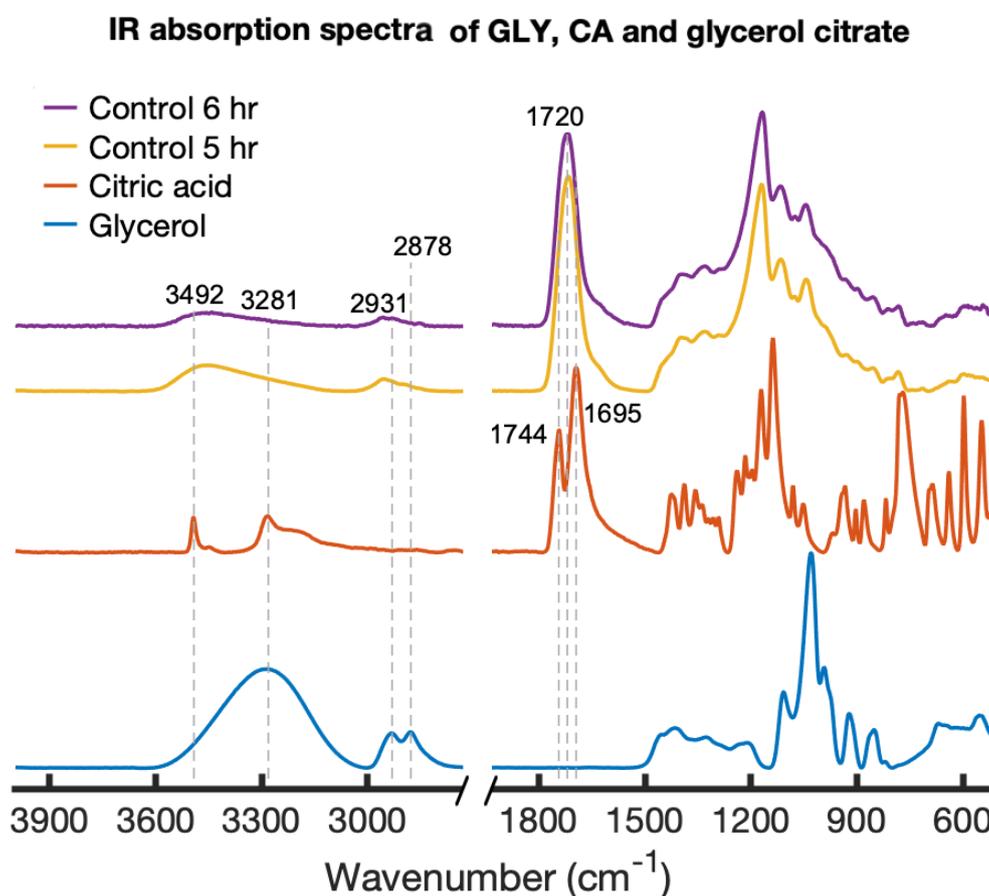

Figure 5.8   Averaged spectra from three measurements each of glycerol, citric acid and glycerol citrate polymers heated for 5 and 6 hours.

Around 3300 cm^{-1} GLY shows a characteristic broad OH peak, and alkyl groups at 2931 and 2878 cm^{-1}. CA shows OH-stretching (carboxyl group) around 3492 and 3281 cm^{-1}, as well as C=O stretching at 1744 and 1695 cm^{-1}. Both glycerol citrate samples have prominent peaks at 1720 cm^{-1} indicating ester formation. The sample heated for 6 hours clearly shows a reduction in OH-stretching around 3300-3500 cm^{-1}, indicating that more of the CA and GLY have reacted.

Figure 5.9 shows the absorption spectra for MF, glycerol citrate heated for 5 hours, and MF-resin films pressed for 1, 15 and 30 minutes. At 3281, 1630 and 1530 cm^{-1} amide A, I and II bands are visible for the pure MF spectrum. The amide I and II bands are visible in the MF-resin film spectra but missing in the glycerol citrate spectrum. For the film pressed for 30 minutes, there is minimal absorption above 1800 cm^{-1}. This indicates that residual carboxylic and OH groups have reacted with the MF or are participating in hydrogen bond formation with the MF. The spectra show that the films pressed for 1 and 15 minutes contain unreacted amides, a contribution from the MF. However, the film pressed for 30 minutes shows a reduction in absorption at 1630 cm^{-1}, indicating that the MF have reacted with the resin. Furthermore, the 1720 cm^{-1} peak is broadened and shifted in the spectrum for the 30 minute film, also an indication that the MF have reacted with the resin.

5.4 Future Work

To the best of the author's knowledge, the thin films formed from glycerol citrate resin and keratin microfibers represent a completely new material. The main goal initially was to synthesize a film that was insoluble in water. Even though this was achieved, much remains to be understood and there is room for further optimization. This project could act as a proof-of-concept and a starting point for future work.

5.4.1 Control samples

One of the main problems with the experimental process was the inability to generate control samples when pressing films. This made it difficult to decisively
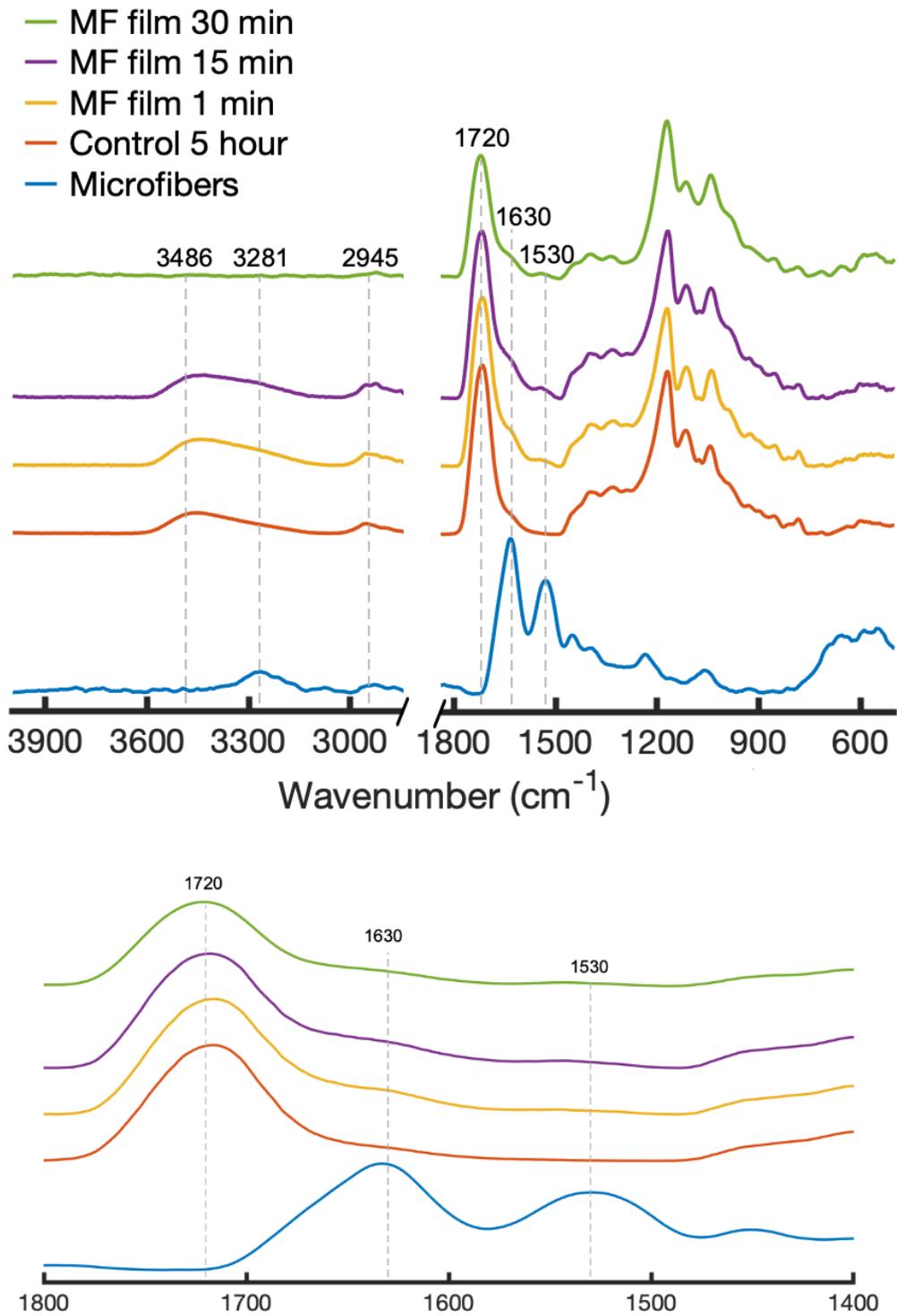
Figure 5.9 Averaged spectra from three measurements each of microfibers, glycerol citrate polymer and MF films pressed for different durations.

conclude the effect of the incorporated microfibers and whether cross-linking had occurred between resin and microfibers. For a future continuation of the project, an alternative approach could be used to generate control samples. One suggestion would be to avoid pressing the MF-resin pastes entirely, and instead heat them in the oven. This process would not produce thin films, however it would make it possible to generate good control samples.

5.4.2 Resin formation

Since the TGA and FTIR results indicate that there is no residual glycerol acting as a plasticizer, resin formation would benefit from reducing the amount of glycerol. The excess glycerol limits the polymerization of the resin and makes fewer COOH groups available for interactions with the MF. Decreasing the amount of glycerol would decrease the duration needed to form the resin, and also improve the interactions with the MF. To further decrease the duration required to form the resin, an acid catalyst can be used.

5.4.3 Further optimization

During this project the experimental process was constantly adapted and altered depending on the day-to-day results. Since there was no previous record of successfully generating similar films, the optimization work had to be adjusted continuously to ensure that at least one insoluble thin film was created. The parameters investigated were all deemed to have significant effects on the resulting film's properties. This means that a future project would benefit from a more rigid design of experiments, choosing a number of parameters to vary that is appropriate for the scope of said project. For example a factorial, fractional factorial, or central composite design could be a suitable starting point.

5.4.4 Additional analysis

In addition to TGA, DSC and FT-IR analysis, there are other relevant techniques that could be used to study the material. Since mechanical properties are of key importance for plastics, a future project could include tensile testing. This could

be done during dry conditions but also during wet conditions, since this project showed that the strength of the material was reduced significantly after immersion in water.

6

Conclusion

Via green conversion of feather waste into keratin microfibers, and upon addition of green modifying agents, a new bio-based and biologically degradable bioplastic has been developed during this project. The required reagents are non-toxic, cheap and derived from renewable sources. Furthermore, the finished product is insoluble in water and is also mechanically flexible. While the minimum requirements are met for a proof-of-concept prototype, there is much left to analyze and to improve.

The initial planning of the experiments revolved around a relatively simple notion of using citric acid as a cross-linker and glycerol as a plasticizer and cross-linking extender. As this project has shown, the reactions between the reagents, and also the practical aspects of film formation, were not so straight forward. Simply mixing the reagents and heating and pressing them into a thin film proved to be difficult. Instead, a glycerol citrate pre-polymerized resin had to act as a carrier for the microfibers. However, the glycerol:citric acid ratio of the resin, as well as the heating duration, had to be optimized so that the resin was in a thermoplastic state but with high enough viscosity to prevent from leaking out during pressing. The preparation of the resin required sufficient time and temperature, as well as a non-adhesive reaction vessel. The adhesion between the resin and the microfibers proved to be strong, in fact it was not possible to press thin films without the addition of microfibers.

The inability to generate thin film control samples without microfibers represents the most prominent challenge of this project. This makes it difficult to conclude whether the system is a two-phase system or not, and exactly how the incorporation of microfibers affect the thermal stability of the films. For a potential continuation of the project, the central focus could be to develop a method which allows for better control samples. Additionally, a design of experiments could be used, varying a suitable amount of process parameters, in order to optimize the process and generate films with improved properties.

The analysis using TGA, DSC and FTIR could be used to confirm some theories and show indications of some specific effects. Again, the analysis would greatly benefit from better control samples. TGA indicates that the addition of MF improves the thermal stability of the samples. The film pressed for 30 minutes shows the highest thermal stability and remains insoluble in water after three months. The films show no weight loss around the degradation temperatures of citric acid and glycerol, indicating that both reagents have reacted and the flexibility of the final films is a result of moisture absoprtion. DSC measurements show an endothermic process for the MF, glycerol citrate and the MF-resin film, at different temperatures for each sample, possibly indicating a one-phase system. FTIR analysis shows the formation of ester bonds in the resin, and a reduction of OH groups in the film pressed for 30 minutes, an indication that all residual monomers have reacted with the MF.

This project can serve as an example of possible challenges that arise when using green alternatives to conventional reagents. To compensate for the low reactivity of the reagents, the developed process requires extensive optimization and probably needs longer time and effort to yield results. Therefore it can be difficult to evaluate the likelihood of success and the amount of time and resources that should be allocated to such a project. For this reason, it is suitable to use an approach that uses readily available and cheap reagents, combined with simple processing methods. For this project, the resulting films can serve as proof-of-concept, however the gaps in the design of experiments affects the quantitative analysis. This project leaves room for future continuation with much left to explore.

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